Olive Oil and Health

Old and thirty olive trees
under the shining sun of the day
dusty olive-tree fields
from the land of Andalucía ...

Antonio Machado (*The olive trees*)
Free translation

Olive Oil and Health

Edited by

José L. Quiles, M. Carmen Ramírez-Tortosa and Parveen Yaqoob

www.cabi.org

To our families

CABI is a trading name of CAB International

CABI Head Office
Nosworthy Way
Wallingford
Oxfordshire OX10 8DE
UK

Tel: +44 (0)1491 832111
Fax: +44 (0)1491 833508
E-mail: cabi@cabi.org
Website: www.cabi.org

CABI North American Office
875 Massachusetts Avenue
7th Floor
Cambridge, MA 02139
USA

Tel: +1 617 395 4056
Fax: +1 617 354 6875
E-mail: cabi-nao@cabi.org

A catalogue record for this book is available from the British Library, London, UK.

A catalogue record for this book is available from the Library of Congress, Washington, DC.

ISBN-10: 1-84593-068-1
ISBN-13: 978-1-84593-068-4

Produced and typeset by Columns Design Ltd, Reading
Printed and bound in the UK by Cromwell Press, Trowbridge

Contents

Contributors

C.M. Aguilera, *Institute of Nutrition and Food Technology, Department of Biochemistry and Molecular Biology, C/ Ramón y Cajal 4, 18071 Granada, Spain.*

F.J. Barbancho, *Nursing School, University of Extremadura, Plasencia University Center, Plasencia, Cáceres, Spain.*

S. Bastida, *Departamento de Nutrición y Bromatología I (Nutrición), Facultad de Farmacia, Plaza Ramón y Cajal s/n, Universidad Complutense, 28040-Madrid, Spain.*

M. Battino, *Institute of Biochemistry, Faculty of Medicine, Marche Polytechnic University, Via Ranieri 65, 60100 Ancona, Italy. Phone: +39 071 2204646. Fax: +39 071 2204398. e-mail: mbattino@univpm.it*

A.B. Blanco Molina, *Department of Medicine, University of Cordoba. Lipid and Atherosclerosis Unit, Reina Sofia University Hospital, Avda Menendez Pidal s/n, 14004 Cordoba, Spain.*

P. Bogani, *University of Milan, Department of Pharmacological Sciences, Via Balzaretti 9, 20133 Milan, Italy.*

E. Cabré, *Section of Gastroenterology, Hospital Universitari Germans Trias i Pujol, Carretera del Canyet s/n, 08916 Badalona, Barcelona, Spain.*

M.A. Carluccio, *Institute of Cardiology, 'G. d'Annunzio' University – Chieti, C/o Ospedale S, Camillo de Lellis, Via Forlanini 50, 66100 Chieti, Italy.*

R. De Caterina, *Institute of Cardiology, 'G. d'Annunzio' University – Chieti, C/o Ospedale S, Camillo de Lellis, Via Forlanini 50, 66100 Chieti, Italy. Tel. +39 0871 41512. FAX: +39 0871 402817. e-mail: rdecater@unich.it*

E. Escrich, *Department of Cell Biology, Physiology and Immunology, Medical Physiology Unit, School of Medicine, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain. e-mail: Eduard.Escrich@uab.es*

C. Galli, *University of Milan, Department of Pharmacological Sciences, Via Balzaretti 9, 20133 Milan, Italy.*

M.A. Gassull, *Section of Gastroenterology, Hospital Universitari Germans Trias i Pujol, Carretera del Canyet s/n, 08916 Badalona, Barcelona, Spain.*

A. Gil, *Institute of Nutrition and Food Technology, Department of Biochemistry and Molecular Biology, C/ Ramón y Cajal 4, 18071 Granada, Spain.*

S. Granados, *Institute of Nutrition and Food Technology, Department of Biochemistry and Molecular Biology, C/ Ramón y Cajal 4, 18071 Granada, Spain.*

S. Grande, *University of Milan, Department of Pharmacological Sciences, Via Balzaretti 9, 20133 Milan, Italy.*

J.R. Huertas, *Institute of Nutrition and Food Technology, Department of Physiology, C/ Ramón y Cajal 4, 18071, Granada, Spain.*

X. Llor, *Section of Gastroenterology, Hospital Universitari Germans Trias i Pujol, Carretera del Canyet s/n, 08916 Badalona, Barcelona, Spain. Tel: +34 93 4651200 Ext. 3716. Fax: +34 93 4978843. e-mail: 23636xlf@comb.es*

M. López-Frías, *Institute of Nutrition and Food Technology, Department of Physiology, C/ Ramón y Cajal 4, 18071, Granada, Spain.*

J.L.J. López Miranda, *Department of Medicine, University of Cordoba. Lipid and Atherosclerosis Unit, Reina Sofia University Hospital, Avda Menendez Pidal s/n, 14004 Cordoba, Spain.*

M. Mañas, *Institute of Nutrition and Food Technology, Department of Physiology, C/ Ramón y Cajal 4, 18071 Granada, Spain.*

E. Martínez-Victoria, *Institute of Nutrition and Food Technology, Department of Physiology, C/ Ramón y Cajal 4, 18071 Granada, Spain. Tel: +34 95 8248321. Fax: +34 95 8248326. e-mail: emiliom@ugr.es*

M. Massaro, *Institute of Cardiology, 'G. d'Annunzio' University – Chieti, C/o Ospedale S, Camillo de Lellis, Via Forlanini 50, 66100 Chieti, Italy.*

J. Mataix, *Institute of Nutrition and Food Technology, Department of Physiology, C/ Ramón y Cajal 4, 18071 Granada, Spain. Tel: +34 95 8244173; Fax: +34 95 8248326; e-mail: jmataix@ugr.es*

M.D. Mesa, *Institute of Nutrition and Food Technology, Department of Biochemistry and Molecular Biology, C/ Ramón y Cajal 4, 18071 Granada, Spain.*

R. Moral, *Department of Cell Biology, Physiology and Immunology, Medical Physiology Unit, School of Medicine, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain.*

J.J. Ochoa, *Institute of Nutrition and Food Technology, Department of Physiology, C/ Ramón y Cajal 4, 18071, Granada, Spain.*

N. Pellegrini, *Department of Public Health, University of Parma, Italy.*

F.P. Pérez-Jiménez, *Department of Medicine, University of Cordoba. Lipid and Atherosclerosis Unit, Reina Sofia University Hospital, Avda Menendez Pidal s/n, 14004 Cordoba, Spain. Tel: +34 957 218250. Fax: +34 957 218229. e-mail: md1pejif@uco.es*

J.S. Perona, *Instituto de la Grasa, CSIC, Av. Padre García Tejero 4, 41012 Sevilla, Spain.*

J.L. Quiles, *Institute of Nutrition and Food Technology, Department of Physiology, C/ Ramón y Cajal 4, 18071 Granada, Spain. Tel: +34 95 8248324; Fax: +34 95 8248326; e-mail: jlquiles@ugr.es*

C.L. Ramírez-Tortosa, *Department of Pathology, Complejo Hospitalario de Jaen, Jaen, Spain.*

M.C. Ramírez-Tortosa, *Institute of Nutrition and Food Technology, Department of Biochemistry and Molecular Biology, C/ Ramón y Cajal 4, 18071 Granada, Spain. Tel: +34 95 8248324; Fax: +34 95 8248326; e-mail: mramirez@ugr.es*

V. Ruiz-Gutiérrez, *Instituto de la Grasa, CSIC, Av. Padre García Tejero 4, 41012 Sevilla, Spain. Tel: +34 95 1641550. e-mail: valruiz@ig.csic.es*

F.J. Sánchez-Muniz, *Departamento de Nutrición y Bromatología I (Nutrición), Facultad de Farmacia, Plaza Ramón y Cajal s/n, Universidad Complutense, 28040-Madrid, Spain. Tel: +34 91 3941828 Fax: +34 91 3941732. e-mail: frasan@farm.ucm.es*

E. Scoditti, *Institute of Cardiology, 'G. d'Annunzio' University – Chieti, C/o Ospedale S, Camillo de Lellis, Via Forlanini 50, 66100 Chieti, Italy.*

M. Solanas, *Department of Cell Biology, Physiology and Immunology, Medical Physiology Unit, School of Medicine, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain.*

F. Visioli, *University of Milan, Department of Pharmacological Sciences, Via Balzaretti 9, 20133 Milan, Italy. Tel: +39 02 50318280. Fax: +39 02 700.426.106. e-mail: francesco.visioli@unimi.it*

J.E. Wright, *School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, UK.*

M.D. Yago, *Institute of Nutrition and Food Technology, Department of Physiology, C/ Ramón y Cajal 4, 18071 Granada, Spain.*

P. Yaqoob, *The Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, The University of Reading, Whiteknights, PO Box 226, Reading RG6 6AP, United Kingdom. Tel: +44 (0)118 3788720. Fax: +44 (0)118 9310080. e-mail: P.Yaqoob@reading.ac.uk*

This page intentionally left blank

Preface

The use of olive oil, particularly in the context of the Mediterranean diet, has been advocated for its health-promoting properties for many years. What better evidence than the fact that Jeanne Calment, who had the longest confirmed lifespan (122 years) for any human being in history, attributed her longevity to olive oil and port wine? Throughout history, passionate individuals have extolled the virtues of olive oil. Giacomo Castelvetro was rescued from the Inquisition in Venice by the British Ambassador, only to face the horror of seemingly enormous quantities of meat and sugar consumed by the British during his 3-year exile. His 1614 manuscript, *A Brief Account of the Fruit, Herbs and Vegetables of Italy*, was a lively account of Italian ingredients and how to use them, and was finally translated and published in 1989. Other writers, including Elizabeth David and Ancel Keys, are well known for their advocacy of the Mediterranean diet.

Despite a wealth of anecdotal evidence, the scientific knowledge for the health benefits associated with consumption of olive oil is still developing. This book is a compilation of chapters from contributors who are experts in their field and it comprehensively evaluates the effects of olive oil in human health and disease. It begins with a fascinating insight, by Mataix and Barbancho, into the use of olive oil in Mediterranean food through the ages, followed by a chapter describing the chemical composition, classification and characteristics of olive oil by Ramírez-Tortosa *et al*. Pellegrini and Battino describe the antioxidant characteristics of olive oil in Chapter 3 and comment on the use of antioxidant assays for measuring the stability of the oil, while Sánchez-Muniz and Bastida in Chapter 4 deal with the effects of frying and thermooxidation on oil quality. This is important because loss of organoleptic quality is secondary to loss of antioxidant capacity. To complement this chapter, Visioli *et al.*, in Chapter 5, put forward evidence for antioxidant effects of olive oil phenolic compounds in the body and suggest that these effects could provide new insights into the mechanisms by which olive oil contributes to lower mortality from cardiovascular diseases. Quiles *et al.* describe the effects of olive oil on mitochondrial oxidative stress in

Chapter 6 and this leads on to Chapter 7, by Massaro *et al.*, evaluating the epidemiological evidence linking olive oil with reduced cardiovascular disease, highlighting both the strengths and limitations of this research.

Blood lipid levels and postprandial lipaemia have long been associated with cardiovascular disease and a number of studies have examined the effects of olive oil consumption on these parameters. Chapter 8, by Perona and Ruiz-Gutiérrez, documents evidence which suggests that olive oil may improve the blood lipid profile and puts forward some mechanistic explanations for the modification of lipoprotein size and structure by oleic acid, the key fatty acid component of olive oil. A number of studies have also suggested that phenolic components of olive oil protect low-density lipoprotein from oxidation, thus providing another potential mechanism for protection against cardiovascular diseases. Mesa *et al.* explore this phenomenon in detail in Chapter 9, developing and extending the notion that these compounds have antioxidant properties *in vivo*, as suggested in the Visioli chapter.

A relatively new area of research is the study of the effects of olive oil on haemostatic factors. As Chapter 10, by López-Miranda *et al.*, testifies, the relative infancy of this area means that more research is required for definitive evidence. In Chapter 11, Wright describes the influence of monounsaturated fatty acids in diabetes, indicating that dietary fatty acids could modify many features of this complex disease. Yaqoob in Chapter 12 critically evaluates the effects of olive oil on immune function, suggesting that while animal studies support immunomodulatory effects of olive oil, they are not wholly supported by human studies, and Chapter 13, by Mañas *et al.*, provides a detailed analysis of the influence of olive oil on the gastrointestinal system and gut-associated regulatory molecules. The lack of clear evidence for anti-inflammatory effects of olive oil is also documented in Chapter 14 by Llor *et al.* on inflammatory bowel disease, where the authors attribute the inconsistency in the evidence to limitations in study design. The final chapter in the book deals with the influence of olive oil on cancer and draws on evidence from experimental models of breast, colorectal and prostate cancers.

In many cultures the olive tree holds an important symbolic value. Pallas Athena, the Greek goddess of peace and wisdom, was said to have competed with her uncle, the sea god Poseidon, for the affections of the Greeks. The assembly of gods dictated that each should provide one gift to the nation and the provider of the most useful invention would be rewarded with the honour of giving a town founded in the 17th century BC a new name. Poseidon provided a wonderful horse, but Athena stamped the ground and sprouted an olive tree, 'capable of giving a flame for lighting up the night; of soothing wounds; of being a precious food, both rich in flavour and a source of energy', and so Athens was named after her.

José L. Quiles, M. Carmen Ramírez-Tortosa,
Carmen Ramírez-Tortosa and Parveen Yaqoob
July 2005

Abbreviations

5-HETE	5-hydroxyeicosatetraenoic acid
5-LOX	5-lipooxygenase
8-OhdG	8-oxo-dehydroxyguanosine
AA	arachidonic acid (20:4n-6)
$ABTS^{\bullet+}$	2,2′-azinobis-(3-thylbenz-thiazoline-6-sulphonic) radical cation
AG	accessory glands
AHA	American Heart Association
ALNA	α-linolenic acid
ANT	adenine nucleotide transporter
AOM	azoxymethane
aP2	fatty acid binding protein adipocyte P2
APC	adenomatous polyposis coil
Apo B-100	apolipoprotein B-100
Apo B-48	apolipoprotein B-48
AR	androgen receptor
BER	base-excision repair
BHT	butyl hydroxy toluene
BMI	body mass index
CaM-K II	Ca^{2+}/Calmoduline-dependent protein kinase II
CCK	cholecystokinin
CcOx	cytochrome c oxidase
Cdk	cyclin-dependent kinases
CE	cholesteryl esters
CETP	cholesteryl ester transfer protein
CFR	cooking-freezing-reheating
CHD	coronary heart disease
CLA	conjugated linoleic acid
CM	chylomicron
COMT	catechol-O-methyl-transferase

COX	ciclooxygenase
CPT	carnitine palmitoyltransferase
CRP	C-reactive protein
CSI	cholesterol saturation index
CVD	cardiovascular diseases
DAG	diacilglycerol
DCFH	dichlorofluorescein
DHA	docosahexaenoic acid (22:6n-3)
DMBA	7,12-dimethylbenz(α)anthracene
DMH	1,2-dimethyllhydrazin
DPPH	2,2′-diphenyl-1-picrylhydrazyl radical
EGFR	epidermal growth factor receptor
eNOS	endothelial nitric oxide synthase
EPA	eicosapentaenoic acid (20:5n-3)
ER_{α}	oestrogen receptor α
ESR	electron spin resonance
ETA	eicosatrienoic acid
EVOO	extra virgin olive oil
FAD	flavin adenine dinucleotide
$FADH_2$	reduced flavin adenine dinucleotide
FFA	free fatty acids
FMN	flavin mononucleotide
$FMNH_2$	reduced flavin mononucleotide
FPP	farnesyl pyrophosphate
GI	glycaemic index
GIP	gastric inhibitory peptide (Chapter 13)
GIP	glucose-dependent insulinotrophic polypeptide (Chapter 11)
GIT	gastrointestinal tract
GJIC	gap junction-mediated intercellular communication
GLA	γ-linolenic acid (18:3n-6)
GPx	glutathione peroxidase
GSH	reduced glutathione
GSSG	oxidized glutathione
H_2O_2	hydrogen peroxide
Hba	haemoglobin A
HB-EGF	heparin bound-epidermic growth factor
HCO	high corn oil diet group
HDL	high density lipoprotein
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
HOSO	high-oleic sunflower oil
HPLC	high performance liquid chromatography
HPSEC	high performance size exclusion
HR	hazard ratios
HSL	hormone sensitive lipase
HT	hydroxytyrosol
I	initiation group
IBD	Inflammatory Bowel Disease

ICAM-1	intercellular adhesion molecule-1
IFN-γ	interferon-γ
IGT	impaired glucose tolerance
IL-1α	interleukin 1α
IL-1β	interleukin 1β
IL-2	Interleukin 2
IL-6	interleukin 6
iNOS	inducible nitric oxide synthase
IP_3	inositol triphosphate (Chapter 15)
LA	linoleic acid (18:2 n-6)
LDL	low density lipoprotein
LDLr	low density lipoprotein receptor
LNA	α-linolenic acid (18:3 n-3)
LOH	loss of heterozygosity
Lp(a)	lipoprotein a
LPL	lipoprotein lipase
LRP	low density lipoprotein receptor-related protein
LT	leukotrienes
LTA_3	leukotriene A_3
LTB_4	leukotriene B_4
MCP-1	monocyte chemotactic protein
MCSF	monocyte colony stimulating factor
MDA	malondialdehyde
MMPs	matrix metalloproteinases
MMR	mismatch repair
MNNG	methylnitrosoguanidine
moxLDL	minimally oxidized LDL
MR	microwave oven-reheating
mtDNA	mitochondrial DNA
mtETC	mitochondrial electron transport chain
MTP	microsomal triacylglycerols transfer protein
MUFA	monounsaturated fatty acids
NAD^+	nicotinamide adenine dinucleotide
NER	nucleotide-excision repair
NK	natural killer
NMU	methylnitrosourea
NO	nitric oxide
NTPase	nucleosidetriphosphatase
$O_2^{\bullet -}$	superoxide anion
OA	oleic acid (18:1 n-9)
OE	oleuropeína
OO	olive oil
OR	odds ratio (Chapter 7)
OR	oven reheating (Chapter 4)
ORAC	oxygen radical absorbance capacity
oxLDL	oxidized LDL
PA	phosphatidic acid

PAI	plasminogen activator inhibitor
PAI-1	plasminogen activator inhibitor-1
PBMNC	peripheral blood mononuclear cells
PDGF	platelet derived growth factor
PEN	polymeric enteral nutrition
PGE	prostaglandins
PGE_2	prostaglandin E_2
PGI	prostacyclines
PGI_2	prostaglandin I_2
PhIP	amine-1-methyl-6-phenylimidazo[4,5-b] piridin
PIP_2	phosphatidylinositol 4,5-diphosphate
PKA	AMPc-dependent protein kinase
PKC	protein kinase C
PLA_2	phospholipase A_2
PLC	phospholipase C
PLD	phospholipase D
PMA	phorbol 12-myristate 13-acetate
PP	pancreatic polypeptide
PPAR	peroxisome proliferators activated receptor
PTPases	tyrosine phosphatases
PUFA	polyunsaturated fatty acids
PYY	peptide YY
$R^{\bullet}$	lipid radical
RCS	reactive chlorous species
RLP	remnant-like particles
RNS	reactive nitrogen species
ROOH	hydroperoxide
ROS	reactive oxygen species
RPTKs	receptor protein-tyrosine kinases
RR	relative risks
RXR	retinoid acid receptor
SER	smooth endoplasmic reticulum
Sf	Svedberg flotation rate
SFA	saturated fatty acids
SFK	Src-family protein tyrosine kinase
SHR	spontaneously hypertensive rats
SMC	smooth muscle cells
SOD	superoxide dismutase
SREBP	sterol regulatory element binding proteins
TAC	total antioxidant capacity
TBARS	thiobarbituric-acid reactive substances
TEBs	terminal end buds
TF	Tissue Factor
TFPI	Tissue Factor pathway inhibitor
TG	triacylglycerols (Chapter 8)
TGF-β	transforming growth factor
TNF	Tumour Necrosis Factor

TNF-α	Tumour Necrosis Factor α
t-PA	tissue plasminogen activator
TRL	triglycerides rich lipoproteins
TX	thromboxanes
TXA_2	thromboxane A_2
TXB_2	thromboxane B_2
TXB_3	thromboxane B_3
u-PA	urokinase plasminogen activator
VCAM-1	vascular adhesion molecule
VDUP1	vitamin D3-upregulated protein 1
VEGF	vascular endothelial growth factor
VHAI	Van Hess activity index
VLDL	very low-density lipoprotein
VSMC	vascular smooth muscle cells
vWF	von Willebrand Factor
ψ_{mt}	mitochondrial membrane potential

This page intentionally left blank

1 Olive Oil in Mediterranean Food

JOSÉ MATAIX[1] AND FRANCISCO JAVIER BARBANCHO[2]

[1]*Institute of Nutrition and Food Technology, Department of Physiology, University of Granada, Granada, Spain*; [2] *Nursing School, University of Extremadura, Plasencia University Center, Plasencia, Cáceres, Spain*

1. Introduction

The diet of Mediterranean populations has followed a stable model that has undergone compositional changes to any degree only in the last three or four decades of the 20th century. This diet is based on products derived from wheat, olive and grape, these constituting the Mediterranean triad of bread, oil and wine. This may perhaps be incomplete, as legumes have carried great weight also, and thus they might be included in a Mediterranean tetrad. The most characteristic element, olive oil, differentiates this diet from other models and has been described in the renowned words of George Duhamel: 'Where the olive tree disappears, the Mediterranean ends' (Toussaint-Samat, 1987). Therefore, the olive tree and the oil of its fruits in large measure mark the Mediterranean setting. The tree is an integral element in the landscape that it transforms, and the oil is the fat that makes a peculiar form of nourishment and cuisine possible. If, as according to Faustino Cordón (Cordón, 1988), 'cooking made the human', then the Mediterranean human, without olive oil in the cuisine, could not be understood as such. Mediterranean food, reduced today to the term 'Mediterranean diet', is fashionable for being credited with healthy virtues and has been taken as a reference for balanced and prudent eating. Although this statement should be refined in different details, it can be stated that the Mediterranean area has the appropriate and sufficient food potential for a pleasurable and healthy dietary model, within which olive oil occupies a special position that transcends its natural framework of production. This study seeks to provide an up-to-date historical synthesis of the production, uses, and consumption of olive oil. The information is not meant to be exhaustive, but it is intended to be adequate and rigorous. A major part is taken directly from existing sources for each historical period while another portion comes from specialists in diverse fields to clarify aspects that until recently have been ignored or cast into doubt.

 Olive Oil and Health
(eds J.L. Quiles, M.C. Ramírez-Tortosa and P. Yaqoob)

2. The Olive and the Wild Olive

The olive tree belongs to the family Oleacea. The genus *Olea* is divided into the subgenera *Tetrapilus* (containing tropical species) and *Olea.* The subgenus *Olea* is divided in turn into two sections: Olea and Ligustroides. The section Olea contains the complex *Olea europaea*, which is currently divided into four subspecies: *O. e.* ssp *cuspidata* (found in China and eastern and southern Africa), *O. e.* ssp *laperrinei* (in eastern Africa and the Sahara), *O. e.* ssp *cerasiformis* (in the Canary Islands), and *O. e.* ssp *europaea* (throughout the Mediterranean Basin). The subspecies *O. e.* ssp *europaea* is even divided into two varieties: *sylvestris* (wild olive) and *macrocarpa* (domesticated olive); the two cannot always be differentiated by strict botanical criteria, and their distinction is based simply on the fact that one is cultivated and the other not (Dubur-Jarrige, 2001).

In theory, it should be easy to distinguish the wild from the domestic olive. The greater size of the domesticated olive, the thick trunk, the rounded canopy, the long branches without thorns, and its ovate and fleshy fruit should eliminate confusion. In addition, the flavour of the olive oil and its abundance is far different from the bitter and meagre oil of the wild olive. However, the wild olive, well pruned or transplanted, can be confused with an abandoned domesticated olive tree. Both are highly resistant to harsh climatic conditions and adapt to similar environments. The Greek Theophrastus (*H. P.* I 14, 4) used this as an example of the difficulty at times to differentiate botanically between a wild plant and a cultivated one. At present, palaeobotanists continue to have problems in distinguishing the botanical vestiges of the wild olive from the domesticated one. The wild olive grows spontaneously in very warm and dry zones of the Mediterranean Basin (within the Sahara, it reaches 2700 m in altitude), places where it has great environmental importance for its capacity to retain water and form plant covers. The olive tolerates hot, dry and cool climates. Its fruits mature during the summer, varying in colour from light green to violet-red to black. Green maturation occurs in the autumn, and complete ripening in the winter. The fruit reaches its optimal size in warm, bright sites with aerated soils. It has a natural alternation in fruit production, which can be regulated with good cultivation practices. Harvest for oil begins with the autumn rains. There are more than 260 varieties (some 25 main varieties); in practice, these are classified according to the destination of the oil. The olives used for oil extraction are medium in size, averaging less than 3.5 g in weight, with a low pulp/stone relationship but a high yield in oil of not less than 16–18%. Table olives, eaten after being marinated, are medium to large, weighing 5 to 6 g (up to 17 g). The pulp/stone relationship is high and yields little oil. Double-use olives have intermediate characteristics.

3. The Origin and Spread of the Olive – a Complex Question

The origin of *O. europaea* is still argued, although palynology has demonstrated that it had appeared in the Tertiary (Palamarev, 1989). Its presence in the Mediterranean Basin, testified to by traces of pollen, goes back some 3.2 million years (Suc, 1984).

The origin of the wild olive (subspecies *O. e.* ssp *europaea* var. *sylvestris*) is still poorly known and continues to stir controversy among naturalists, botanists and prehistorians. It appears that it is the result of a hybridization between *O. e.* ssp *laperrinei* as the female and *O. e.* ssp *cuspidata* as the male, and would have appeared some 500,000 years ago in eastern Africa, spreading from this focal point throughout the Mediterranean Basin (Bervillé *et al.*, 2001). The oldest remains of carbonized wild-olive wood have been found in Israel in the Negev Desert and could be 43,000 years old (Liphschitz *et al.*, 1991). In the Aegean, the most ancient find is from Thera (Santorin), this being fossil prints of olive leaves found in layers of lava dated to 37,000 BC (Friedrich and Pichler, 1976).

The origin of the domesticated olive is even more disputed and continues to be obscure. Botanists contend that the olive comes from the domestication of the wild olive, a fact corroborated by genetic analysis (Besnard and Bervillé, 2000). Domestication is universally accepted to have begun during the Neolithic in the eastern Mediterranean, although recent palaeobotanical, climatic and genetic studies hold that the domestication also occurred practically at the same time in the western basin, and thus the process is more complex than previously thought (Bervillé *et al.*, 2001; Dubur-Jarrige, 2001). Morphological studies made on carbonized fragments of wood and stones of olive fruits in eastern Spain, in La Cova de les Cendres, Alicante (Terral, 1996), and in southern France, en La Cova de l'Espérit of the western Pyrenees (Terral and Arnold-Simard, 1996), provide evidence for the presence and use of the olive since the Neolithic, with a clear increase in its exploitation during the Bronze Age. Genetic research by DNA identification of chloroplasts and mitochondria belonging to cells of olive-stone fragments revealed that the genetic structure of olive varieties found in Spain and France show the presence of genes unknown in the eastern Mediterranean, implying local domestication independently of the western Mediterranean (Bervillé *et al.*, 2001; Dubur-Jarrige, 2001). These studies challenge the classical theory that the cultivation of the olive spread from its original focus of the eastern Mediterranean to the western territories by a stepped expansion with human migration from the east to the west.

In Spain, before these new findings, the oldest remains were stones from olives found in several caves. Olive stones from the cave of Nerja (Malaga) have been dated by C-14 to 3115 BC, a date which, after calibration, would stretch to 3800 BC. Its small size (5–9 mm as opposed to 9–20 mm today) suggests that the olive was still close to the wild olive in the domestication process (Blanco, 1994). The Tartessians and Turdetans perhaps used wild-olive fruits for dietary purposes. The cultivation of the olive was instrumental in expanding the agricultural horizon, as it is compatible with cereals, given not only its adaptation to soils less appropriate for grain but also its winter harvest (Blanco, 1994).

The appearance of the olive in Greece is highly controversial, as to date the oldest palaeobotanical findings date to the third millennium, and therefore some researchers argue that its cultivation is much more recent than in other places and that it did not begin until the Early Bronze Age, first in Crete and the Cyclades and later in continental Greece (Renfrew, 1972; Blitzer, 1993).

Others believe that the beginning of cultivation in these zones was later, not until the Late Bronze Age or even the Archaic Period (Runnels and Hansen, 1986; Hansen, 1994). However, many authors contend that in the Greek territories the olive must have been cultivated before, citing that high percentages of olive pollen are found since the Neolithic both in Crete and in continental Greece, and, furthermore, they suggest that the lack of botanical remains is due to the fact that previous excavations were not adequate, lacking precise technological means to find and examine the remains in the laboratory. Finally, it has been reasoned that it would be illogical for olive cultivation not to have taken hold early in a territory so prone to cultural contact, having an advantageous geographical position between the Near East and the western Mediterranean, where olive cultivation since the Neolithic has been documented. For all of these reasons, it appears plausible that the first experiences of olive cultivation began in the Neolithic and that in the Early Bronze Age it would have had taken hold in insular as well as continental Greece (Zohary and Hopf, 1993; Dubur-Jarrige, 2001; Amouretti and Brun, 2002).

Recent palaeobotanical and genetic research has shown that the origin of the olive is even more complex than previously believed. Nevertheless, it continues to be feasible that cultivation began in the eastern Mediterranean during the Neolithic, some 6000 years BC (Bervillé *et al.*, 2001). The first irrefutable evidence of olive domestication and of oil extraction is more recent, dating from the Chalcolithic (4500–3500 BC) (Liphschitz, 1987). At the site of Teleilat el-Ghassul, near the Jordan valley, dumps of olive remains have been found, these corresponding morphologically to cultivated olives (Liphschitz *et al.*, 1991). Also, olive-stone fragments, similar to those found near Abu Hamid, presented fractures characteristic of grinding the fruits. These marks found on the stones imply the production of oil at these localities (Neef, 1990; Zohary and Hopf, 1993). In Kfa Samir, on the slopes of Mount Carmel, definitive archaeological proof was found for the production of olive oil: a vessel, excavated in a clay stratum, full of olive stones and organic matter (Galili *et al.*, 1989; Liphschitz *et al.*, 1991; Frankel, 1994). The fruits were crushed in the vessel with a stone and the oil was extracted by washing it with water. This method is called washed oil (*shemen rahutz*, in ancient Hebrew) or 'skimmed-off oil' (*zeit tafah*, in Arabic) (Eitam, 1993a, 1996). During the fourth millennium BC, the olive continued to be cultivated on the edges of the Golan Heights and on the hills of Samaria, and the advances of oil-production technology are confirmed by the finding of dozens of stone mortars to crush the fruits as well as containers to collect the juices. Such rudimentary techniques gave a low yield in oil (some 6%), and would have been for home consumption (estimated at a minimum of somewhat more than 5 litres per person per year). This would correspond to *shemen zeit zah katit* (pure oil from milled olives) of *Leviticus* (Eitam, 1993a). The cultivation of the olive was essential for the settlement of the agricultural communities, and the tree, becoming a permanent part of the landscape, required years of maintenance to become profitable. These characteristics differentiated it from cereals and legumes, which are grown annually and can be cultivated in a different area from year to year. The biological traits of the olive tied the farmer to one spot.

4. Olive Cultivation in the Earliest Historical Times

Botanical traces of the olive continue to be found from the third millennium BC, together with the remains of facilities and vessels for producing oil in the Near East and Anatolia. As the urbanization of communities increased, olive growing developed and the groves enlarged with the use of more efficient tools (Frankel, 1994; Eitam, 1996). Dating from the middle of the third millennium, the first documents emerge in the region of Canaan (northern Syria) which demonstrate the capacity of a community to produce surpluses of oil for trade (Heltzer, 1987, 1993; Callot, 1993). The documentation is found in the archives of three independent city-states: Ebla, Mari and Ugarit. In Ebla (near modern-day Aleppo), a collection of clay tablets was found, these mentioning the expansion of the olive (the crop third in importance) and notable oil production. One document notes the olive trees existing in three fields: one had a thousand while the other two had five hundred trees. Another record mentions the exportation of olive oil of highest quality (Remesal, 1996). For the second millennium BC, data on olive growing and oil trade increase (Heltzer, 1987; Callot, 1993). The valuable texts of Mari report that this city imported olive oil from Aleppo, which, with its proximity to ancient Ebla, reflects the great production of this region over a thousand years and reveals data on the price of oil, which was five times higher than wine and two and a half times as expensive as sesame or flaxseed oil. The documentation from Ugarit (present-day Ras Shamra) demonstrates local olive-growing prosperity. For the extraction of oil, the fruits were trampled with wooden clogs or squashed with stone wheels and then pressed using a beam as a handspike, as evidenced in the archaeology of Ugarit and the palace of Tel Hazor (1500 BC). The system most similar to the beam press was invented in northern Syria, spreading quickly to neighbouring territories (Callot, 1993). Large-scale production gave Ugarit 5500 tonnes of oil annually, and the archives of this city record exportation to Egypt and Cyprus (Heltzer, 1987; Callot, 1993). Also, information exists from ancient Assyria of long-distance trade. One document from Karum of Kanish, the trade centre of Anatolia, reports that a merchant requested highest-quality oil from Ashur, the capital, or from a city called Hahlum (Remesal, 1996). It is known that the oil was highly appreciated and in Ugarit it served to pay tributes to the palace, compensation and salaries (Callot, 1993).

In the 13th to the 11th centuries in the region of Syro-Palestine, olive production declined, although at the beginning of the first millennium a recovery began, reflected in the archaeological finds of many grinding wheels and beam presses. This recovery strengthened in the Iron Age (7th century BC), a period in which truly specialized oil-growing centres developed, such as the industrial enclaves of Tel-Migne-Ekrom, Klá and Kh. Khadash (Eitam, 1987, 1993b, 1996). Findings from Ekrom (city-state of Philistea, which for centuries maintained high production) have enabled the reconstruction of the type of mill used. It consisted of a large clay storage jar to grind the fruits with a millstone, and a large lever press to extract the oil (one end of a beam was fixed to the wall and the other was weighted with perforated stones hung as a counterweight block). For faster production, two presses functioned at the same time, and the extracted oil was poured into vessels (Eitam, 1993b, 1996). Together with the great

centres, other smaller ones coexisted, these having much smaller presses and weights. There were also many rudimentary operations where oil was produced for private consumption. One site found in Galilea consisted of a milling vat, a foot press that spilled into a settling tank and a decanting jar. This type of press was also found in Phoenician colonies (Eitam, 1996). The spread of olive growing from the eastern Mediterranean to Europe is still not clear, although it is traditionally accepted that it spread through Syro-Palestine to Turkey and the Aegean islands and from there continued to expand eastwards and westwards throughout the Mediterranean Basin (Renfrew, 1973; Bottema, 1994).

5. Olive Cultivation in the Great Civilizations: Mesopotamia, Egypt, Israel, Phoenicia, Crete and Mycenae

In Mesopotamia and Egypt, olive raising and the use of oil has been a topic of controversy. Today we know that cultivation was very reduced and that the oil had no uses or only insignificant ones in food.

In Mesopotamia, botanical findings testify to the existence of the olive, although it was always a minor crop and very limited by the geoclimatic characteristics of the region. As pointed out by Margueron (1991), there was a desire to acclimatize the olive to the fertile lands of lower Mesopotamia, since its oil was used for pharmacopaeia and perfumes (Zaragoza, 1972), being cultivated in the westernmost regions. In the first millennium BC, Sennacharib tried, although without success, to introduce the olive into southern Mesopotamia (Margueron, 1991). Graeco-Roman sources report the scarcity of the olive, and Herodotus (I 193, 4) even denies that it was cultivated, praising instead sesame and mentioning the use of its oil: 'sesame reaches approximately the height of a tree ... the Babylonians, certainly, do not use olive oil but make one of sesame'. This implies that olive oil had no role in the food of Mesopotamia.

Egypt's climate is not suitable, either, for the development of the olive, and thus it was necessary to acclimatize the crop. However, it would produce substantial quantities of other oleaginous crops. The evidence available shows that the olive did not acclimatize until the 12th Dynasty (1985–1795 BC) or even until the 18th Dynasty (1550–1295 BC), very late dates compared with the eastern zones (Liphschitz *et al.*, 1991; Frankel, 1994). The oldest botanical vestiges come from Memphis (12th Dynasty), but it cannot be determined whether they are autochthonous (Meeks, 1993). Up to the Amarna Period (1372–1314 BC), botanical remains of the olive are not frequent and the tree does not appear in paintings or reliefs. In Amarna and Deir el-Medina, olive stones have been found as evidence for their consumption as food (Renfrew, 1985). In the area of olive groves, documents are scarce up to the Graeco-Roman period. The information available indicates that in the Old Kingdom, the oldest were situated at Hermopolis-Amarna, in Panopolis-Akhmim, in the Fayum oasis (the most important and largest, reaching to the province of Oxyrhynchus) and, possibly, in Siwa and Kharga. During the New Kingdom (1550–1069 BC) cultivation increased in Fayum, Kharga and Siwa and groves appeared in Alexandria, Heliopolis, Thebes and Qantir, although in these last two places they could have had ornamental

functions. In the Ptolemaic Dynasty (323 BC–AD 30) the greatest expansion occurred (Meeks, 1993). Greek sources mention olive orchards in Alexandria, la Tebaida and, above all, in Fayum. Theophrastus (*H. P.* IV 2, 9) cited those of Tebaida and stated that Egyptian oil was not inferior to the Greek, although it had a less pleasant smell. The Harris I papyrus, the best source of information for the New Kingdom, indicates that for three decades Rameses III (1184–1153 BC) offered the temples a quantity of oil equivalent to the production of 2735 ha of olive trees, a number, according to Meeks (1993), that would involve a good part of the total cultivated surface area, and which was used primarily for the illumination of religious edifices. In Heliopolis, Rameses III himself dedicated an olive grove of 15 ha to his temple. The great value of the olive oil as a fuel for sacred lamps is evidenced by the numerous lamps found in the pharaonic tombs. A fresco of a funerary chamber of the tomb of Ramses III in the Valley of the Kings (Luxor-Thebes) represents the characteristic Cretan vessels used for transporting the olive oil, this demonstrating that the pharaoh valued it so much that he did not wish to deprive himself of it in the afterlife. The insufficient production of oil in Egypt always obligated importation (Zohary and Spiegel-Roy, 1975). In the third millennium BC (1st Dynasty), jars containing Palestinian oil appeared in the royal tombs, but it has not been determined whether it was olive oil. In the Second Intermediate Period (1650–1550 BC) the Minoan containers found at Tell el-Dab'a indicate the importation of olive oil, although the number of vessels is insufficient to represent large quantities (Meeks, 1993). The arrival of Syro-Palestinian olive oil is reported in the record of the 15th century BC which indicates an order for 30,000 l from Canaan, and the Anastasi papyrus indicates the presence of olive oils at the port. In the *Historia de Sinuhé* (1191–1178 BC), the protagonist, who lived in a Syro-Palestinian environment, speaks of the fine oil in the house of the son of the king and of the land of Yaa in the east 'rich in oil' (Eitam, 1996).

For all the above, it appears that olive oil was used and appreciated in Egypt. But, apart from illumination, what other uses did it have? From the Amarna Period (1372–1314 BC) it formed part of the liturgical and funerary cult, uses that accentuated during the 18th Dynasty. As in Mesopotamia, it was used in pharmacopeia and cosmetics to produce essential oils and unguents, and it was applied in the treatment of burns and cracks in the skin, uses that justified its importation but suggest that it was treated as a relatively luxurious article (Ghaliongui, 1972). Its value, as observed by Meeks (1993), is revealed 'when the head of the workforce on the pharaoh's tomb receives from the pharaoh, among other things, two jars of olive oil; this represents, in real terms, a royal gift'. The olive oil had to compete with other, more abundant and cheaper, ones for which there were as many as 21 different names. Vegetable oil was called *merhet*, and animal *adj* (Strouhal, 1992). Oil was obtained from sesame, castor bean, flaxseed, radish and the tree *ben* (*Moringa oleífera)*. The oil from *ben* was highly esteemed for being odourless and clear and for not turning rancid. It was used in perfumes and it was exported to Greece and Rome. Olive oil was not used in Egyptian food, even in the Graeco-Roman period, except by the foreign population accustomed to its flavour, and continued to be a product of luxury, as testified by a letter written by a member of the Syrian community of Elephantina and the commentaries of Plato in his travels to Egypt (Meeks, 1993).

In the Judeo-Palestine territories, the cultivation and use of the olive underwent notable development as both the olive and its oil took on a strong symbolic character. The Biblical texts often cite the olive, as a few examples will show. Noah knew that the waters of the Great Flood had receded because a dove returned to the Ark with carrying 'a green olive branch in its mouth' (*Gen.* 8, 11). Israel is 'the land of olives and oil' (*Dt.* 8, 8). 'You shall have olives throughout your territory' (*Dt.* 28, 40). The olive, as shown in the texts, symbolized beauty (*Jer.* 11, 16), vigour and fertility (*Sal.* 52, 10). According to the apology of Jotam, it was the first of the trees (*Ju.* 8, 8–9). Olive oil was essential and omnipresent in Jewish food and without it Hebrew cuisine would be inconceivable. From the beginning, it carried great weight in *Leviticus* (2, 4–5; 5, 14–15), on including it in the everyday dishes, whether cooked or not, such as the preparations with cereal flours that were always made into dough or sprinkled with oil. Also, the flesh of sheep and cattle, before and after being cooked, were doused with ample oil. As observed by David Eitam (1996), it was added even to drinks such as *khilmi* and *alontit* or to the wine *anigron*, for which it was estimated that the consumption of oil would have been high, some 20 l per inhabitant per year. Similar figures of annual consumption are provided by Zertal (1987), who calculated a consumption of 17 to 20 l per person, and by Safrai (1987), who estimated that each family had 130 l of olive oil. All these calculations are far higher than the quantity of a *log* (equivalent to 0.5 l), the quantity indicated in the *Mishnah ketubot* (Eitam, 1996).

Olive oil was used to illuminate the temples and sanctuaries and to make aromatic oils and essences that had a privileged role in the liturgy (Weinfeld, 1987). *Exodus* (25, 31–40) describes the most famous oil lamp, the sacred menorah (the candelabrum having seven arms of gold), whose manufacture for the temple was assigned by God to Moses and which had to be illuminated with pure olive oil, the same that fuelled the permanent candle of the Temple of Jerusalem. It was used to consecrate holy objects, altars and buildings. Since remote times, olive oil was used for another fundamental rite: the anointment of kings. It is well known that the name of the Messiah means 'the anointed one' and that unction by antonomasia is that of the body of Christ at the sepulchre (Schäfer-Schuchardt, 1996). The sick were anointed and oil was used for the cult of the dead, but also in festivities (births and weddings). Unction was a daily act in which the body was lightly anointed with oil, although the rich poured it copiously onto their head and beard and bathed their feet, or received a general massage. During the Roman period, olive oil was supplied in the public baths by the *oliyar* (Eitam, 1996). It was the basis for cosmetics and perfumes, considered authentic treasures, which were manufactured with aromatic plants by impregnation under heat. The perfume makers were designated *rokkhim* or *mefatmin* (the impregnator) in Biblical texts (*Neh.* 3, 8; *I Sam.* 8, 13) and during the Graeco-Roman period were highly respected specialists (Eitam, 1996). Some plants, such as the much esteemed myrrh, were imported while others were cultivated locally in orchards such as those of Gilead and Ein Gedi (*Cant.* 1, 14). Here, archaeology has revealed a perfumery workshop from the 7th and 6th centuries BC, which functioned for a long period with high economic output (Eitam, 1996). In the Roman period (AD 70), according to Pliny the Elder, Titus and the rebellious Jews fought for its control.

Olive oil had medicinal uses and was used to treat throat pain and injuries (*Is.* 1, 6). The oil production of Hebrew Palestine enabled the exportation to other countries, mainly Phoenicia and Egypt. Tyre and Sidon traded their cedar wood for the Hebrew oil (Blázquez, 1996).

In Phoenicia, the olive was cultivated but its low production necessitated the importation of olive oil. The Galilean presses found in Tyre and Tell Shiqmona (Eitam, 1987, 1996) show increasing interest in olive growing that would crystallize in the pre-eminence of Phoenicians in its spread (Chabal *et al.*, 1999). The active Phoenician commerce favoured the development of olive cultivation and the consumption of oil throughout the Mediterranean (Blázquez, 1992; Heltzer, 1993). In Spain, olive growing took hold in the Guadalquivir and Genil valleys, while in the localities of Toscanos, Chorreras and Mezquitilla amphoras to hold the olive oil were made from the 8th century BC. The people of this region developed the custom of depositing the oil amphoras in the tombs of their necropoleis (Blázquez, 1992).

In Crete, the most ancient remains of olive (stones and wood) date from the Bronze Age (Early Minoan 3500–2100 BC). During the Middle Minoan (2100–1700 BC) and Late Minoan (1700–1200 BC), botanical evidence accumulates, as does archaeological (utensils and writing tablets in Linear A and B) as well as ethnographic findings at more than twenty sites that demonstrate the development of olive growing (Renfrew, 1973; Blitzer, 1993). Carpology has revealed two types of olive stone (wild olive and primitive cultivated olives), a duality that concurs with the two types of ideogram represented in Linear B (13th century BC) and shows that the fruits of both were probably used (Chadwick, 1976; Melena, 1983; Blitzer, 1993). The olives, above all in brine, were heavily consumed (Fidanza, 1979; Blitzer, 1993). The production of oil is reflected by the utensils for grinding the olives and the large jars (*pithoi*) to store the oil that was kept in villas and palaces (Ruzé and Amouretti, 1978; Blitzer, 1993). The palace of Knossos contained numerous *pithoi*, which all together could hold between 75,000 and 250,000 l. The tablets indicated great allotments, some more than 10,000 l being sent from the fields to the palace. The *pithoi* of Mallia stored up to 23,000 l. Mallia was a very rich region that produced 200,000 l per year (Ruzé and Amouretti, 1978). Olive oil was used for illumination, for the making of unguents and probably for food. The wild olive was used for perfumes, oils and aromatic essences. The surplus oil was exported to the Cyclades, Mycenae and Egypt (Blitzer, 1993). The oil for food was shipped in amphoras and *pithoi* while the aromatic oils were sent in beautifully adorned flasks, sometimes with handles (Chadwick, 1976; Blitzer, 1993). In no case are data available on the cultivation of the olive nor on the technology of oil production. Olive growing is reflected in other islands of the Aegean and it prospered on Cyprus in the second millennium BC (Heltzer, 1993; Callot, 1993).

Olive cultivation may possibly have been introduced to Cyprus from Crete (Renfrew, 1973; Chadwick, 1976). In Mycenaean civilization, the first great European culture and the basis for Greek civilization, the olive was cultivated, as testified by Linear B tablets (13th century BC) which record cities that produced olives (Chadwick, 1976; Melena, 1983). Olives must have been important, as the oil was exported in stirrup jars such as those found in the house of

the oil maker in Mycenae (Chadwick, 1976; Melena, 1983). The accounting of the palaces and temples demonstrates the liturgical use of oil. In the palace of Pilos, dozens of tablets were found registering oil deposits dedicated above all to Zeus, Poseidon and Athena (Chadwick, 1976; Melena, 1983; Amouretti and Brun, 2002). Afterwards, sacred objects and statues were anointed with oil; for example, the *omphalos* (the 'stone navel' which supposedly marked the centre of the earth) at Delphi was rubbed every day with perfumed oil. Texts also testify to olive cultivation. Homer evoked the olive (*elaíe*) cultivated by grafting to a wild one: 'two bushes sprouting from the same place, one of the wild olive and the other domesticated' (*Od.* V 480), and during the Mycenaean period it expanded by planting and grafting, beginning to form part of the landscape (Chadwick, 1976). In Hesiod (*Obras.* 523) the use of olive oil (*élaion*) is confirmed, and Xenophon (*Ec.* XIX 13) provides some details on its cultivation. Planting increased in the 8th and 7th centuries BC, and from the 5th century BC the olive rose to become the tree par excellence of the Hellenic world (Esqu., *Pers.* 881–2), consecrating Athena, the eponymous goddess of Athens, who, according to legend, caused the olive to sprout from the soil on disputing the possession of Attica with Poseidon. For a long time, this olive tree was preserved in a fenced area on the Acropolis. The oil served to honour the dead: the body of Hector was anointed with rose oil by Aphrodite (Hom., *Il.* XXIV 585), and until the 4th century AD, flasks of perfumed oils were deposited in tombs. This tree, divine and venerated, provided substantial economic benefits, as the surplus oil was exported to offset the heavy importation of cereals needed by the Greek population (Plut., *Vit. Sol.* 24). The success of the olive did not cause the ancient Greeks to leave behind the wild olive, as they preferred its wood to that of the domesticated olive, and they made perfumes from its oil, although it was hardly used for food. So that the wild tree would yield more, they pruned it and transplanted seedlings. The value of the wild olive in Greece is reflected in the fame of the one at Olympia, which rivalled the olive tree in Athens (Amouretti, 1986).

Outside the sphere of the Aegean, according to classical texts, the olive was unknown and Greece would have the merit of expanding it throughout the Mediterranean. In the year 581 BC, according to Pliny the Elder (XV 1, 17), the tree did not exist in Italy, Spain, Africa or Gaul. In Italy, it was introduced from Greek cities of southern Sicily. Rome understood its utility and olives were planted in all the areas where it could acclimatize. Columella (V 8) wrote of its climatic characteristics: 'Many believe that this tree does not live, or is not fertile, at a distance from the sea of greater than sixty miles, but in some areas which are farther away, it thrives.' The varieties introduced into the Mediterranean are of Greek origin, as denoted by the etymology of the Latin terms that designate them (André, 1956, 1961, 1985). According to Varro (*L. L.* V 108), *olea* (olive) comes from *elaía*, and likewise the names of the olives *orchitis, circites* and *radius* had Greek origins. Broadly, it can be said that olive growing began in Syria and Palestine and later appeared in Crete and Cyprus. In Greece, the agricultural techniques were improved and the tree spread throughout the Mediterranean. In Rome, the cultivation was intensified and expanded to all possible places, resulting in an appreciable advance in olive cultivation.

6. The Olive Tree and Olive Oil in the Classical Graeco-Roman World

6.1. The olive harvest

This task continues to be familiar in the Mediterranean area, having far-reaching social importance, as it provides the income of many families. In Greece the table olive was harvested in September and the oil olive between October and December (Theophr., *C. P.* VI 19, 3), with the same systems as used today: manually by knocking with a pole and collection after fall from ripeness. Hand picking was indispensable to obtain good olives for the table or quality oil (Plin. XV 6, 9; Pal. XII 17, 1), but it is practical only for olive trees that are not very high and are well maintained and pruned. It is difficult work as it requires strength to pluck the fruits and carry baskets filled with fruits. The quantity of olives picked by hand per person is some 7 to 10 kg per hour, and thus a day's work represents some 60 to 80 kg. The Greeks, in contrast to the Romans, used this approach little. Columella (XII 52, 9) advised collecting the fruits without damage and with the desired ripeness. Palladius (XII 17, 1) recommended 'harvesting only the quantity of olives that can be pressed the following night', asserting that this was necessary to produce quality oil. This work was performed by women. Knocking was necessary for high olive trees and demanded a careful technique so as not to damage the fruits. Farmers advise knocking only from the inside towards the outside of the tree. Good pole workers can knock down all the fruits from the tree in 30 to 60 minutes and afterwards gather the fruits from the soil and then winnow them to separate the impurities. This was the usual method in Greece but criticized by the Romans (Varr. *R. R.* I 55).

The harvest required hiring labourers, as the work of one family, no matter how intense, was insufficient (Aristophanes, *Vesp.* 712). In Greece, if labourers were scarce, the ripe olives were collected by windfall. However, this system was not common in Italy, as the fallen fruits always presented variable degrees of deterioration that worsened during the storage that was necessary until sufficient quantity had been collected to take to the mill. With the passage of time, the harvest has varied little and only some years ago were nylon screens installed between trees and shakers used as well as other machines that are not completely satisfactory (and that can be applied only in olive orchards that have been planted with this system in mind). The mechanization of the olive harvest poses grave problems with regard to the hiring of labourers.

6.2. Milling

Olives grown for oil are left overnight before pressing. Among the milling systems, one of the first was to crush the fruits with a stone roller moving back and forth over a stone table. The juice was collected by soaking masses of wool that were then pressed by hand into containers. For more thoroughness, the residues were washed in water and the oil was floated off. This was done by Greek women, the procedure being archaeologically demonstrated in Crete from the second millennium BC (Amouretti, 1986; Foxal, 1993).

Another system, used in Israel, was to grind the fruits with a mortar and pestle until creating a paste that was deposited in a large tub into which hot water was added. The paste was repeatedly kneaded with the hands, and the oil was collected from the surface with the palms of the hand and placed in another container. This method, mentioned in Biblical texts, is still used by women in Kabylia (north Africa), and is called *zit* Uberray (Amouretti, 1996).

A widely used system consisted of placing a large bag of ripe fruits in a wooden trough and trampling them with bare feet or with a type of wooden clog (*kroupezai*) and then tightly twisting the sack. On occasions, large wooden bars were moved over the edges of the sack, using loops, to increase the pressure. The oil was filtered through a lower aperture in the trough and collected in a container. To extract the largest possible quantity, hot water was poured over the remaining paste, the sack was twisted again, and weights were placed on top of the sack. This method, used with wine, is represented in Egypt in the mastaba of Ptahhotep (Sakkara-north, 5th Dynasty) (Meeks, 1993) and continues to be used in Corsica and Turkey (Amouretti, 1996).

In Rome, the fruits were trampled with clogs (*solcae*) in large tanks and the oil was collected in the channels (*canalis*). Afterwards, simple cylindrical millstones appeared that inevitably broke the olive stone and therefore the oil was less valued. In Syria, Africa and Spain, the millstones were conical and lasted for centuries.

The systems in Rome improved with the introduction of the proper mills. An important innovation was the perpendicular millstone, using for the first time the rotation of the mechanism in a machine used for transformation. The earliest testimony (4th century BC) comes from the Greek city of Olynthus (Amouretti, 1986; Foxal, 1993). Another notable advance was the *trapetum* (Cat., *Agr.* 23–25), a more complex mill consisting of a *mortarium*, where the fruits were placed, which was handled by two men using wooden handles (*modioli*) that passed through two semi-spherical millstones, flat on the inside and convex on the outside (*orbes*), which were inserted into an iron axle (*columella*) joined to the *mortarium* by a stone column (*milliarium*). On turning, the millstones crushed the olives against the walls without affecting the olive stones. Later, cylindrical millstones (*mola olearia*) perpendicular to a rounded tub turned on themselves, crushing the fruits. The system later used animal power. It spread widely in the Roman period and lasted for centuries. The system is well represented in Roman iconography (Amouretti, 1986).

6.3. Pressing

For a long time, olive oil was extracted without presses, by the systems described above using trampling, torsion in sacks, or compression of the pulp in a basket with a stone weight (systems used predominantly from the 7th to the 3rd centuries BC and documented by iconography and archaeology) (Amouretti, 1986; Foxal, 1993). From the 6th century BC, the handspike press (beam) and simple lever-and-weight press were used for centuries, coexisting with the primitive and successive techniques. This new system is represented most famously on the

black-ground Greek pot (*skyphos*) in the Boston Museum. In the middle of the 3rd century BC, handspike presses and iron capstan (Catonian and Pompeian types) predominated, as reflected in contemporary texts.

A subsequent advance was the introduction of the press (*torcular*), composed of two wooden pillars (*arbores*) sunk into the ground framing a heavy beam (*praelum*) that exerted pressure by means of a circular plate (*orbis olearius*) over a press table where the olives were placed (*area*) in a basket (*fiscina*), and later several baskets. Thus, the oil was obtained from the first pressing, and then the paste was bathed in hot water followed by a second and even a third pressing. Archaeology confirms the appearance of the handspike press with a capstan over the counterweight block from the beginning of the 2nd century BC. More evolved and efficient, this system would predominate until the 6th century. The handspike press with a screw over the counterweight emerged at the end of the 2nd century BC, gaining great success with its endurance, reliability and saving in work. It was widespread in the late Empire and throughout the Middle Ages. The direct-screw press, which, according to the texts of Vitruvius and Heron, was a Hellenic invention, arrived in Italy in the last quarter of the 1st century BC, as demonstrated in the archaeology of Pompeii. This press, with either one or two screws, was valued for its precision and regularity, but it demanded more manpower and was more fragile. It spread during the 1st century AD to Galia, Dalmatia, Italy, and especially Spain and Portugal. It was used for centuries, with small details being improved, although its fundamentals remained unaltered (Amouretti, 1986; Foxal, 1993). A special press, with wedges, was used in perfume making. The wooden wedges were driven between horizontal planks that, on opening, pressed the olive paste in the baskets. This is represented in the paintings of Herculaneum and Pompeii (Mattingly, 1990), and has been found at Delos and Paestum (Brun, 1998, 2000). This system spread during the Hellenistic period.

6.4. Decanting of the oil

Olive oil must be decanted to effect a natural separation of the oil from the remains (*amurca*, *faeces*) and the slurry called alpechin. This took place in containers where the remains settled to the bottom and the oil floated to the top, to be collected in *capulatores* with a kind of spoon (*patella*). For purification, the oil was drawn off and deposited successively, and finally stored in large clay jars (*dolia olearia*). At times, salt and nitre were added to thin the oil (Col. XII 50; Plin. XV 18). Decantation tanks were also used, these consisting of a series of connected clay storage jars such as the magnificent specimens found in North Africa (Amouretti, 1996).

6.5. Types of oil

In antiquity, depending on the time period, the harvest system and the preparation procedure, several qualities of olive oil were distinguished.

The best oils were from the first pressing, without mixing (André, 1961; Amouretti, 1986; Blanc and Nercessian, 1992). The oil called *omphákinon* (Theophr., *De odor.* 115; Aten. II 67b) came from green fruits at the end of summer, hardly profitable in oil, and in Greece was used for perfumes and medicine. This would correspond to the *oleum acerbum* of the Romans, which was also called *oleum aestivum* and *omphacium* (Diosc., I 30), highly appreciated for its excellent flavour (Col. XII 52). The green oil (*viride*) was extracted in October at the point of ripening of the fruits on turning from green to black and richest in oil (Col. XII 50, 52; Pal. XII 4, 1). This oil was acidic, less bitter, and fruity. It was used raw to prepare foods. In the *Edict of Diocletian*, it is called *oleum flos* (flower oil) and cost 40 denarii a sextary (roughly a half litre, the salary of an unskilled labourer). In Greece, with the November fruits, well ripened, black and recently harvested, an oil with a light taste was produced, this being widely used and much appreciated (Theophr., *C. P.* XIX 4). In Italy, it was called *primum oleum* and it was more profitable but less flavourful (Varr., *R. R.* I 55; Pal. XII 4, 1). It was used in food by the families that produced it. In Italy, with the fruits at the end of winter, another good oil was made, this one being light coloured, not very fruity and similar to *viride* (Col. XII 50).

Other more abundant and common oils also existed, these being pressed from over-ripe fruits, crushed and collected without care. The most usual in Greece was *élaion koinón*, very popular in cooking and preserving foods. In the *Edict of Diocletian*, it is called *oleum sequens* and cost 24 denarii. It was usual to extract other lower-quality oils (*deúteron geúmatos*) by means of a second and third pressing, and even worse (*khydaion*, *oleum cibarium*), wetting and grinding the marc, which had primarily industrial uses (Hor., *Sat.* I 6, 124), and its price was 12 denarii (Blanc and Nercessian, 1992). A by-product could even be produced from the stalks and the dregs, and this cost eight denarii (Juv. V 86–91; Hor., *Sat.* II 2, 59).

Like today, there were oils with denomination of origin (*appellation d'origine*), these being prized by Roman gastronomes but rarely cited in Greek texts (Amouretti, 1986; Blanc and Nercessian, 1992). Athenaeus (I 66f; 30b) eulogized those of Samos and Turios. Romans appreciated the exquisite and perfumed oil from Liburnia (southern Istria) (Plin. XV, 8; XV 16; Marc. XII 63, 2) as well as the Italian ones of Picene and Venafrum (Cat., *Agr.* 146; Varr., *R. R.* I 2, 6; Estr. V 3, 10). Pliny the Elder (XVII 93–94), Strabo (III 2, 6) and Martial (XII 63, 1) praise the quality and abundance of the oil from Baetica. Pliny the Elder (XV 17) also extols the oil from Lusitania, which was made from very sweet olives. Apicium (I 4) and Palladius (XII 18) disclosed tricks for improving the oils of Hispania. According to Palladius, this could be done in a way similar to that of Liburnia, adding a marinade of ground and toasted salt with dry elecampane, laurel and rushes, mixing it all well and letting it settle for a few days, when it would be ready.

Archaeology has documented the abundance of oil from Hispania in Rome. In the first centuries of the Roman Empire, this was evidenced by painted inscriptions (*tituli picti*) on millions of amphoras deposited in the Mount Testaccio (Rodríguez-Almeida, 1984). It is estimated that this mount currently contains close to 25 million amphoras that would have contained a minimum of

1,732,500,000 kg of oil. These data have been used to calculate that during the first three centuries of the Christian era, each inhabitant of Rome consumed a yearly average of 6 kg of oil from Baetica, a quantity that has probably been underestimated, as it estimates that the city of Rome would have had a constant population of one million inhabitants, a figure that appears to be exaggerated (Blázquez and Cabrero, 2001). Most of the oil was allocated to the imperial *annona*, which distributed it among the plebians (H. A., *Aur.* 48, 1; *AP.* 8, 11; *AS.* 22, 1–3) and the army (H. A., *Gord.* 28, 2). The zenith of exportation of oil from Hispania was reached between AD 140 and 165, especially in the period of Antoninus Pius, and declined between AD 160 and 200, times in which Septimus Severus sought 'forever to assure the Roman public of a daily ration of oil, free and very abundant', leaving behind at his death the funds necessary for this (H. A., *Sev.* 18, 3; 23, 2). The oil was also exported massively to Britain, Gaul, Germania and North Africa (Remesal, 1986; Curchin, 1991). In the second half of the 3rd century AD, the exportation declined to a minimum (Remesal, 1986; Curchin, 1991). In addition to the oil from Hispania, Rome imported great quantities of oil from North Africa (Camps-Fabrer, 1996; Ponsich, 1996).

The quality oil was used by the upper classes for food and the normal oil was used by the rest of the population. The worst oils, and even those made from by-products, were for the poor, and thus the miserable masses that had no resources were obliged to use these inferior oils, as emphasized by Juvenal (V 85–88): 'He will bathe his fish in cheap oil; but this discoloured cabbage that, sadly, they serve you will smell like a lamp, since they fill your cruets with oil transported here in ships ...'.

According to Juvenal (V 90), some oils had such a foul odour that they scared away 'black serpents'. Similarly Horace (*Sat.* I 6, 124) complained about the disgusting oils. To correct the bad oils, or so that they would take a certain perfume, the oils were aromatized with herbs or flowers, according to formulas given by the growers (Cat., *Agr.* 145; Varr., *R. R.* I 51; Pal. XII 19–21).

6.6. Food products from the olive

In the ancient Mediterranean world, the olive fruit played a role of the highest magnitude. We shall examine its consumption with oil, olives and derivatives.

Olive oil was by antonomasia the food fat of Greece, afterwards of Rome, and finally of the entire Mediterranean Basin, becoming a distinctive cultural sign of civilization, since the barbarians used other fats (Str. III 3, 7; XVI 4, 24). Since then, it has not ceased to be used up to the present and constitutes the most characteristic element of the Mediterranean diet. The different oils had different uses. The best, kept in the oil dispenser (*lagoena*), was placed on the table to drizzle food. Archaeology has uncovered containers in the form of bottles with two spouts and two different reservoirs, one for oil and the other for vinegar or *garum* (Bovon and Bruneau, 1966). The mixtures of oil and vinegar (*oxélaion*) and of oil and *garum* (*garélaion, oleogarum*) were widely used. Virgin oil served above all for dressing salads and raw vegetables (*acetaria*) (Amouretti, 1986; Blanc and Nercessian, 1992), highly prized and consumed by the Graeco-Romans (Hor., *Sat.* II 3, 125). The

quality oil was essential for preparing the most exquisite dishes and the most refined sauces (Hor., *Sat.* II 4, 64–69). Another very common use was to drip the oil onto bread, a custom that has survived to the present. The Greeks and Romans ate a great number of boiled foods, particularly soups and stews, the flavours of which were heightened with a splash of oil, which was also dribbled on meats and fish (Hor., *Sat.* II 4, 50) or the popular *moretum* described by Virgil (*Moret.* 112). Also, olive oil was frequently used in pastry to lighten the dough. Apicium used it profusely, and statistically it is the third most used ingredient in his book of recipes, appearing in three-quarters of all the recipes and being surpassed only by pepper and *garum* (Blanc and Nercessian, 1992). Also, it was used to fry foods, but frying was much less common than today. It should be clarified, as observed by André (1961), that despite everything said about oil in Graeco-Roman antiquity, it was much worse than today's because the production procedures were rudimentary, being performed in closed places with heat and firewood smoke, and the oil was often salted to maintain fluidity. It quickly lost its aroma, it easily went rancid and it acquired an acrid flavour.

6.7. Olives: preparation methods

Olives were an everyday food and indispensable for their nutritive and gustative virtues, their resistance to deterioration, and their ease of preservation and transport. They were taken home and prepared in a basket or in a bag of esparto, or they were bought already prepared from street vendors or the market at a modest price of 2 drachmas the medimnum (55 litres) (Amouretti, 1986). In ancient Greece, olives were essential in the daily fare of the country people, workers, artisans, soldiers, participants in the assemblies, etc., as reflected in the comedies of Aristophanes (*Ran.* 989; *Asambl.* 309; *Acar.* 550). According to Plato (*Leg.* VI, 782b; *Rep.* 372c), olives constituted one of the traditional foods that should not be absent from habitual meals. In Rome, they were everyday fare for humble people, and Cato (*Agr.* 58) recommends them for the food of those who worked the fields, being an excellent accompaniment for bread. The 'true Romans' also consumed them, since, despite their wealth, they never renounced the healthy traditional foods (Hor., *Sat.* II 2, 46). They were eaten freshly cured or in conserves and were not simply hors d'oeuvres but rather a basic food that was eaten before, during and at the end of a meal, as reflected in the epigram by Martial (XIII 36) dedicated to the much appreciated olives in Rome: 'These olives that come from the mills of Picene, they themselves announce the beginning and end of banquets.' They were famed to stimulate the appetite: 'many persons without appetite,' says Plutarch (*Mor.* VI 687d), 'if they take a cured olive, on trying it regain their appetite ... ' and '... prompt a certain good disposition to receive food.' The rich and the poor ate olives daily, although of different quality. The best were for those with money, as seen in the *Satyricon* (Petr., 31 9), where Trimalcion serves his guests exquisite olives; the worse ones were for the humble.

For the preservation of such an important food over the entire year, there were methods that are still in practice. As is well known, the olive cannot be eaten directly from the tree, for it has a disagreeable texture and bitterness

(which today we know is due to its glucosides) and should be sweetened, soaking it in water, which is renewed periodically, and different products are added to the final water for the curing. The preparations were very diverse, as they were for green, black or chopped fruits, or in paste. The Greek recipes were laconic and scarce (Athen., II 56b-f) but from these come the Roman ones that have come down to us (Amouretti, 1986; Blanc and Nercessian, 1992).

To preserve green olives of autumn (*colymbades*), according to Columella (XII 47–49), they are smashed or slit (to facilitate soaking and save time) and are submerged for a while in hot water, then dill seed is added, lentisk, and toasted salt, and then they are squeezed. They are placed in a clay pot and covered with fresh new wine or grape wine or vinegar and green fennel is added. Also, they can be placed in an amphora with dry fennel, lentisk seeds and fennel and covered with brine; before they are eaten, they are seasoned with leek, rue, celery, mint and vinegar and dusted with pepper, *mulsum* and green oil. Apicium (I 14) gives a very simple formula: 'place the olives picked from the tree in oil; they will be preserved the same as if they were recently collected'; furthermore, 'they can be used to make green oil whenever desired'. So that they last a long time without rotting, the container should be closed well. It is a good recommendation, as observes Amouretti (1986), as today we know that this slows down oxidation and fermentation, so that the olives do not turn rancid.

Well-ripened marinated black olives (*drypepeîs*) were well liked in Greece. In Rome, less ripened olives were preferred, just on turning from green to black (Plin., XV 6). Palladius (XII 22, 1) recommends preparation according to the 'Greek method'. First, the alpechin is eliminated and the olives are preserved in brine, or they are kept for a month in layers alternating with layers of salt, and afterwards are dried and oiled. According to Columella (XII 48), it suffices to cut them and place them in cooked wine, vinegar and honey.

The preparations of chopped olives, according to Diphilos of Syphnos (Athen., I 56a–d), were considered in Greece to be very digestive and flavourful. The paste of black olives was sold in the market prepared to be eaten, the recipes having survived through Latin authors. Cato (*Agr.* 119) cites the *epityrum*, which Varro (*L. L.* VII 86) attributed to the Greeks of Sicily. According to Columella (XII 47), it was done with *pausias* or *orquites*, picked by hand, pitted and chopped, seasoned with oil, vinegar, coriander, cumin, fennel, rue and peppermint, and left in a clay pot covered with oil. The *sirape* or *samsa* was another preserve that lasted up to two months and was made by pressing the best fruits. According to Columella (XII 49), very ripe black olives were used after being washed and screened. They were placed in a hemp bag and pressed overnight. The next day, they were milled without breaking the olive stone; the mush was turned with crushed salt, fenugreek, cumin, fennel and Egyptian anis, adding a *hemina* of salt for each *modium* of olives, and so that they did not dry, oil was poured over the mass. The current equivalent is the olive paste *tapenade provençal* (Amouretti, 1986; Blanc and Nercessian, 1992), which is made with capers, anchovies and pitted black olives crushed in a mortar and seasoned with olive oil, aromatic herbs and lemon juice. In Provence, this paste is used to accompany raw vegetables, to spread onto slices of toast or to fill hard-boiled eggs, mixing the paste with the yolk. It also accompanies roasted meats and fish.

6.8. Non-dietary uses of olive oil

These uses include hygiene and body unction, perfumery, industry and religion. A fundamental use, in the sphere of hygiene and medical therapy, was body care and cleanliness. The ancient Greeks cleaned themselves with water and a sponge, without soap (only in the 1st century BC was some sort of soapy emulsion obtained from oil), and on leaving the bath, they rubbed their body with oil, as the friction warmed and softened the body, preventing drying by the calcareous water (Amouretti, 1986). The unctions appear frequently in Homer, and they were given by women and servants. Ulysses tells Naussica (*Od.* VI 96): 'I will know without your help how to wash myself with foam and rub myself down with this oil that, after so much time, my skin does not know.' Each person took a flask of oil (*lecythus*) to the baths, these vessels being frequently illustrated on Attic vases (6th and 5th centuries BC). Massage was true therapy, and Plutarch (*Mor.* VI 695d) pointed out that its limit is that it should not upset the senses nor cause pain. It was applied for the care of newborns, as indicated by Soranus of Ephesus in *On Diseases of Women* (II, 11, 12): 'the wise woman sits, spreads a linen towel or other cloth over her hips and knees, then places there the newborn, undresses it, and rubs its body with tepid olive oil.' If someone could not bathe, Hippocrates (*Dis.* 42) prescribed friction with warm oil and wine. The gymnasts before exercising were given massages to ready the muscles, avoid injuries and to protect their skin against differences in temperature. According to Hippocrates (*Vict.* II 65), it was fundamental against the cold and the sun: 'during the winter, oil aids development by keeping the cold from disturbing anything in the body.' He stated that 'the rubbing of oil mixed with water softens and prevents dangerous overheating'. He also credited it with helping against blows, advising the practice of 'prolonged wrestling, with the body oiled' (*Vict.* III 68). After the exercise, the body was covered in sand and dust of the arena, which stuck to the oil, this layer then being scraped off with a strigil (curved metal scraper), and it was used for medicine (Diosc. I 30, 6) and illumination. According to Pliny the Elder (XV 19; XXVIII 50), its sale made the gymnasts rich. The athletes, after their event, bathed and were rubbed down with oil again, at times hot, to relax and avoid soreness (Hip.; *Vict.* II 66).

The oil served as a base for making perfumes (*myron*), although the aroma of the olive oil itself was considered a perfume. Galen, as noted by Amouretti (1986), differentiated clearly between the perfume and perfumed oils. Archaeology (Mycenaean tablets), wall paintings of Pompeii, and the texts of Dioscurides (I 30–65), Theophrastus (*De los Olores*), and Pliny the Elder (XIII; XV 24–32; XXIII 83–96), provide rich information. Recent archaeological findings in Delos and Paestum highlight their economic importance (Brun, 1998, 2000). A perfume was composed of the excipient (the fatty matter), the essence (the plant that impregnated the excipient with aroma), and the complements (colorants, fixatives, preservatives). The best excipient was olive oil, especially the bitter oil of green olives (which rendered little oil), for being receptive, preserving better and resisting heat. Columella (XII 52) recommended the use of *licinia* and *regia*. Perfume makers extracted the oil with the mortar and the wedge press (represented in the paintings in the House of the Vetii in Pompeii) (Mattingly,

1990). They also used the oil from *ben*, from bitter almond and from sesame. The essence of the plant was extracted by squeezing (mincing and mashing it in the mortar), by heat exhaustion (maceration in oil heated to 60–70 °C) and by cold exhaustion. Some oils served as the excipient and essence at the same time: laurel, myrtle, rose, narcissus, cider, quince, spurge laurel, lentisk and henna (Diosc. I 34–35). For colourants, plants or minerals were used (henna for green, alkanet for red). As fixatives, gum and resin were used. The preservatives (salt and alkanet) prevented the perfume from going rancid (Amouretti, 1986).

Olive oil was used in illumination, the textile industry, impermeabilization, lubrication, etc. The basic fuel for domestic, public and industrial lighting used the poorest grade of oils and residues. The domestic ceramic or bronze lamp lasted about two and a half hours and those for mines lasted 10 hours. One litre filled 10 to 12 lamps, translating as 250 to 300 hours of illumination (Amouretti, 1986).

Since Homeric times (*Od.* VII 107), oil has been used for wool (for greasing and impregnating), linen (moistening the threads of the warp) and in fulling mills (cleaning of fabric). Olive oil was used for maintaining metal and wood used in ships. It was used to coat containers (clay wine jars, tubs and storage jars). For assurance of hermetic seals, the joints of water pipes were covered with a layer of lime soaked in oil (Vitr., VIII 6, 8; Pal., IX 9). *Amurca* was used to grease wood, leather, metals and axles, to impermeablilize containers, and to finish walls, floors, threshing floors and silos.

In medicine, it was prescribed for treating ulcers and wounds. In veterinary medicine, it was used to heal cuts; and in agriculture, it was used as an insecticide and fertilizer (Cat., *Agr.* 95, 97; Col., V 10; Plin., XVII 47, 259). Furthermore, the olive stones were used as a fuel (Amouretti, 1993).

Olive oil was used for libations to the gods and spread on meats to be sacrificed (Plut., *Vit. Arist*. 21), although this use in Greece and Rome was more moderate than among eastern peoples. It served as a prize for winning athletes at the Pan-Athenian festivals dedicated to Athena. This oil came from trees that descended from the olive tree consecrated to the goddess, and it was demanded as a contribution from their owners. 'The archon, after collecting the oil produced that year,' states Aristotle (*Const. At.* 60 3), 'delivers it to the treasurers on the Acropolis, and in the Pan-Athenian festivals gives measures of it to the arbiters of the games, and these in turn to the victorious participants.' The winners received their oil in Pan-Athenian amphoras, of which about 700 were distributed, although, according to Amouretti (1986), the number could reach 1300. Each one made a *metreta* (some 30 litres) with a value of 12 drachmas in the 4th century BC. A victory provided 1680 drachmas, a fortune in an epoch in which the salary of a labourer was 1 drachma per day.

6.9. Nutritional, dietary and therapeutic properties of olive oil

Olive oil, from Hippocrates to Galen, was considered to be nutritious and to provide energy as well as to promote reconstitution. It supplied a notable amount of lipids to the diet, basically vegetal in composition, balancing the scant intake of

other fats and boosting energy. According to Amouretti (1986), a free Greek citizen ingested some 20 litres of olive oil per year (55 ml daily, providing 490 calories). But certainly this figure would be less for women and children, slaves and the poor. Some calculations estimate that the mean daily caloric intake would be some 3200 to 4300 calories, and thus the oil would supply some 12 to 15% of the total energy. Its dietetic properties were valuable. Dioscurides (I-30) affirmed that it was 'very gratifying to the stomach' and cleaned the digestive tract. It was a usual condiment of dietary regimes to improve the flavour of dishes and for its power to reconstitute the body (Hip., *Dis.* 55). Hippocratic treatises frequently stress that cereals, legumes and fresh vegetables are served 'well oiled'.

Dioscurides (I-30) states that olive oil itself possesses curative virtues, warming the body and fortifying its capacity to react. He recommended unction for intense physical activity for its effectiveness against muscular fatigue and pain, indicating that the best is *onfacino*, 'fresh, fragrant and not at all acidic'. However, for the medical preparations, he preferred mature fruits. He also praised the medicinal properties of aromatic oils (laurel, myrtle, cedar, henna, rose, lily, iris, etc.), prepared by perfume makers that performed the duties of pharmacists (Brun, 2000; Amouretti and Brun, 2002).

Olive oil was used in skin care. It was believed to be excellent for maintaining healthy, lustrous hair as well as reducing dandruff, grey hair, female hair and hair loss (Diosc. I 30; Hip., *Mul.* II 189, 186). Dioscurides held that it was useful against scabies and dry patches of the body, giving facial lustre to women. The rub-downs, general or local, were applied in the therapy of internal diseases, the temperature often indicated at which the oil should be applied. Hippocrates prescribed the general unction for a type of typhus (*Int.* 39), which clinically resembled typhoid fever, and recommended that the patient apply warm oil and 'go to bed'. He also prescribed 'hot washes with oil' for tetanus (*Int.* 52). Local unction was used for ulcers on the head, which were first rubbed with oils and afterwards covered with a layer of oil (Hip., *Morb.* II 13), and as an analgesic for odinophagia, cephaly, pleural or intestinal pain. It was frequent to cook legume and cereal flours with oil and wine and apply them in cataplasms to the affected zones. It was used in eye and ear maladies. According to Dioscurides, 'it clears the eyes', while Hippocrates counselled rubbing the eyes with 'mild oil'. Instilled tepid into the ear, it was useful in deafness and to eliminated wax plugs (Hip., *Epid.* VII 63), a use still in vogue. It was effective for disinfecting and healing over of injuries, burns and ulcers. Olive oil was administered in complex preparations, injecting (into the lung) to treat the 'empyema that appears after a pneumonia'. It was used in enemas, as, according to Hippocrates (*Int.* 20), oil, wine, and honey 'are very mild things, in an enema, for the nature of the human', advising for 'emptying the bowels' of children to use a mild washing of wheat flour, honey and oil (*Mul.* I 92). Dioscurides (I-30) claimed that olive oil expelled worms (an ailment that must have been very common from the abundant citations in the texts). It was used to submerge cauterizing irons (*Int.* 28). It also was included in the therapy of the respiratory system in inhaled mixtures (*Morb.* II 26). An expectorant compound was taken orally. In Hippocratic gynaecology, it was used profusely as a lubricant: 'If during childbirth the woman is dry and does not moisten, she should drink oil and moisten her parts with hot oil ... and liquid wax is rubbed in

and goose grease with oil is poured on' (Hip., *Mul.* I 34). It helped expel afterbirth and speed delivery (*Mul.* I 46, 48, 77), served as an emetic (*Mul.* I 52), helped the galactophores (*Mul.* I 44) and acted as an anti-inflammatory (*Mul.* I 77). It was useful in irrigation for the 'saturation of bile', in shrinking the pituitary (*Mul.* I 80, 109), and in fumigation for the suffocation and flows (*Mul.* II 195, 200).

It has been said that the oily layer removed from the skin of wrestlers had medical uses. Dioscurides said that this 'mud' was very useful as a plaster. Shredded or cooked olive leaves were effective as a febrifuge, a use that has come down to us, and were used against ophthalmological maladies, inflammations, ulcers and to stop periods (uses described from Hippocrates to Galen).

6.10. Other vegetable oils and animal fats

The importance olive oil in the Graeco-Roman world defined the ranking of other fats. It was a symbol of civilization and the classical authors criticized the barbarians for not using it. Other vegetable oils used were from almond, walnut, castor bean, sesame, cardamom, radish and *ben* (Theophr., *De Odor.* IV 14, 15). Occasionally, these oils were used in food, but above all in perfumes and medicine. According to Dioscurides (I, 30–32), the most useful was from bitter almond (*metopio*), as it was an analgesic, diuretic, antiseptic and emollient. Oil from the castor bean was used against worms, wounds and otalgia; from radish for lice; from sesame for the throat and to clarify the voice; from *ben* was for anti-inflammatory properties; and oil from walnut was used only for illumination (Diosc. I 30–32).

The Graeco-Romans used few animal fats in their food. The fat from duck, chicken, goose, goat, sheep and cow did not appear in culinary preparations, and their use was medicinal and industrial. However, they used pork lard (*adeps*). Apicium (II 4) uses pork lard fresh, mixed with meat, in sausage making (*lucanicae*), and, especially to coat pots where he made *patina*. As André (1961) observed, it was used in preparing certain dishes (*puls, catillus ornatus*), certain pastries (*globi, encytus, mustacei*) and in a special type of bread called *artolaganon* (although this was also made with olive oil).

A product highly esteemed for its good preserving properties was bacon fat. It was consumed with moderation by all social classes, as testified by Horace (*Sat.* II 6, 64), who supped on broad beans and vegetables 'sautéed with just the right amount of bacon fat', and Juvenal (XI 82–85), who remembered that the consul Curius ate it only at festivities. It was a basic food of country people and soldiers, who carried it with them (H. A., *Adr.* 10), enabling them to reduce their intake of olive oil. For its importance, some emperors lowered its price so that it could be acquired by the poorest people. Alexander Severus lowered the price eight times and even distributed it free to the plebians in distributions by the state (H. A., *Aur.* 48, 1). It was eaten salted, cured, and often smoked (Col. X 53), although at the tables of the rich it was cooked with dill and seasoned with salt and oil 'drop by drop' (Apic. VII 11). Animal fats were a rare culinary ingredient.

Dioscurides (II-76) ascribed medicinal properties to fats, claiming that they had warming virtues, softened the skin and opened pores. Goat and sheep were said to be useful for podagra and dysentery; pork fat helped burns and inflammation; fat

from fowl was used for otalgia and facial lustre; and cow fat for wasp stings. Animal fat was used by the followers of Hippocrates in gynaecology (Hip., *Mul.* I 15; 64–6; *Steril.* 9, 30; *Superf.* 32; *Nat. Mul.* 83) and to treat open blisters, burns, haemorrhoids and fistulas (Hip., *Ulc.* 21–23; *Haem.* 9; *Fist.* 7).

7. The Olive and Olive Oil in the Medieval and Modern Eras

During the Middle Ages and the modern era, cultivation of the olive and the making of olive oil underwent no notable changes, and the production of oils and their uses hardly altered their course since antiquity. The archaeological sources and writings are scarce for the medieval period, becoming somewhat more abundant in the recent past. Medieval olive cultivation in the eastern Mediterranean is known from Graeco-Byzantine and Arab texts. Similarly, rich information is available for Moorish Spain but it is more sketchy for the rest of the western Mediterranean. The information for the modern period is hardly novel. However, during medieval and modern times, certain facts deserve comment.

After the fall of Rome, the political and socio-economic situation was not propitious for agricultural, technological or commercial development, resulting in a regression in the cultivation of olives in the western Mediterranean, although in Byzantium it appears to have maintained acceptable levels (Fiorino and Nizzi, 1992).

In Italy, despite the existence of olive orchards throughout almost the entire country (even in northern areas) (Iorio, 1985), the olive did not remain preeminent in the first centuries of the medieval period (except in some southern zones), and there was a general waning during the 10th and 11th centuries. At the end of the 13th century, there was a revival in the south and during the 14th century the olive recovered its dominance in the landscape, with a boost in oil production and trade (Comet, 1993; Grieco, 1993; Cherubini, 1996; Cortonesi, 2002). In central Italy, the recovery was strong only after the 16th century (Cortonesi, 2002). Notable oil trade centres included Gaeta (with a long tradition), Apulia (from the 13th century), and, from the 13th to the 14th centuries, Brindisi, Bari, Giovinazzo, Molfetta and Manfredonia, as recorded in the *Pratica di mercatura* by Francesco Balducci Pegolitti (Cherubini, 1996; Comet, 1996; Cortonesi, 2002). Nevertheless, in Italy, it would not be until the modern era when olive cultivation and oil making would be firmly developed (Grieco, 1993; Cherubini, 1996; Cortonesi, 2002).

In France, there is no mention of olive orchards until the 11th and 12th centuries, these being located in Provence (Marseilles, Nice and Draguignan) (Stouff, 1988; Comet, 1993, 1996), but the progression had to wait until the 17th century, a time in which olive cultivation and oil making were becoming established in Provence, constituting one of the main resources (Stouff, 1988; Comet, 1993; Boulanger, 1996, 2001). Marseilles and Nice turned into important trade centres for olive oil (Comet, 1993; Boulanger, 1996, 2001).

In Visigoth Spain, olive cultivation receded, but it is presumed to have retained a certain importance in the south. The situation improved in the Moorish period, and from the 8th to the 9th centuries, olive cultivation developed and

remained stable for the successive centuries (Vallvé, 1982). In the 10th century, the chronicler Rasis (XXXI) testified to the great oil production of *Al-Andalus*, which was sustained without interruption, although with highs and lows, until the 15th century (Vallvé, 1982; Bolens, 1996; García Sánchez 1996, 1997). Outstanding among olive-growing regions was Andalusia, above all the zone of hills to the west of Seville, called *al-©araf* (Aljarafe), considered the kingdom of the olive (Ibn al-Awwam VII 1), for its extent and production of optimum-quality oil, which enjoyed great prestige over the centuries (Vallvé, 1982). Together with Seville, the chroniclers of the 10th to the 14th centuries cited notable zones of Andalusian olives: Priego, Baena, Cabra, Jaén, Arcos, Guadix, Baza, Jerez, Morón, Niebla and Jódar. Outside Andalusia, the most important areas for olives were: Lérida, Valencia, Crevillente, Xátiva, Jaca, Fraga, Mequinenza, Barbastro, Badajoz and Coimbra (Vallvé, 1982). Olive growing in Seville declined in the 15th century, and the oil from Aljarafe lost prestige, the preferred oils at that time being from Écija, Loja and Antequera (De Castro, 1996b).

At the beginning of the modern era, olive cultivation was consolidated and expanded due to the repopulation of olive-growing zones, to general demographic growth, to the surge in oil exports to traditional European markets, and to the recently created markets in America (Céspedes del Castillo, 1961; Reglá, 1961), since, although Spain had taken the olive to the New World with the intent of expanding its cultivation, its development was later slowed down for the interests of Spanish growers, who, to avoid competition, persuaded Philip II and Philip III to prohibit its cultivation abroad (Céspedes del Castillo, 1961; Parra, 1990). From the 16th century on, numerous olive orchards were planted in Andalusia, Castille and Extremadura (Reglá, 1961) as a consequence of a sharp rise in the price of olive oil, which tripled from 1511 to 1559 (Tió, 1982). With the beginning of the 17th century, olive cultivation stagnated and converted to cereals, until the emergence of a crisis for major fluctuations in yield and prices. The new expansion of olive growing was aided by the expulsion of the Moors, which seriously damaged horticulture and thus an effort was made to compensate for the damage by strengthening dryland farming (Tió, 1982). At the end of the century, another downturn lasted until the middle of the 18th century, when a new, vigorous surge swept Andalusia and, to a lesser degree Catalonia, Aragon and central Spain (Mercader and Domínguez, 1961), related to profound economic and political changes: the disappearance of land-owner privileges, the cultivation of lands disentailed from the church, and an increase in trade for the boost in interior and exterior demand for oil (Mercader and Domínguez, 1961; Lovera, 1993; Zambrana, 2000).

Information on agricultural techniques is scarce, but indicates that they hardly evolved. The western Mediterranean followed the Roman agricultural tradition while the east followed the Graeco-Byzantine (begun by Vindanio de Beirut, 4th to 5th centuries AD) and Aramean (*Nabatean Agriculture* by Ibn Wahsiyya) (Carabaza Bravo, 1996; Fahd, 1996). Olive cultivation in Moorish Spain is a special case, as it was under triple influence: Roman, Graeco-Byzantine (through Casiano Basso Escolástico, 6th century of *Geoponica*, 10th century) and Aramean (*Nabatean Agriculture*); and, with hardly any new developments, it was widely practised and valued, as shown by the rich geoponic literature, which

states that the olive trees could live up to 3000 years (Abu l-Jayr, fol. 69v; Ibn al-Awwam VIII 15) (Carabaza Bravo, 1996). For the modern era, the information available reveals that olive cultivation evolved with respect to the previous periods. Thus, in Spain, for example, the *Agricultura General* by Alonso de Herrera at the beginning of the 16th century served as a model for several centuries.

Of the olive varieties, little information is available, although for the medieval period, a half dozen are known in Spain, and more than twice that number in Italy, some of which lasted in both countries and are still among the leading varieties. This is the case in Italy with the olives *celine* (known in the Norman period in the land of Bari), *frantoio* and *moraiolo* (cited in Florence in the 15th century) (Cherubini, 1996). In Spain, such varieties include *lagin* (*licinia* in Latin, today's *lechín*), *mansanal* (*manzanilla* today) and *warkat* (*gordial* or *sevillana*) (García-Sánchez 1996, 1997). The varieties multiplied over the modern centuries throughout the Mediterranean.

In the preparation of olive oil, no notable variations occurred with respect to antiquity, and in some areas, the most rudimentary systems persisted into the modern era. The means to collect olives remained the same and, depending on the region, knocking predominated over hand picking. For example, in central Italy, picking was preferred (*brucatura*) while knocking is more usual in the south (*bacchiatura*). However, as observed by Cortonesi (2002), the work is facilitated by spreading a tarp (*racana*) under the tree. In Spain, in medieval times, the preference was careful hand picking, as recommended by the Andalusian geoponic writers (Abu l-Jayr, fol. 131r), this continuing in the modern epoch (Herrera XXXV), but knocking was also practised (Ibn al-Awwam VII 4). The time of collection of the olive usually depended on its ripeness, but in some places, there were fixed dates or the authorities made the determinations (Cortonesi, 2002).

The fruits were usually transported to the mill by pack animals. The extraction systems followed the same steps as always. In the family setting, the same procedures were used, disregarding the mills and presses. In the mills, whether Italian, Spanish, Byzantine, Magrebi or Syrian, practically the same mechanisms were used, although improvements were slowly introduced for increasing yield. The milling was done with millstones that perpetuated the Roman *trapetum*, generally powered by animals. There were hardly any differences between the Italian *trappeti* (Cortonesi, 2002) and the Spanish *alfraje*: over a circular stone structure turned another stone that was moved around a tree by an axle that connected the tree to the animal (Rodríguez-Molina, 1991; Chalmeta, 1996). It was very rare to use hydraulic energy, and it was adopted only from the 15th century on, but was never widespread (Chalmeta, 1996; Cortonesi, 2002). Over the modern age, the *trapetum* was replaced by a millstone of greater dimensions, but the cylinders remained (Amouretti, 1996). The presses throughout the Mediterranean continued to be lever or screw types. The most usual medieval lever press consisted of a large revolving horizontal timber that was tightened in the middle by a screw, or threaded wooden column, which supported the beam and by which the stone that squeezed the esparto pressing bags full of olives exerted pressure. The oil fell into a well or vat where it was decanted and afterwards drawn off into other containers (Rodríguez-Molina, 1991). In the modern

era, an attempt was made to improve productivity of the lever press by systems of heavy trees that used a timber of 12 to 20 m long with a frame formed by large beams connected by iron bands that were activated by hand, reaching 30 tonnes of pressure. This required a great deal of labour time, as in one lot only 20 to 30 bags could be put into place, and, furthermore, a building was needed to lodge the press (Zambrana, 1981, 1987; Amouretti, 1996). In short, it was slow and expensive and therefore it began to be replaced by the direct screw column, quite widespread in Italy, in which the pressure on the plank was exerted directly by the screw. It was also manual, but strong and fast. In time, efforts were made to perfect this system: improving the size of the screw column, adding the capstan to turn the screw, manufacturing the screw in iron rather than wood, etc. The improvements increased the number of bags per lot, raising yield and lowering costs (Zambrana, 1981, 1987). Nevertheless, at the end of the 18th century and the beginning of the 19th, almost all the press systems invented in antiquity coexisted, each with its advantages and disadvantages (Zambrana, 1987; Amouretti, 1996).

The information available suggests that the quality of the olives obtained remained the same as in antiquity. Thus, in *al-Andalus*, 'water oil' (*zayt al-mā'*) was the best, as was the 'washed oil' of the Neolithic, which was made in a rudimentary way for home consumption mashing the fruits in a mortar and washing the mush with hot water; the oil separated by flotation and poured off (Eitam, 1987, 1993a, 1996). This virgin oil, for its laborious preparation, was expensive and appeared only in small quantities on the market (Chalmeta, 1996). The second in quality was *zayt al-ma'sara* or *zayt al-badd* ('milled oil', as press in Arabic was *ma'sara* or *baad*), this being the most common for food and obtained by milling and pressing the fruits at air temperature (Bolens, 1996; Chalmeta, 1996; García Sánchez, 1996). The worst, 'cooked oil' (*zayt al-matbūh*), which was extracted from olive marc after scalding, grinding and pressing it, was put to industrial uses (Bolens, 1996; Chalmeta, 1996; García-Sánchez, 1996). As in antiquity, an oil of great quality, *zayt al-unfāq* (*onfacino* for the ancients) or *rīkāb*, which was used in the pharmacy and in perfume making but rarely in food. It was extracted from optimal green, hand-picked olives (Bolens, 1996; García-Sánchez 1996, 1997). Efforts were made to improve defective oils by following Graeco-Roman procedures (Abu l-Jayr fol. 131v, 132r).

For preservation, the oil was protected from light and heat, keeping it in clean clay storage jars in places facing north (Ibn al-Awwam VII 1). During the modern age, oil quality was hardly improved, as the main preoccupation was profitability. As noted by Amouretti (1996), the abbot Couture recorded at the end of the 18th century that 'most of the direct producers were concerned less with the quality than the quantity obtained', using all the means possible to increase production. They allowed the fruits to drain for days or they were boiled, ground, and pressed to the utmost and then mixed with broths, rendering products of abysmal quality. Nevertheless, in France, especially, and in Italy, attention was given to increasing quality by scrupulously following all the steps, from the harvest to the pressing, differentiating the juices of the first cold pressing from those extracted with scalding (Amouretti, 1996; Boulanger, 1996; Ramón, 2003). In Spain, quality oils were also produced in Aragon and Catalonia that

could compete with Italy and France; however, in Andalusia, production was directed towards profitability without attention to quality, although the oils continued to be exported by the growing industrial demand at the end of the 18th century (Zambrana, 2000, 2003; Ramón, 2003).

7.1. Products from the olive in medieval and modern food

With the exception of the Arab Mediterranean zones of North Africa, of the Far East, and of Spain, and some areas of Europe, the food consumption of olive oil fell with respect to antiquity, although it continued to be used for illumination, religion, perfume making, medicine, textiles, soap, etc. (Stouff, 1988; Bolens, 1996; Boulanger, 1996; García-Sánchez, 1996).

The fall of the Roman Empire wrought profound socio-cultural change with sweeping economic and dietary implications. The Germanic domain that succeeded the Latin one revalued the forest and pasture economy to the detriment of agriculture, and in the sphere of food placed animal products, especially pork and fat, over vegetables and olive oil (Montanari, 1979, 1997). Nevertheless, the changes were not rapid, so that, in the 6th century, olive oil was still preferred. As pointed out by Montanari (1997), Antimus, the first medieval dietician, stated that bacon fat could be used as a condiment for vegetables and other foods 'when oil was lacking'. Gradually, the dominant classes accepted the consumption of the new leaders and ended up pleasurably consuming meat and fat products from animals, as was in fashion. Pig raising became dominant in the large lay properties and its fat instead of olive oil filled the pantries. Lard became accepted even in the monastic diet to stew vegetables and greens. Thus, the Latin imprint gradually faded from food in favour of the Nordic one, and in this way the barbarians, whom Pliny the Elder and Strabo had denigrated for their taste for animal fat, had their revenge, launching a fight that would have no end (Montanari, 1993, 1997). Afterwards, when Christian values predominated in Europe, the Latin and Germanic systems would blend, favouring the alternation of the products of both. The ecclesiastic guideline, on prohibiting animal products on certain dates, facilitated the diversification of the fats and a homogenization of the dietary model. In this way, under the guidance of religion, olive oil was assured symbolic power by which the south would prevail over the north (Montanari, 1993, 1997).

Throughout Christian Europe, except in a few Mediterranean zones, the daily consumption of olive oil was scarce, and its culinary use was restricted, using it to fry broad beans, eggs and, especially, fish. It was taken raw to dress salads, a function that would be long lasting (Flandrin, 1983, 1993; Stouff, 1988). However, it was obligatory in the days of abstinence and Lent ('lean days'), that all together made up a third of the year – some 140 to 160 days (Stouff, 1988; Montanari, 1993). The most usual fats were animal, as observed in books on cooking and recipe books of the period, and there were also other vegetable oils that were more abundant and cheaper than olive oil (Hémardinquer, 1970; Stouff, 1970, 1988; Flandrin, 1983). However, in the eastern and Islamic Mediterranean, the daily use of olive oil was frequent

(Rodinson, 1949; Ashtor, 1968), this being the paradigm, as will be seen, in Moorish Spain (Bolens, 1991, 1996). In Europe, animal fat was so entrenched that even in the 'lean days' ways were sought to consume it by ecclesiastical privileges, as were granted to the sick (Flandrin, 1983; Nigro, 1997). Olive oil was noteworthy for its absence in the preparation of everyday dishes and even in sauces in Spanish recipe books (*Libre del Sent Soví*, *Libre del Coch*), French ones (*Le Ménagier de Paris*, *Le Viandier de Taillevent*), and Italian ones (*Anónimo Toscano*) as well as in the treatises of the Master Martin of Messibugo and of Scappi. On 'lean days', acids instead of fats were used (wine, vinegar, verjuice, citric juices, etc.), adding spices and thickeners (usually breadcrumbs and nuts such as almonds or walnuts), egg yolk and fowl liver. In the few cases where fat appeared, it was usually animal, especially bacon fat and lard (Flandrin and Redon, 1981; Flandrin, 1983, 1984; Montanari, 1993, 1997).

The meagre use for olive oil in northern Europe was not only a sociocultural question but also an economic and gastronomical one, as the oil was scarce, expensive, and was usually bad. The good oil could be consumed only by the privileged classes and the producers in the olive-growing regions; otherwise acceptable olive oil was difficult to find (Flandrin, 1983, 1984; Montanari, 1993). However, religious rules demanded its use on 'lean days', obligating its acquisition at a high price. Most oil that reached the north was mediocre to bad and, given also its high price, provoked complete rejection. Some examples illustrate this. In Provence, although the production of olive oil was low, good use was made of it. The first pressing was consumed there, while the rest, according to Thomas Platter, as quoted by Flandrin (1983), was sent north, where it was priced as quality oil; and it must have been bad, since in England in the 15th century a saying arose: 'black as olive oil'. Flandrin (1983) asks whether the unhappy northern Europeans had known at one time what good olive oil was, as by their inexperience they were easily cheated. Montanari (1997) tells that the Germans appreciated the Italian oil from Garda, which was clearer, lighter and smoother than that of the south, and therefore imported that oil for their food. The German market was very attractive for the Italian exporters, among which were the Venetians, who began to sell oil from Garda, mixing it with oil from Puglia, which was more abundant and less expensive. As the sales increased, the mixture contained growing quantities of Puglien oil, thereby gradually shifting the palate of the Germans. Garda accused Venice of ruining their market, and the main reason for their accusation was based on the unscrupulous mixing of oils by the Venetian merchants, offering a cheaper product to the detriment of its quality. Only the rich customers continued to buy fine oil from Garda.

The obligatory consumption of olive oil during the 'lean days' posed a serious problem of supply, as the good was very expensive and scarce and could be acquired only by the rich, while the bad did not abound, either, and was still expensive and therefore was rejected, particularly in northern Europe. The solution lay in replacing it by a fat allowed by ecclesiastic exemption that had to be paid for. Thus, the Church filled its treasury while the faithful, without compunction, consumed other, cheaper and more pleasant oils. In the year 818, the Council of Aix decided to replace olive oil with melted bacon fat, which rhetorically was called *oleum lardinum* – that is, 'lard oil'. The result was successful and

spread. Later, Pope Gregory XI made a comparable concession to king Charles V of France, and the bull called *Cruzade* authorized the Spanish to use lard (Flandrin, 1983). Also, other vegetable oils were used as replacements, a more problematic change for the scarcity of oils having acceptable organoleptic characteristics. The walnut, colourless, odourless and almost insipid, was the one with the best success in Europe in medieval and modern times. At the end of the Middle Ages, the sources diversified. In the 16th century, Agostino Gallo recovered flaxseed as a culinary oil, and later sunflower and grapeseed were used with little success, although they were successful centuries later. Also, acorn oil was used, as was sycamore, some legumes, turnip and iris, the oils of which were consumed only by the poor (Montanari, 1997).

With the passage of time, the greatest success as a substitute for olive oil was gained by butter. Although this occurred in the time of Charlemagne, it was used in 'lean days' and its use was habitual in northern Europe. However, it did not become a culinary fat because the dominant classes did not consider it a worthy food (Flandrin, 1983, 1984; Montanari, 1993). Butter began to be used with distrust when the ecclesiastic authorities awarded exemptions to several northern European communities for 'lean days'. Afterwards its use spread and it became generalized on being fully accepted by the upper classes, thereby changing its status. The acceptance of butter in the north is logical, given that the olive oil that arrived was expensive and often bad, suggesting that it was taken more for obligation than for pleasure or conviction (Flandrin, 1983, 1984; Montanari, 1997). The decline of olive oil in the north favoured by the ecclesiastic exemptions intensified with the rise of butter in the French cuisine of the modern age. In 17th-century France, a revolution of taste occurred and butter became fashionable in the refined kitchen, becoming a note of culture and prestige. Medieval tastes were abandoned and the basics of sauces as well as the underpinnings of cuisine changed as sour sauces were replaced by others which were milder and delicate and which contained fat, in this case butter (Flandrin, 1983; Montanari, 1997). Because of the strong influence of France, its taste soon crossed borders, arriving even in Mediterranean zones. The religious changes prompted by the Reformation also affected food and dealt the *coup de grâce* to the oil cuisine in northern Europe (Flandrin, 1983; Montanari, 1993).

Changes in the consumption of fats meant a fall in the market for the olive-growing regions, although exports persisted, since oil continued to be consumed and, furthermore, it was irreplaceable for industrial and medicinal use (Boulanger, 1996; Comet, 2001). The decline in olive oil was accompanied by major productive and economic shifts that have lasted to the present. The acceptance of butter and the taste for meat products, as observed by Flandrin (1983), strengthened livestock growing, particularly cattle, which, for its biological traits adapted very well to the geo-ecological setting of northern and central Europe (Netherlands, England, Germany, etc.), where butter triumphed overwhelmingly. In Christian Spain, however, it would not have much success except in small, powerful circles with a proclivity for fashion; its influence would be weak and bacon fat and lard would continue to be used with a notable presence of the traditional olive oil (De Castro, 1996a; García-Sánchez, 1996). In Italy, things would be even more complex, as the use of fats would differ depending on the

geographical zone. Butter broke in at the end of the 15th century and the beginning of the 16th with no perceptible consequences, although the upper classes of the northern regions began to use it in the periods of abstinence relegated to olive oil. However, other zones resisted and combined oil with bacon fat and lard, and in some areas the oil even appears to have gained ground (Flandrin, 1983, 1984; Montanari, 1997). This was observed, for example, in Tuscany, where a turnabout was seen in the consumption of fats, with a notable increase in oil, coinciding with the expansion of olive growing. Grieco (1993) reports the opinions of the English traveller, Dallington, who in his stories usually describes the dietary habits of the zones he visited. In 1605, he wrote, with astonishment, that olive oil was almost indispensable for Tuscans and that it seemed that if they lacked olive oil, they would find it impossible to live. Nevertheless, in Tuscany itself, the rich continued the fashion of using butter as well. Thus, in the *Arte di ben cucinare* (1662), a recipe book by Bartolomeo Stefani, the cook for the Gonzaga family in Mantua, butter was enthusiastically used in many dishes in which it had not been used before (Montanari, 1997). Over the modern age, butter became dominant in the north (Lombardy and Padania), while in central and southern Italy, olive oil or pork lard was preferred. Thus, in Tuscany, olive oil prevailed and in Emilia pork fat (Montanari, 1997).

In Moorish Spain, the main culinary fat was olive oil, appreciated for its perfume, flavour and smoothness. It was so omnipresent that, as stated by Bolens, it was rarely absent, although other fats appeared in the recipes (Bolens, 1991). It was the only fat used to fry or to stew fish; it was used to fry pastries and eggs or sauté vegetables and greens. It was added to casseroles and to roasts and always used for rolling dough and greasing turnovers (Bolens, 1996; De Castro, 1996b; García-Sánchez, 1996, 1997). To determine its role as an ingredient, and its manner of being employed, we analysed the composition of 290 highly varied recipes (229 of the *Anónimo Andaluz* and 61 from the recipe book of Ibn Razin). In 120 recipes for meat, game and fowl, oil was present in 86%: in 18 recipes for fish 94%, in 31 cereal recipes (breads and pastry) 52%, in 32 legume and vegetable recipes 88%, in seven egg 86%, in 14 cheese 67%, and in 68 fruit and confectionery 66%. The results confirm its overwhelming presence in all types of dishes. However, the texts did not clarify the type of oil used, as it appears designated in three ways: 'oil', 'sweet oil' and 'good oil'. It seems clear that the term 'oil' refers always to olive oil (*zayt al-zaytum*) and not to other vegetable oil (almond or sesame) or animal fat (lard or butter) (Bolens, 1991). Nothing is mentioned concerning its quality, either, although it is probable that it is meant to be first cold press (*zayt al-ma'sara*) or water oil (*zayt al-mā'*).

With respect to the manner of using the oil, frying is notable for its nutritional and cultural importance, this invariably using the characteristic Andalusí preparations of ground meat (*banadiq*, *mirkas*, *laqaniq*, *isfiriya*) and, to a lesser degree, chunks of meat, fowl and game. Fried fish was very popular, and breaded foods were also fried, as were vegetables, greens, cheeses, eggs and pastries (doughnuts, pastry rings, almond pastries etc.). In *al-Andalus*, the technique of frying was so masterful that the volume of oil needed could be calculated according to the food and the desired result, the fire could be regulated for the appropriate oil temperature, and the degree frying could be controlled (browning or

thorough frying). Doctors often attended to this, according to Avenzoar (E 148r), stating that it was used for fish, meat, cheese, eggs and bread (*tara'id*) as well as doughs, but it should not be done in copper pans, the foods 'become noxious ... and are easily altered'. This danger was also mentioned in the treatises of *hisba*, as the inspectors of the market prohibited these pans for frying in the souk (Lévy-Provençal, 2001). Olive oil was habitual in stews. It was used in cereals, such as *asida* (basic dish, similar to *puls* of ancient Rome, consisting of a thick porridge of flour or *semola* with vegetables and legumes). It was also used in elementary preparations such as wheat flour or *semola* dishes, and always in cooking pastries (*fidaws*, *atriya*...). It gave its flavour to casseroles, stews and soups, variable quantities being added according to the dish. Thus, it was added to recipes of boiled meat (*zirbaja*); in various ragouts, typical Hispano-Arab meat dishes (*tafaya*, *maruziyya*, *fartum, suà* and *tharida*); stews of legumes, vegetables and greens (alone or mixed with meats). Its presence was constant and abundant in stewed fish to enrich the cooking and flavour, and it was added to sauces and seasoning for roasted fish. It was necessary for kneading dough to make filo dough, pastry and other sweets and for dressing turnovers and fish or meat pies (Arié, 1974–1975; Bolens, 1991; De Castro, 1996b; García-Sánchez, 1996, 1997).

For the Middle Ages and the modern period, there are few data on the quantitative consumption of olive oil. A review of all previous studies (Eiras Roel, 1974) calculated the mean consumption of fat between the 14th and 18th centuries for three groups of the European population: military expeditions and fleets; cities; and groups (castles, hospitals, colleges). Fat consumption per person and day was estimated at 34 g, representing an energy supply of 28% of the dietary calories. It should be noted that the fat calculated includes butter and oil jointly and thus cannot be differentiated.

7.2. Nutritional, dietary and therapeutic properties of olive oil

Hispano-Arab medicine, strongly influenced by the Graeco-Roman tradition, valued olive oil for its nutritional and dietary virtues. The texts evidence the interest in healthy and prudent eating: '... all diseases suffered by man, or at least the great majority,' wrote Maimonides, 'are the consequence of a deficient or unbalanced diet'. Averroes affirmed that eating in accord with rules 'prevents disease'. Maimonides counselled his children to abhor harmful foods as though they were someone trying to kill you, stating that the sage 'does not cure with medicine when he can do so with an adequate diet' (Pérez-Jiménez *et al.*, 2000). The importance of oil did not escape the doctors. Averroes indicated that if the oil is from 'ripe and healthy olives and its properties have not been artificially altered, it can be perfectly assimilated by the human constitution' (García-Sánchez, 1996 1997). He also stated that foods are nutritive if they are seasoned with oil which is 'fresh and not acidic', which he considered a very effective food-medicine to evacuate the wastes of digestion. According to Al-Arbuli, 'olive oil is the most appropriate for the human body', noting that 'it possesses excellent nutritive value and is lighter than other oils' (García-Sánchez, 1996, 1997). Specialists spoke of cooking with olive oil, pointing out some of the disadvan-

tages, as in frying, although they stated that, well done, it has positive effects: '... the doctors,' said Avenzoar (E 148r), 'prefer fish to be cooked in oil so that its soft meat becomes balanced', and recommends that mutton be seasoned with 'abundant oil', so that it becomes more balanced. 'The quality of fried eggs improves,' according to Averroes, 'if recent olive oil of little acidity is used' and that the best way to temper meat is by the technique called 'browning' (Pérez-Jiménez *et al.*, 2000). Together with the oil, they appreciated olives. Marinated according to methods similar to those used by Graeco-Roman farmers, they had a reputation of being nutritious and healthy and were very popular, although they proved harmful if eaten in large quantities. They were prohibited in cases of diarrhoea and were prescribed for constipation (Ibn Awwam VII 4).

The Arabs used animal fat in the kitchen with a certain frequency, though they attributed it with negative effects on the circulatory system. Maimonides made the observation that '... fats from the bowels of animals are too nutritive and produce cold, thick blood' (Pérez-Jiménez *et al.*, 2000). It is evident that this was an empirical observation, but it remains striking, taking into account that the texts contain data on the existence of coronary cardiopathy, and it is plausible that the son of Almanzor, Abd-al-Malik al Muzzafar, died of an acute myocardial heart attack (Arjona-Castro, 1997; Pérez-Jiménez *et al.*, 2000).

7.3. Non-dietary uses of olive oil

During the Middle Ages and the modern era, olive oil continued to be used for industry and medicine, basically as in antiquity: illumination, soap production, textiles, lubrication, perfumes and essential oils. For all of these, olive oil was advantageous and difficult to replace, and therefore demand persisted (Boulanger, 1996, 2001; Cherubini, 1996; Comet, 1996).

Certainly, since antiquity, through the modern age, the evolution of olive cultivation and the technology of oil production was not significant, although certain agricultural and technological advances were made in the interest of boosting yield. It was in recent centuries that authentic innovations were made (Amouretti, 1996).

8. Olive Oil in Contemporary Times

In the 19th and 20th centuries, olive cultivation underwent a general expansion due to the strong demand for olive oil in industrialized countries. Its spread was massive in the eastern Mediterranean and North Africa, first in Greece and Tunisia and then in Algeria and Turkey, while in the eastern part the traditional olive-growing countries of Italy, Spain and France constructed terraces to increase the land appropriate for cultivation (Amouretti, 1996). The expansion was constant in Spain (Zambrana, 2000; Ramón, 2003), and very controlled in Italy and France, where the surface area of olive orchards even shrank; the reduction was early and pronounced in France (Bevilacqua, 1988; Boulanger, 1996, 2001). The agricultural techniques of the 19th century, and the

innovations, had to wait until the mechanization of the countryside, this occurring later in the 20th century. Nevertheless, cultivation was improved by working the soil, cleaning and appropriate cutting, renewing the old trees, reducing the association with other crops and increasing the number of olives, selecting varieties suited to local agroclimates and planting new single-variety orchards. These improvements occurred above all in Italy and France, which preferred selective olive cultivation (Zambrana, 2000). In other countries, such as Spain, only some regions (Catalonia, Aragon) opted for this model, while the rest chose massive cultivation. Only at the end of the 19th century, when the olive-oil sector entered a crisis, did the cultivation of the olive tree become rational (Zambrana, 2000, 2003).

The procedures for producing oil were improved in all the phases. The olives were picked by hand or carefully knocked down, transported, rapidly milled and pressed, separating the oil of the first cold pressing from the rest. The oil was decanted into metal containers in the extraction and was clarified much more (Zambrana, 1987, 2000, 2003). The increase in the speed of the extraction and in the yield was achieved in the 19th century thanks to technological innovations. Milling improved when the base-block orthostats were replaced by three or four conical rollers. Pressing was improved during the first decades of the 19th century by the introduction of a hydraulic press that replaced the screw column and the traditional lever, still widely used (Zambrana, 1987, 2003; Amouretti, 1996). The new press consisted basically of a pump of great diameter with very strong walls, in the interior of which was a cylinder or massive steel or cast-iron piston with a close fit, supporting a plate over which the pressure was applied (up to 400 tonnes, i.e. 10–15 times that of the lever). The system was very fast, making one pressing in four hours (less than half the time of the lever system). If the press was powered by steam or electricity, its speed increased (handling cargoes of 60–75 bags). The mill with the new system and steam pressure notably improved the essential phases of the production, thereby lowering costs (Zambrana, 1987, 2003).

The new oil-production technology was introduced into olive-growing zones of France and Italy (Ramón, 2003) and later in some Spanish areas, such as Catalonia and Aragon (Ramón, 2000; Zambrana, 2000, 2003). However, in the rest of the country, with some exceptions in Andalusia, the renewal would be later, as traditional systems continued (Zambrana, 2000). The eastern and African countries were left farther behind. During the 19th century and the first few decades of the 20th, throughout the Mediterranean the most rudimentary systems to produce oil coexisted alongside the most innovative, as the replacement of the old materials was slow because of its high cost (Zambrana, 1987, 2000, 2003; Amouretti, 1996). Thus, in Spain, at the beginning of the 20th century, there were various types of press that went from the traditional and very widespread lever press to the most modern hydraulic presses, the screw column constituting the intermediate stage. Contemporary olive cultivation and oil production corresponded to two basic models. One was oriented to producing oils of quality, mainly in France and Italy, and the other bent on mass production, primarily in Spain and the other Mediterranean countries (Tió, 1982; Zambrana, 2000; Ramón, 2003).

The interest in producing quality olive oil was a response to the arrival on the market of other products, mineral and vegetable, that could replace olive oil for industrial use. Thereafter, olive oil was oriented to dietary uses, as its organoleptic and culinary qualities were difficult to replace (Tió, 1982; Zambrana, 2000). The use of new fuels (gas, petroleum, tar, bitumen) began in the 19th century and became generalized in the second half, first in industrialized countries and afterwards in Mediterranean countries. At this time, oleaginous seeds and plants (cotton, sesame, palm and coconut) also reached the market, cultivated in undeveloped countries, and cheaper oil with similar uses became available. Industrialized countries imported the oils or the raw material to manufacture oils. The great bargain fuels and oils slowed down the olive-oil sector that supplied industry, although the suppliers of the food sector maintained their market as the seed oils, with their organoleptic properties, could not compete at that time with olive oil (Loussert and Brousse, 1978; Tió, 1982; Zambrana, 2000). Olive-growing countries responded in a different way in the face of this new situation, deeply marking the future for the production and world trade of olive oil. Spain, although trying to improve its oils, continued to increase its production, of mainly low-quality oil, and in the second half of the 19th century it equalled Italy and later surpassed it (Zambrana, 2000; Ramón, 2003). Italy and France chose to diminish their production, increase the quality of their oils, for local consumption and exportation, and increase the purchase of crude, especially Spanish, oil with the aim of transforming it, mixing it and packaging it for export as its own food product (Zambrana, 2000, 2003; Ramón, 2003; Stumpo, 2003). The French market was of high quality but quantitatively not important (Ramón, 2003); the Italian market was voluminous and dominated international commerce with great exportation to the United States and Argentina, countries that had a large population of Italians (Zambrana, 2000; Ramón, 2003; Stumpo, 2003). The twist that Italy gave to its oil production, based on the importation of Spanish oil, started a triangular trade that consolidated its position in the international market and its triumph in the oil war (Ramón, 2003; Stumpo, 2003). At the beginning of the 20th century, other countries, such as Greece and Tunisia, and to a lesser extent Algeria and Turkey, began to boost their production and to export oil (Ramón, 2003). Tunisia began a strong expansion of cultivation in the last decades of the 19th century and in the first third of the 20th, doubling its production intended for France and Italy. Algeria followed an opposite path, directed its market at France, and its production, which at the end of the 19th century surpassed that of Tunisia, underwent a notable reduction from 1920 on, to the point that Tunisian production in the decade of the 1930s was treble that of Algeria. Greece had an evolution similar to that of Tunisia, with an almost incessant increase in cultivation and production from the end of the 19th century, converting it into the third largest producer in the world, and in the third decade of the 20th century it produced half as much as Italy (Zambrana, 1987, 2000).

The First World War brought a great change in the production and commerce of oil involving most of the oil-producing countries. Spain, which did not go to war, increased its production and succeeded in dominating the international market, and even increased its exports spectacularly to the USA and

Argentina, countries traditionally linked to Italy (Ramón, 2000, 2003). Thus, in 1917, the USA imported 420 tonnes from Italy and 5351 tonnes from Spain; in 1919 the figures were 1053 tonnes from Italy and 35,782 from Spain (Zambrana, 1987). After the war, Italy re-established its trade and displaced Spain from its privileged situation in the select markets abroad characterized by consuming quality oils or registered marks. Italy returned to its policy of buying large quantities of Spanish oil in bulk and selling it with Italian labels (Zambrana, 2000; Ramón, 2003).

The market of fats again suffered convulsions in the first decades of the 20th century with the eruption of those of oleaginous seeds and plants (soy, colza, sunflower, peanut), which rendered cheap oils suitable for cooking. In their extraction and production, machinery was used: cleaners, mills, presses, hullers, laminators; and the most advanced chemical procedures: refining, deodorizing, etc. (Loussert and Brousse, 1978; Zambrana, 2000, 2003). The oils were separated according to quality of the raw material, degree of pressure, and use or not of chemical procedures to increase the extraction or to improve the organoleptic characteristics. To achieve maximum extraction, solvents were used, and to eliminate the sensorial defects, it was refined, deodorized, mixed with olive oil, etc. Thus, for example, for culinary purposes, coconut oil was used with a deodorizer, or refined cotton was used mixed with olive oil. Good preparation and the elimination of undesirable characteristics reduced the differences with respect to olive oil, which, together with the massive production and reasonable prices, created diversified and attractive offerings for consumers (Zambrana, 2000).

In this situation, olive oil could be competitive if it was of good quality and had a good price, but this was difficult to achieve (Tió, 1982; Zambrana, 2000). The Italian and French oils found a place in the market because of their lower production costs, their careful preparation, and their traditional good reputation (Ramón, 2003). However, most of the olive oils from other countries, such as most of the Spanish oils, left much to be desired, being sold in bulk and often rancid, acidic broths that were too expensive to compete with the new vegetable oils. Major rectifications were required to remove these defects (Zambrana, 1987). Chemical treatment of the oil began at the end of the 19th century and intensified during the first decades of the 20th century, including the use of acids, alkalis, oxides, metal salts, air, ozone and light. Refinement eliminated the undesired colours and odours, the rancidness and the acidity. Thus, in the second and third decades of the 20th century, diverse types of olive oil were able to reach the market: virgin, with careful preparation and new refinement, which, on being cheaper and suitable for culinary use, achieved strong international demand (Tió, 1982; Zambrana, 1987). But at this time, other, new, fatty materials began to arrive on the market that were sourced from vegetable and animal oils – abundant and cheap. They were the hardened oils and solid greases developed from liquid glycerides through hydrogenation, which competed with olive oil but, above all, were replaced by lard and butter (Zambrana, 1987). The technological innovations had revolutionized the market of dietary fats, now diverse and competitive. Vegetable oils and solid fats dominated normal olive oil with their lower prices and slight qualitative differences, and the only oil to survive was good quality; this prestige was enjoyed only by those sold by Italy and France

(Ramón, 2003). Spain, subordinated to the power of Italian oil, reduced its market, a situation aggravated by the Spanish Civil War in 1936, which devastated any attempt at recovery (Capel, 1992; Zambrana, 2000, 2003).

The middle decades of the 20th century were bad for olive oil. The oil producers, especially of soy and seeds, and of hydrogenated fats, energetically promoted their products at the time, dismissing olive oil as antiquated and not very healthy. The international market, including the Mediterranean countries, was assaulted from all sides by oils and fats of doubtful dietary virtue and poor gastronomical value (Capel, 1992). In the 1960s and at the beginning of the 1970s a radical change occurred when the results were published of research on the relationship between food and health and the nutritional role of dietary-fat quality (Nestle, 1995). Olive oil began to be presented as a product whose biological properties and physiological action could play a preventive role in chronic diseases (cardiovascular, cancer, diabetes and arterial hypertension), the main causes of death in industrialized countries. The research in countries with little inclination towards olive oil recognized its nutritional value and began to create a state of opinion that would contradict accepted theories based on superficial knowledge of the nutritional behaviour of fats. The researcher Ancel Keys (Keys, 1970, 1995) most praised and publicized Mediterranean food, which he considered a prudent and healthy dietary model. Thus, olive oil, its most characteristic component, took centre stage and its nutritional values influenced public opinion.

Taking advantage of such a favourable situation and the technological advances of the last few years, the oil-producing sector invested heavily in the production of high-quality oils prepared from optimal fruits, carefully harvested and transported. The milling of the olive was performed rapidly by mechanical hammers that achieve a homogeneous paste that is later submitted to slow thermobeating and, afterwards, centrifugation. The first centrifuges were called three-phase, for rendering three products: marc, alpechin and oil (Loussert and Brousse, 1978). In the 1990s, the two-phase centrifuges were introduced, capable of rendering, on the one hand, oil, and on the other a marc containing the alpechin (Díaz, 1993; Alba and Martínez, 2001). The oil extracted was passed through small decanters and stored in vitrified polyester or stainless-steel tanks in cellars kept at 15 to 18°C and with minimal luminosity (Díaz, 1993; Alba and Martínez, 2001).

Because the olive is very sensitive to the natural environment in which it develops, its fruits and consequently its oil present distinct characteristics depending on its origin. Thus, there are different virgin olive oils, according to the geoclimatic setting, the different varieties and the form of cultivation. These particularities gave rise to the creation of the denomination of origin with the aim of promoting the production of oils of great quality in the different oil-producing zones. The oils that enjoy the qualification of denomination of origin must fulfil certain requirements: (i) that they belong to a circumscribed geographic zone having its own climatic and edaphological conditions in which oils of the same characteristics were traditionally obtained; (ii) that there be definite cultivation practices and that the same varieties of olive be produced for the preparation of the oil; (iii) that uniform preparation methods for maximum-quality virgin oil be employed.

Each denomination of origin, by a Regulating Council, establishes the conditions to be fulfilled by olive growers and oil producers, as well as the requirements that the oil must present in the organoleptic characteristics, as well as in the physico-chemical parameters used in the quality indices. The denomination of origin is a guarantee of quality for the consumer, as the oils must have been produced by a rigorous process controlled from the harvest of the fruits to packaging in the production zone.

The Mediterranean diet owes its gastronomical and health value largely to these good olive oils that enhance the palatability of food and encourage the consumption of raw vegetables, hot or cold soups and legume dishes; it provides incomparable frying and can be drizzled on bread or toast. The virtues of the Mediterranean diet buttress the production and consumption of olive oil, which again has achieved pre-eminence among culinary fats.

Currently, worldwide production of olive oil exceeds 2,500,000 tonnes, of which Spain produces 48.1%, Italy 28.8%, Greece 21%, Tunisia 5.8%, Turkey 4.7%, Syria 4%, Morocco 2%, Portugal 1.8%, Algeria 1.4% and Jordan 0.7%. However, exportation is dominated by Italy, reaching 36% of world exports, followed by Tunisia at 21.9%, Spain 19%, Turkey 11.9%, Portugal 3.4%, Greece 1.8%, Syria 1.2%, Argentina 1.1%, the USA 1.1% and Morocco 0.9%. The importers are led by the USA, which accounts for 36.4% of world imports, followed by Italy with 19.6%, Japan 5.8%, Spain 5.6%, Australia 5.3%, Brazil 4.8%, Israel 1.6%, Switzerland 1.6%, Saudi Arabia 1% and Mexico 0.9% (International Olive Oil Council, 2004).

The consumption of olive oil in kg per inhabitant and year is led by Greece with 24, followed by Spain at 13.1, Italy 12.5, Portugal 6.4, Syria 6.2, Tunisia 5.3, Israel 2.2, Morocco 1.9, Jordan 1.7 and France 1.6 (International Olive Oil Council, 2004). Consumption has grown annually at between 5 and 7%, although in some countries where it was hardly or not at all habitual, such as the USA and Japan, growth has been 12% and 30%, respectively. Despite all, the war of fats continues, and in the last few years in all industrialized countries, including Mediterranean ones, a high consumption of saturated and hydrogenated fats has been detected, particularly in the young sector of the population (Helsing, 1995). The battle between fats flares again and does not appear to have an end.

9. References

Alba, J. and Martínez, L. (2001) Elaboración de aceites de oliva. In: Mataix, J. (ed.) *Aceite de Oliva Virgen: nuestro patrimonio alimentario.* Universidad de Granada, Granada, pp. 43–61.

Amouretti, M.C. (1986) *Le pain et l'huile dans la Grèce antique. Évolution des techniques agraires d' Hesiode à Théophraste.* Les Belles Lettres, Paris, pp. 41–45, 158–192.

Amouretti, M.C. (1993) Les sous-produits de la fabrication de l'huile et du vin dans l'Antiquité. In: Amouretti, M.C. and Brun, J.P. (eds) *La production du vin et de l'huile en Mediterranée.* École Française d'Athènes, Athens, pp. 463–476.

Amouretti, M.C. (1996) La fabricación del aceite de oliva: una historia técnica original. In: Consejo Oleícola Internacional del Olivo

(ed.). *Enciclopedia Mundial del Olivo*. Plaza y Janés, Barcelona, pp. 26–29.

Amouretti, M.C. and Brun, J.P. (2002) Oliviers et huile dans l'Antiquité: découvertes archéologiques récentes. In: Amouretti, M.C. and Brun, J.P. (eds) *Agriculture Méditerranéenne. Varieté des techniques anciennes. Cahier d'histoire des techniques* 5. Publications de l'Université de Provence, Aix-en-Provence, pp. 127–140.

André, J. (1956) *Léxique des termes de botanique en latin.* Klincksieck, Paris. pp. 225–226.

André, J. (1961) *L' alimentation et la cuisine à Rome*, 2nd edn. (1981). Les Belles Lettres, Paris, pp. 90–91, 180–185.

André, J. (1985) *Le noms de plantes dans la Rome antique*. Les Belles Lettres, Paris, pp. 176

Arié, R. (1974–1975) Remarques sur l'alimentation des musulmans d'Espagne au cours de bas Moyen Age. *Cuadernos de Estudios medievales* II–III, 299–312.

Arjona-Castro, A. (1977) Aspectos médicos de la obra de Al-Muzzafar, el sucesor de Almanzor. *Corduba* 6, 179–183.

Ashtor, E. (1968) Essai sur l'alimentation des diverses classes sociales dans l'Orient medieval. *Annales. Economies, Societés, Civilisations* 5, 1017–1053.

Bervillé, A., Besnard, G., Baradat, Ph., Khadari, B. and Breton, C. (2001) Origine et domestication de l'olivier. In: Actes des 1éres Rencontres Internationales de l'olivier (19 et 20 octobre 2000) *L'olivier dans l'espace et dans le temps.* Institut du monde de l'olivier, Nyons, pp. 8–9.

Besnard, G. and Bervillé, A. (2000) Multiple origins for Mediterranean olive (Olea europaea L. ssp. europaea) based upon mitochondrial DNA polymorphims. *Comptes Rendus de l'Académie des Sciences, Sciences de la Vie* 323, 173–181.

Bevilacqua, P. (1988) Il paesaggio degli alberi nel Mezzogiornio d' Italia e in Sicilia (fra XVIII e XX secolo). *Annali dell'Istituto Alcide Cervi* 10, 259–306.

Blanc, N. and Nercessian, A. (1992) *La cuisine romaine antique.* Glénat, Grenoble, pp. 24–28.

Blanco, A. (1994) Los primeros españoles. *Historia 16* 1, 20–31.

Blázquez, J.M. (1992) *Fenicios, griegos y cartagineses en Occidente.* Cátedra, Madrid, pp. 70.

Blázquez, J.M. (1996) Origen y difusión del cultivo. In: Consejo Oleícola Internacional del Olivo (ed.). *Enciclopedia Mundial del Olivo*. Plaza y Janés, Barcelona, pp. 19–20.

Blázquez, J.M. and Cabrero, J. (2001) Un monte de aceite andaluz. *La aventura de la Historia*, 29, 68–73.

Blitzer, H. (1993) Olive cultivation and oil production in Minoan Crete. In: Amouretti, M.C. and Brun, J.P. (eds) *La production du vin et de l'huile en Mediterranée.* École Française d'Athènes, Athens, pp. 163–176.

Bolens, L. (1991) *La cocina andaluza un arte de vivir.* Edaf, Madrid, pp. 173–175.

Bolens, L. (1996) Riqueza de la tierra andaluza y primacía del aceite de oliva en la sociedad y la civilización de al-Andalus. *Agricultura y Sociedad* 80–81, 181–216.

Bottema, S. (1994) The prehistoric environment of Greece: a review of the palynological record. In: Kardulias, P. (ed.) *Beyond the Site. Regional Studies in the Aegean Area.* University Press of America, Lanham, pp. 45–68.

Boulanger, P. (1996) *Marseille, marché international de l'huile d'olive: un produit et des hommes de 1725 à 1825.* Institut Historique de Provence, Marseille, pp. 41–72.

Boulanger, P. (2001) Huile d'olive et savon de Marseille. In: Actes des 1éres Rencontres Internationales de l'olivier (19 et 20 octobre 2000) *L'olivier dans l'espace et dans le temps.* Institut du monde de l'olivier, Nyons, pp. 56–62.

Bovon, A. and Bruneau, Ph. (1966) Huiliers hellénistiques. *Bulletin de Correspondance Hellénique* 1, 131–134.

Brun, J.P. (1998) Une parfumerie sur le Forum de Paestum. *Mélanges d'Archeologie et d'Histoire de l'Ecole Francaise de Rome* 110, 119–472.

Brun, J.P. (2000) The production of perfumes in antiquity: the cases of Delos and Paestum. *American Journal of Archaeology* 104, 277–308.

Callot, O. (1993) Les huileries et l'huile au Bronce Récent: quelques exemples syriens et chypriotes. In: Amouretti, M.C. and Brun, J.P. (eds) *La production du vin et de l'huile en Mediterranée.* École Française d'Athènes, Athens, pp. 55–64.

Camps-Fabrer, H. (1996) El cultivo del olivo en el norte de África. In: Consejo Oleícola Internacional del Olivo (ed.) *Enciclopedia Mundial del Olivo*. Plaza y Janés, Barcelona, pp. 30–33.

Capel, C. (1992) *Aceite de oliva.* El País-Aguilar, Madrid, pp. 29–31.

Carabaza Bravo, J.M. (1996) El olivo en los tratados agronómicos clásicos y andalusíes. In: Alvarez de Morales, C. (ed.) *Ciencias de la Naturaleza en al-Andalus, Textos y Estudios IV.* Consejo Superior de Investigaciones Científicas, Granada, pp. 11–39.

Céspedes del Castillo, G. (1961) La sociedad colonial americana en los siglos XVI y XVII: las realizaciones económicas. In: Vicens Vives, J. (ed.) *Historia Social y Económica de España y América* III, 2nd edn (1972) Vicens Bolsillo, Barcelona, pp. 399–422.

Chabal, L., Fabre, L., Terral, J. and Théry-Parisot, I. (1999) L'anthracologie. In: Ferdière (dir.) *La botanique.* Errance, Paris, pp. 43–104.

Chadwick, J. (1976) *The Mycenaean World.* Cambridge: Cambridge University Press (tr. de Melena, J.L. (1998) *El mundo micénico.* Alianza, Madrid), pp. 98, 143, 160–161, 198.

Chalmeta, P. (1996) Aceites, almazaras y etimologías. *Anaquel de Estudios Árabes* VII, 57–68.

Cherubini, G. (1996) *L'Italia rurale del basso Medioevo.* Laterza, Roma-Bari, pp. 184–193.

Comet, G. (1993) Le vin et l'huile en Provenze mediévale: essai de bilan. In: Amouretti, M.C. and Brun, J.P. (eds) *La production du vin et de l'huile en Mediterranée.* École Française d'Athènes, Athens, pp. 343–358.

Comet, G. (1996) Economía oleícola en la Edad Media. In: Consejo Oleícola Internacional del Olivo (ed.) *Enciclopedia Mundial del Olivo*. Plaza y Janés, Barcelona, pp. 50–52.

Comet, G. (2001) L'olivier dans la Provence Médiévale: extension et techniques. In: Actes des 1éres Rencontres Internationales de l'olivier (19 et 20 octobre 2000) *L'olivier dans l'espace et dans le temps.* Institut du monde de l'olivier, Nyons, pp. 31–37.

Cordón, F. (1988) *Cocinar hizo al hombre.* Tusquets, Barcelona.

Cortonesi, A. (2002) Vinificazione e oleficazione nell'Italia medievale. In: Amouretti, M.C. and Brun, J.P. (eds) *Agriculture Méditerranéenne. Varieté des techniques anciennes. Cahier d'histoire des techniques* 5. Publications de l'Université de Provence, Aix-en-Provence, pp. 143–160.

Curchin, L.A. (1991) *Roman Spain. Conquest and assimilation.* Routledge, London–New York (tr. De Calonge, J. (1996) *España Romana. Conquista y Asimilación.* Gredos, Madrid), pp. 196–200.

De Castro, T. (1966a) La alimentación castellana e hispanomusulmana bajomedieval ¿Dos códigos opuestos? *Estudios de Historia y de Arqueología Medievales*, XI, 35–65.

De Castro, T. (1966b) *La alimentación en las crónicas castellanas bajomedievales.* Disponible en: www.geocities.com/tdcastros/Hystoryserver/Tes2/aceite.htm

Díaz, A.L. (1993) Tecnologías para la extracción del aceite de oliva de calidad. In: Díaz, A.L., Lovera, C. and Lobillo, C. (eds) *Nuestro aceite de oliva.* Caja Provincial de Ahorro de Córdoba, Córdoba, pp. 87–123.

Dubur-Jarrige, M.A. (2001) Les origines de la culture de l'olivier en Méditerranée: le point sur les découvertes paléobotaniques et leurs interprétations. In: Actes des 1éres Rencontres Internationales de l'olivier (19 et 20 octobre 2000) *L'olivier dans l'espace et dans le temps.* Institut du monde de l'olivier, Nyons, pp. 10–22.

Eiras Roel, A. (1974) Historia cuantitativa del consumo alimentario: estado actual de las investigaciones. *Hispania* XXXIV 126, 105–148.

Eitam, D. (1987) Olive production during the Biblical Period. In: Heltzer, M. and Eitam, D. (eds) *Olive oil in Antiquity*. University of Haifa Press, Haifa, pp. 15–56.

Eitam, D. (1993a) Between the (olive) rows, oil will be produced, presses will be trod ... (Job. 24, 11). In: Amouretti, M.C. and Brun, J.P. (eds) *La production du vin et de l'huile en Mediterranée.* École Française d'Athènes, Athens, pp. 65–89

Eitam, D. (1993b) Selected oil and wine instalations in Ancient Israel. In: Amouretti, M.C. and Brun, J.P. (eds) *La production du vin et de l'huile en Mediterranée.* École Française d'Athènes, Athens, pp. 91–106.

Eitam, D. (1996) El cultivo del olivo en la Antigua Israel. In: Consejo Oleícola Internacional del Olivo (ed.) *Enciclopedia Mundial del Olivo*. Plaza y Janés, Barcelona, pp. 36–41.

Fahd, T. (1996) L'agriculture nabatéenne en Andalousie. In: Alvarez de Morales, C. (ed.) *Ciencias de la Naturaleza en al-Andalus, Textos y Estudios IV.* Consejo Superior de Investigaciones Científicas, Granada, pp, 41–52.

Fidanza, F. (1979) Diets and dietary recommendations in Ancient Greece and Rome and the School of Salerno. *Progress in Food & Nutrition Science* 3, 79–99.

Fiorino, P. and Nizzi, F. (1992) La oleicultura y su expansión. *Olivae* 44, 9–12.

Flandrin, J.L. (1983) Le gout et la nécessité: sur l'usage des graisses dans les cusines d'Europe occidentale (XIV^{e}–XVIII^{e} siécle). *Annales. Economies, Societes, Civilisations* XXXVIII, 369–401.

Flandrin, J.L. (1984) Internationalisme, nationalisme et régionalisme dans la cuisine des XIV^{e}–XV^{e} siécles: le témoignage des livres de cuisine. In: *Manger et boire au Moyen Age (Actes du Colloque de Nice, 15–17 octobre 1982, vol. 2)* Les Belles Lettres, Paris, pp. 75–91.

Flandrin, J.L. and Redon, O. (1981) Les livres de cuisine italiens des XIV^{e}–XV^{e} siécles. *Archeologia Medievale* VII, 393–408.

Foxal, L. (1993) Oil extraction and processing equipment in Classical Greece. In: Amouretti, M.C. and Brun, J.P. (eds) *La production du vin et de l'huile en Mediterranée.* École Française d'Athènes, Athens, pp. 183–200.

Frankel, R. (1994) Ancient oil mills and presses in the land of Israel. In: Ayalon, E. (ed.) *History and technology of olive oil in the Holy Land.* Arlington VA: Olearius-Tel Aviv: Eretz Israel Museum, Tel Aviv, pp. 19–89.

Friedrich, W.L. and Pichler, H. (1976) Radiocarbon dates of Santorini Volcanics. *Nature* 262, 373–374.

Galili, E., Weinstein-Evron, M. and Zohary, D. (1989) Appearance of olives in submerged neolithic sites along the Carmel Coast. *Journal of the Israel Prehistoric Society*, 22, 95–97.

García Sánchez, E. (1996) El consumo de aceite de oliva y otras grasas vegetales en Al-Andalus. In: Institut d'Estudis Baleàrics (ed.) *XIV Jornades d'Estudis Històrics Locals. La mediterrània area de convergencia de sistemes alimentaris (ss. V–XVIII).* Institut d'Estudis Baleàrics. Palma de Mallorca, pp. 15–38.

García Sánchez, E. (1997) La tríada mediterránea en Al-Andalus. In: San Martín, C. and Ramos Lizana, M. (coord.) *Con pan, aceite y vino ... La tríada mediterránea a través de la Historia.* Fundación Caja Granada, Granada, pp. 97–127.

Ghaliongui, P. (1972) La medicina en el Egipto faraónico. In: Laín Entralgo, P. (ed.) *Historia Universal de la Medicina*, 3rd edn. Salvat, Barcelona, pp. 95–124.

Grieco, A.J. (1993) Olive tree cultivation and the alimentary use of olive oil in Late medieval Italy (ca. 1300–1500). In: Amouretti, M.C. and Brun, J.P. (eds) *La production du vin et de l'huile en Mediterranée.* École Française d'Athènes, Athens, pp. 297–306.

Hansen, J. (1994) Palaeoethnobotany in regional perspective. In: Kardulias, P. (ed.) *Beyond the Site. Regional Studies in the Aegean Area.* University Press of America, Lanham, pp. 173–190.

Helsing, E. (1995) Traditional diets and disease patterns of the Mediterranean, *circa* 1960. *American Journal of Clinical Nutrition* 61, 1.329–1.337.

Heltzer, M. (1987) Olive growing and olive oil in Ugarit. In: Heltzer, M. and Eitam, D. (eds) *Olive oil in Antiquity*. University of Haifa Press, Haifa, pp. 106–120.

Heltzer, M. (1993) Olive oil and wine production in Phoenicia and in Mediterranean trade. In: Amouretti, M.C. and Brun, J.P. (eds) *La production du vin et de l'huile en Mediterranée.* École Française d'Athènes, Athens, pp. 49–54.

Hémardinquer, J.J. (1970) Les graisses de cuisine en France. Essai de cartes. In: Hémardinquer, J.J. (dir.) *Pour une historie de l'alimentation.* Armand Colin, Paris, pp. 254–271.

International Olive Oil Council (2004). http://www. internationaloliveoil.org (15-07-04)

Iorio, R. (1985) Olivo e olio in Terra di Bari in età normanno-sveva. *Quaderni Medievali* 20, 67–102.

Keys, A. (1970) Coronary heart disease in seven countries. *Circulation* 41 (Suppl. 1), 1–211.

Keys, A. (1995) Mediterranean diet and public health: personal reflections. *American Journal of Clinical Nutrition* 61, 1321–1323.

Lévy-Provençal, E. (2001) *Séville musulmane au début du XII^e siecle. Le traité d'Ibn 'Abdun sur la vie urbaine et les corps de métiers.* Maisonneuve & Larose, Paris, pp. 11–101.

Liphschitz, N. (1987) Olives in Ancient Israel in view of dendroarchaeological investigations. In: Heltzer, M. and Eitam, D. (eds) *Olive oil in Antiquity*. University of Haifa Press, Haifa, pp. 139–145.

Liphschitz, N., Gophna, R., Hartman, M. and Biger, G. (1991) The beginning of olive (*Olea Europaea*) cultivation in the Old World: a reassessment. *Journal of Archaeological Science* 18, 441–453.

Loussert, R. and Brousse, G. (1978) *L'olivier.* Maisonneuve et Larose, Paris: (tr. de Montero, F. and González, J.M. (1980) *El olivo.* Mundi Prensa, Madrid, pp. 31–66, 407–442.

Lovera, C. (1993) El olivo, un árbol para la historia y la leyenda. In: Díaz, A.L., Lovera, C. and Lobillo, C. (eds) *Nuestro aceite de oliva.* Caja Provincial de Ahorro de Córdoba, Córdoba, pp. 35–56.

Margueron, J.C. (1991) *Les Mesopotamiens.* Armand Colin, 1991 (tr. de Rozas, J.L. (1996)) *Los Mesopotámicos.* Cátedra, Madrid, pp. 128–129.

Mattingly, D.J. (1990) Painting, presses and perfume production at Pompeii. *Oxford Journal of Archaeology* 9, 71–90.

Meeks, D. (1993) La production de l'huile et du vin dans l'Egypte pharaonique. In: Amouretti, M.C. and Brun, J.P. (eds) *La production du vin et de l'huile en Mediterranée.* École Française d'Athènes, Athens, pp. 3–38.

Melena, J.L. (1983) Olive oil and other sorts of oil in the Mycenean Tablets. *Minos* 18, 89–123.

Mercader, J. and Domínguez, A. (1961) La época del Despotismo Ilustrado: las realizaciones económicas. In: Vicens Vives, J. (ed.) *Historia Social y Económica de España y América* IV, 2nd edn (1972). Vicens Bolsillo, Barcelona, pp. 128–180.

Montanari, M. (1979) *L' alimentazione contadina nell'alto Medioevo.* Liguori, Nápoles, pp. 388–407.

Montanari, M. (1993) *La fame e l'abondanza. Storia dell' alimentazione in Europa.* Roma-Bari (tr. De Vivanco, J. (1993) *El hambre y la abundancia. Historia y cultura de la alimentación en Europa.* Crítica, Barcelona), pp. 16–38, 115–119

Montanari, M. (1997) Condimento, fondamento. Le materie grassa nella tradizione alimentare europea. In: Cavaciocchi, S. (ed.) *Alimentazione e Nutricione. Secc XIII–XVIII. Atti della 28 Settimane di Studi del Istituto Francesco Datini.* Le Monnier, Prato, pp. 27–51.

Neef, R. (1990) Introduction, Development, and Environmental Implications of Olive Culture: The Evidence from Jordan. In: Bottema, S., Entjes-Nieborg, G. and van Zeist, W. (eds) *Man's Role in the Shaping of the Eastern Mediterranean Landscape.* Balkema, Rotterdam, pp. 295–306.

Nestle, M. (1995) Mediterranean diets: historical and research overview. *American Journal of Clinical Nutrition* 61, 1313–1320.

Nigro, G. (1997) Mangiare di grasso, mangiare di magro: il consumo di carni e pesci tra Medioevo ed Età moderna. 113–146. In: Cavaciocchi, S. (ed.) *Alimentazione e Nutricione. Secc XIII–XVIII. Atti della 28 Settimane di Studi del Istituto Francesco Datini.* Le Monnier, Prato, pp. 27–51.

Palamarev, E. (1989) Paleobotanical evidences of the Tertiary history and origin of the Mediterranean sclerophyll dendroflora. *Plant Systematics and Evolution* 162, 93–107.

Parra, F. (1990) *La dehesa y el olivar.* Debate, Madrid, pp. 77–111

Pérez-Jiménez, F., Fernández Dueñas, A., López Miranda, J. and Jiménez-Perepérez, A. (2000) El aceite de oliva: alimento saludable desde la época califal al umbral del nuevo milenio. *Medicina Clínica* 114, 212–221.

Ponsich, M. (1996) El aceite y el olivo en Tingitania. In: Consejo Oleícola Internacional del Olivo (ed.) *Enciclopedia*

Mundial del Olivo. Plaza y Janés, Barcelona, pp. 34–36.

Ramón, R. (2000) La exportación española de aceite de oliva antes de la Guerra Civil: empresas, mercados y estrategias comerciales. *Revista de Historia Industrial* 17, 97–151.

Ramón, R. (2003) El comercio exterior del aceite de oliva en Italia y España, 1850–1936. In: Barciela, C. and Di Vittorio, A. (eds) *Las industrias agroalimentarias en Italia y España durante los siglos XIX y XX.* Publicaciones de la Universidad de Alicante, Alicante, pp. 497–555.

Reglá, J. (1961) La época de los tres primeros Austrias: las realizaciones económicas. In: Vicens Vives, J. (ed.) *Historia Social y Económica de España y América* III, 2nd edn (1972). Vicens Bolsillo, Barcelona, pp. 120–158.

Remesal, J. (1986) La *annona militaris y la exportación de aceite bético a Germania*. Universidad Complutense, Madrid, pp. 95–108.

Remesal, J. (1996) Economía oleícola en la Antigüedad. In: Consejo Oleícola Internacional del Olivo (ed.) *Enciclopedia Mundial del Olivo*. Plaza y Janés, Barcelona, pp. 47–49.

Renfrew, C. (1972) *The emergence of civilisation. The Cyclades and the Aaegen in the Third Millennium B.C.* Methuen, London, pp. 278–285.

Renfrew, J.M. (1973) *Palaeoethnobotany. The prehistoric foods plants of the Near East and Europe.* Methuen, London, pp. 132–133.

Renfrew, J.M. (1985) *Preliminary report on the botanical remains.* In: Kemp, B.J. (ed.) Amarna Reports II, London, pp. 175–190.

Rodinson, M. (1949) Recherches sur les documents arabes relativs a la cuisine. *Revue des Études Islamiques* 17, 95–165.

Rodríguez-Almeida, E. (1984) *Il Monte Testaccio. Ambiente, storia, materiali.* Quasar, Roma, pp. 118.

Rodríguez-Molina, J. (1991) Los molinos de aceite medievales andaluces. In: Institut d'Estudis Baleàrics (ed.) *IX Jornades d'Estudis Històrics Locals. La manufactura urbana i els menestrals (ss. XIII–XVI)*. Institut d'Estudis Baleàrics, Palma de Mallorca, pp. 159–175.

Runnels, C. and Hansen, J. (1986) The olive in the Prehistoric Aegean: the evidence for domestication in the Early Bronze Age. *Oxford Journal of Archaeology* 53, 299–308.

Ruzé, F. and Amouretti, M.C. (1978) *Le monde grec antique.* Paris: Hachette (tr. de Fatás, G. (1987) *El mundo griego antiguo.* Akal, Madrid), pp. 29–36.

Safrai, Z. (1987) The economic implication of olive oil production in the Mishnah and Talmud periods. In: Heltzer, M. and Eitam, D. (eds) *Olive oil in Antiquity*. University of Haifa Press, Haifa, pp. 176–182.

Schäfer-Schuchardt, H. (1996) Expansión cultural y artística. In: Consejo Oleícola Internacional del Olivo (ed.) *Enciclopedia Mundial del Olivo*. Plaza y Janés, Barcelona, pp. 21–26.

Stouff, L. (1970) *Ravitaillement et alimentation en Provence aux XIV^e^ et XV^e^ siécles.* Mouton, Paris-La Haye, pp. 261.

Stouff, L. (1988) L'olivier et l' huile en Provenze aux derniers siècles du Moyen Age. *Provenze Historique* XXXVIII, 152, 181–191.

Strouhal, E. (1992) *Life in Ancient Egypt* (tr. de López, I. (1994) *La vida en el Antiguo Egipto).* Folio, Barcelona, pp. 21–251.

Stumpo, E. (2003) Per una storia dell'industria dell'ollio di oliva in Italia: i casi della Toscana e della Liguria. In: Barciela, C. and Di Vittorio, A. (eds) *Las industrias agroalimentarias en Italia y España durante los siglos XIX y XX.* Publicaciones de la Universidad de Alicante, Alicante, pp. 137–153.

Suc, J.P. (1984) Origin and Evolution of the Mediterranean Vegetation and Climate in Europe. *Nature* 307, 429–432.

Terral, J.F. (1996) Wild and cultivated olive (*Olea europea L*): a new approach to an old problem using inorganic analyses of modern wood and archaeological charcoal. *Review of Palaeobotany and Palynology* 91, 383–397.

Terral, J.F. and Arnold-Simard, G. (1996) Beginnings of olive cultivation in Eastern Spain in relation to Holocene bioclimatic changes. *Quaternary Research* 46, 176–185.

Tió, C. (1982) *La política de aceites comestibles en la España del siglo XX.* Ministerio de Agricultura, Pesca y Alimentación, Madrid.

Toussaint-Samat. M. (1987) *Histoire naturelle et morale de la nourriture*. Bordas, Paris (tr. de

González, C. (1991) *Historia natural y moral de los alimentos* II: El aceite, el pan y el vino. Alianza, Madrid), pp. 13–34

Vallvé, E. (1982) La agricultura en al-Andalus. *Al-Qantara*, III, 1–2, 261–298.

Weinfeld, M. (1987) The use of oil in the cult of Ancient Israel. In: Heltzer, M. and Eitam, D. (eds) *Olive oil in Antiquity*. University of Haifa Press, Haifa, pp. 192–195.

Zambrana, J.F. (1981) La fabricación de aceite de oliva en España 1870–1930. *Agricultura y Sociedad* 19, 267–290.

Zambrana, J.F. (1987) *Crisis y modernización del olivar.* Ministerio de Agricultura, Pesca y Alimentación, Madrid, 137–153, 256, 288–298.

Zambrana, J.F. (2000) De grasa industrial a producto de mantel: transformaciones y cambios en el sector de oleícola español, 1830–1986. *Revista de Historia Industrial* 18, 13–38.

Zambrana, J.F. (2003) Continuidad y cambio en las industrias del aceite de oliva español, 1830–1996. In: Barciela, C. and Di Vittorio, A. (eds) *Las industrias agroalimentarias en Italia y España durante los siglos XIX y XX.* Publicaciones de la Universidad de Alicante, Alicante, pp. 279–312.

Zaragoza, J.R. (1972) La medicina de los Pueblos Mesopotámicos. In: Laín Entralgo, P. (ed.) *Historia Universal de la Medicina*, 3rd edn, Salvat, Barcelona, pp. 67–92.

Zertal, A. (1987) The cultivation and the Economy of olives during the Iron Age I in the Hill Country of Manasseh. In: Heltzer, M. and Eitam, D. (eds) *Olive oil in Antiquity*. University of Haifa Press, Haifa, pp. 196–202.

Zohary, D. and Hopf, M. (1993) *Domestication of plants in the Old World. The origin and spread of cultivated plants in West Asia and the Nile Valley.* Clarendon Press, Oxford, pp. 137, 143, 235.

Zohary, D. and Spiegel-Roy, P. (1975) The beginning of fruit in the Old World. *Science* 187, 319–327.

SOURCE EDITIONS

Abu l-Jayr (1991) *Kitab al-Filaha* (Tratado de agricultura) Carabaza Bravo, J.M. (ed.) ICMA, Madrid.

Abu Marwan Abd al-Malik b. Zuhr (Avenzoar) (1992) *Kitab al-agdiya* (Tratado de los alimentos) García Sánchez, E. (ed.) Fuentes Arábico-Hispanas 4, Madrid.

Alonso de Herrera, G. (1966) Agricultura General. Terrón, E. (ed.). Ministerio de Agricultura, Pesca y Alimentación. Servicio de Publicaciones, Madrid.

Anónimo (1966) *Kitab al-tabij fi l-Magrib wa-l-Andalus fi 'asr al-Muwahhidin* (Traducción española de un manuscrito anónimo del siglo XIII sobre la cocina hispano-magribí) Huici Miranda, A. (ed.) Maestre, Madrid 1966.

Apicium (1995) Gastronomía en la Antigua Roma Imperial (*De re coquinaria*) (tr. de Ibáñez Artica, M.) R & B. San Sebastián.

Aristophanes (1995) Las nubes. Las Ranas. Pluto. Rodríguez Adrados, F. and Rodríguez Somolinos, J. (eds) Cátedra Letras Universales, Madrid.

Aristophanes (1996) Los acarnienses. Los caballeros. Las Tesmoforias. La asamblea de las mujeres. Rodríguez Adrados, F. (ed.) Cátedra Letras Universales, Madrid.

Aristophanes (1997) Las avispas. La paz. Las aves. Lisístrata. Rodríguez Adrados, F. (ed.) Cátedra Letras Universales, Madrid.

Aristotle (1984) Constitución de los atenienses. Pseudo Aristóteles. Económicos (intr., tr. y notas de García Valdés, M.) Biblioteca Clásica Gredos, Madrid.

Athenaeus (1998) Banquete de los Eruditos. Libros I–V. (intr., tr. y notas de Rodríguez-Noriega Guillén, L. 2 vols) Biblioteca Clásica Gredos, Madrid.

Biblia de Jerusalén (1976) Ubieta, J.A. (ed.) Desclée de Brouwer, Bilbao.

Cato (1975) De l'agriculture (*De agri cultura*). Goujard, R. (ed.) Les Belles Lettres, Paris.

Celso (1996) Los ocho libros de la Medicina (*De Medicina*) (tr. de Blázquez, A. 2 vols.) Iberia Obras Maestras, Barcelona.

Columella (1959) Los doce libros de agricultura (*De re rustica*) (tr. y notas de Castro, C.J. 2 vols) Iberia Obras Maestras, Barcelona.

Crónica del Moro Rasis (1975) Seminario Menéndez Pidal (ed.) Gredos, Madrid.

Dioscurides (1998) Plantas y remedios medicinales (*De Materia Medica*) (tr. Guzmán Guerra, A. 2 vols.) Biblioteca Clásica Gredos, Madrid.

Esquilo (1986) Tragedias. Los persas. Los siete contra Tebas. Las suplicantes. Agamenón. Las coéforas. Las euménides. Prometeo encadenado (tr. y notas de Perea Morales, B.) Biblioteca Clásica Gredos, Madrid.

Galen (1951) Galens' hygiene De sanitate tuenda (transl. by Montraville Green, R. intr. by Sigerist, H.E.) Thomas cop, Springfield.

Galen (2000) Galen on food and diet (transl. and notes by Grant, M.) Routledge, London.

Giacchero, M. (1974) *Edictum Diocletiani et collegarum de pretiis rerum venalium, in integrum fere restitutum e latinis graecisque fragmentis* I. Pubb. dell' Ist. di Storia Antica e Science Ausiliare dell' Universita di Genova, VIII, Genoa.

Herodotus (1997–1998) Historia, libros I–IX (tr. y notas de Schrader, C. 5 vols) Biblioteca Clásica Gredos, Madrid.

Hesiod (1978) Obras y fragmentos. Teogonía, Trabajos y días. Escudo. Fragmentos. Certamen (intr., tr. y notas de Pérez Jiménez, A. and Martínez Díez, A.) Biblioteca Clásica Gredos, Madrid.

Hippocrates (1983) Tratados Hipocráticos I (intr., tr. y notas de García Gual, C., Lara Nava, Mª.D., López Férez, J.A. and Cabellos Alvarez, B.) Biblioteca Clásica Gredos, Madrid.

Hippocrates (1986a) Tratados Hipocráticos II (intr., tr. y notas de López Férez, J.A. and García Novo, E.) Biblioteca Clásica Gredos, Madrid.

Hippocrates (1986b) Tratados Hipocráticos III (intr., tr. y notas de García Gual, C., Lucas de Dios, J.Mª., Cabellos Alvarez, B. and Rodríguez Alfajeme, I.) Biblioteca Clásica Gredos, Madrid.

Hippocrates (1988) Tratados Hipocráticos IV (tr. y notas de Sanz Mingote, L., intr. e índices de Ochoa Anadón, J.A.) Biblioteca Clásica Gredos, Madrid.

Hippocrates (1989) Tratados Hipocráticos V (tr., intr. y notas de Esteban, A., García Novo, E. and Cabellos, B.) Biblioteca Clásica Gredos, Madrid.

Hippocrates (1990) Tratados Hipocráticos VI (tr., intr. y notas de Alamillo Sanz, A. and Lara Nava, D.) Biblioteca Clásica Gredos, Madrid.

Hippocrates (1993) Tratados Hipocráticos VII (tr., intr. y notas de Lara, Mª.D., Torres, H. and Cabellos, B.) Biblioteca Clásica Gredos, Madrid.

Historia Augusta (1989) Picón, V. and Cascón, A. (eds) Akal Clásica, Madrid.

Homer (1991) Ilíada (tr. y notas de Crespo Güemes, E.) Biblioteca Clásica Gredos, Madrid.

Homer (1993) Odisea (tr. de Pabón, J.M.) Biblioteca Clásica Gredos, Madrid.

Horace (1996) Sátiras. Epístolas. Arte poética. Silvestre, H. (ed.) Cátedra Letras Universales, Madrid.

Ibn Abdun (2001) *Séville musulmane au début du XII siècle. Le traité d'Ibn Abdun sur la vie urbaine et les corps de métiers*. Lévy-Provençal, E. (ed.) Maisonneuve et Larose, Paris.

Ibn al-Awwam (1991) *Kitab al-Filaha* (Libro de agricultura) Banqueri, J.A. (ed.) ICMA, Madrid.

Ibn Razi al-Tubuyi (1960) *Fadalat-al-Jiwan fi tayyibat al-ta'am wa-l-alwan* (Relieves de la mesa, sobre manjares y guisos) La cocina arábigoandaluza según un manuscrito inédito. Granja Santamaría, F. Tesis doctoral (extracto) Facultad de Filosofía y Letras, Madrid.

Juvenal (1991) Sátiras (tr. y notas de Balasch, M.) Biblioteca Clásica Gredos, Madrid.

Libre de Sent Soví (1982) (tr. Grewe, R.) Barcino, Barcelona.

Martial (1991) Epigramas completos. Estefanía, D. (ed.) Cátedra Letras Universales, Madrid.

Mestre, Robert (1996) Libre del Coch. Curial Editions Catalanes, Barcelona.

Palladius (1990) Tratado de agricultura. Medicina veterinaria. Poema de los injertos (tr., intr. y notas de Moure Casas, A.) Biblioteca Clásica Gredos, Madrid.

Petronius (1988) El Satiricón (intr., tr. y notas de Rubio Fernández, L.) Biblioteca Clásica Gredos, Madrid.

Plato (1983) Leyes (tr. de Pabón, J.M. and Fernández-Galiano, M.) Centro de Estudios Constitucionales, Madrid.

Plato (1988) La República (tr. de Pabón, J.M. and Fernández-Galiano, M.) Alianza, Madrid.

Pliny the Elder (1956) Histoire Naturelle. Libre XIII. Ernout, A. (ed.) Les Belles Lettres, Paris.

Pliny the Elder (1960) Histoire Naturelle. Libre XV. André, J. (ed.) Les Belles Lettres, Paris.

Pliny the Elder (1964) Histoire Naturelle. Libre XVII. André, J. (ed.) Les Belles Lettres, Paris.

Pliny the Elder (1966) Historia Natural. Libros I–XXXVII (tr. y notas de Hernández, F. 3 vols) Universidad Nacional de Méjico, Méjico.

Plutarch (1986) Vidas paralelas (tr. de Ranz Romanillos, A. 4 vols) Orbis, Barcelona.

Plutarch (1995) Obras morales y de costumbres (*Moralia*) VI. (tr. De Fernández, J.A. and Pordomingo, F.) Biblioteca Clásica Gredos, Madrid.

Soranus (1956) Gynecology (transl. by Owsei Temkin) Johns Hopkins University Press, Baltimore.

Strabo (1992) Geografía. Libros III–IV (tr. y notas de Meana, Mª.J. and Piñero, F.) Biblioteca Clásica Gredos, Madrid.

Strabo (2001) Geografía. Libros V–VII (tr. y notas de Vela Tejada, J. and Gracia Artal, J.) Biblioteca Clásica Gredos, Madrid.

Theophrastus (1976) *De Causis plantarum* (tr. Einardson, B. and Link, G.K.R.) Loeb Classical Library, London.

Theophrastus (1988) Historia de las plantas (intr., tr. y notas de Díaz-Regañón López, J.M.) Biblioteca Clásica Gredos, Madrid.

Theophrastus (1989) Sobre las sensaciones. Anthropos, Barcelona.

Varro (1978) Economie rurale (*De re Rustica*) Livre I. Heurgon, J. (ed.) Les Belles Lettres, Paris.

Varro (1985) Economie rurale (*De re Rustica*) Livre II. Guiraud, Ch. (ed.) Les Belles Lettres, Paris.

Varro (1998) La lengua latina. Libros V–X (intr., tr. y notas de Hernández Miguel, L.A. 2 vols) Biblioteca Clásica Gredos, Madrid.

Virgil (1990) Bucólicas. Geórgicas. Apéndice Virgiliano (tr., intr. y notas de De la Ascensión Recio García, T. and Soler Ruíz, A.) Biblioteca Clásica Gredos, Madrid.

Vitruvius Polius, M. (1992) Los diez libros de arquitectura (tr. Ortiz, J.). Akal, Madrid.

Xenophon (1993) Recuerdos de Sócrates. Económico. Banquete. Apología de Sócrates. (intr., tr. y notas de Zaragoza, J.) Biblioteca Clásica Gredos, Madrid.

2 Chemical Composition, Types and Characteristics of Olive Oil

M. Carmen Ramírez-Tortosa,[1,2] Sergio Granados[1,2] and José L. Quiles[1,3]

[1]*Institute of Nutrition and Food Technology*; [2]*Department of Biochemistry and Molecular Biology*; [3]*Department of Physiology, University of Granada, Granada, Spain*

1. Introduction

The olive tree belongs to the *Oleaceae* family (made up of 20–29 main genera), to the *Olea* genus (including more than 30 species and sub-species), and to the *Olea europaea* family – the only one with edible fruit.

The olive tree (*Olea europaea L.*) is a polymorphous, medium-sized tree (maximum 10 m) with a furrowed trunk. It has fusiform coriaceous greyish-green leaves (generally about 5–6 cm long and about 1–1.5 cm wide at the middle of the leaf) with smooth edges and a short peduncle. A tree of the Mediterranean area, of the dry sub-tropical climatic zone, it is well adapted to extreme environmental conditions, but it requires high-intensity light and aerated soil. The olive tree is a tree that alternates its fruit production, so if it gives a lot of fruit one year, it gives less the next.

The development of the fertilized fruits of the olive tree (olives) is similar to that of the majority of fruits with pits; it begins with the phase of the appearance of floral buds, followed by pollination, fertilization, fruit-bearing and ripening. The length and nature of each phase depends heavily on environmental conditions, but under normal conditions, it begins in April and ends in November with the changing of the colour of the fruit from chlorophyll green to brownish red, to black.

The olive is a drupe (fruit) similar to others of the vegetable kingdom (peach, apricot, cherry, plum, etc.) whose constitutive elements are: the epicarp or skin, the mesocarp or pulp and the endocarp or pit, a woody shell that holds a seed. Although morphologically the olive is not different from other fruits, its chemical composition and organoleptic qualities are very different.

It is known that olive oil has been used for food since prehistory. A transparent, yellowish and aromatic liquid is extracted from the olive by simple pressure and it has been considered as an unusual luxury by cardiologists and nutritionists, being the only vegetal fat that is obtained from the fruit (olive juice), whereas the rest are obtained from seeds (Ruiz-Gutierrez *et al.*, 2000).

 Olive Oil and Health
(eds J.L. Quiles, M.C. Ramírez-Tortosa and P. Yaqoob)

2. Types and Characteristics of Olive Oil

Types and definition of olive oil have been established in the EC Regulation 136/66/EEC (1966), the EC Regulation 356/92 (1992), the EC Regulation 163/98 (1998) and the EC Regulation 1513/2001 (2001), and are described below. To include a particular olive oil in a specific type, that oil must match a series of characteristics established by the same regulations and summarized in Table 2.1, adapted from the EC Regulation 2568/91 (1991) and the EC Regulation 1989/2003 (2003).

2.1. Virgin olive oils

Oils obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, which have not undergone any treatment other than washing, decantation, centrifugation and filtration, to the exclusion of oils obtained using solvents or re-esterification processes and any mixture with oils of other kinds. Virgin olive oils are classified and described as follows:

- *Extra virgin olive oil*: virgin olive oil having a maximum free acidity, in terms of oleic acid, of 1 g per 100 g, the other characteristics of which comply with those laid down for this category.
- *Virgin olive oil* (the expression 'fine virgin oil' may be used at the production and wholesale stage): virgin olive oil having a maximum free acidity, in terms of oleic acid, of 2 g per 100 g, the other characteristics of which comply with those laid down for this category.
- *Ordinary virgin olive oil*: virgin olive oil having a maximum free acidity, in terms of oleic acid of 3.3 g per 100 g, the other characteristics of which comply with those laid down for this category.
- *Lampante virgin olive oil*: virgin olive oil having a free acidity, in terms of oleic acid, of more than 3.3 g per 100 g and/or the other characteristics of which comply with those laid down for this category.

2.2. Refined olive oil

Olive oil obtained by refining virgin olive oil, having a free acid content expressed as oleic acid of not more than 0.5 g per 100 g and the other characteristics which comply with those laid down for this category.

2.3. Olive oil

Olive oil obtained by blending refined olive oil and virgin olive oil other than lampante oil, having a free acid content expressed as oleic acid of not more than 1.5 g per 100 g and the other characteristics which comply with those laid down for this category.

Table 2.1. Characteristics of olive oils.

Characteristic	Category (Oil type)							
	Extra virgin olive oil	Virgin olive oil	Lampante olive oil	Refined olive oil	Olive oil (virgin + refined)	Crude olive-pomace oil	Refined olive-pomace oil	Olive-pomace oil
Acidity (%)*	≤ 0.8	≤ 2.0	> 2.0	≤ 0.3	≤ 1.0	–	≤ 0.3	≤ 1.0
Peroxide value mEq O_2/kg*	≤ 20	≤ 20	–	≤ 5	≤ 15	–	≤ 5	≤ 15
Waxes mg/kg**	≤ 250	≤ 250	≤ 300[3]	≤ 350	≤ 350	> 350[4]	> 350	> 350
Saturated acids in 2-position of the triglyceride (%)	≤ 1.5	≤ 1.5	≤ 1.5	≤ 1.8	≤ 1.8	≤ 2.2	≤ 2.2	≤ 2.2
Stigmasta-dienes mg/kg[1]	≤ 0.15	≤ 0.15	≤ 0.50	–	–	–	–	–
Difference between HPLC ECN42 and theoretical ECN42	≤ 0.2	≤ 0.2	≤ 0.3	≤ 0.3	≤ 0.3	≤ 0.6	≤ 0.5	≤ 0.5
K_{232}*	≤ 2.50	≤ 2.60	–	–	–	–	–	–
K_{270}*	≤ 0.22	≤ 0.25	–	≤ 1.10	≤ 0.90	–	≤ 2.00	≤ 1.70
ΔK*	≤ 0.01	≤ 0.01	–	≤ 0.16	≤ 0.15	–	≤ 0.20	≤ 0.18
Organoleptic assessment								
Median of defects (Md)*	Md = 0	Md ≤ 2.5	Md > 2.5[2]	–	–	–	–	–
Organoleptic assessment								
Median of fruity (Mf)*	Mf > 0	Mf > 0	–	–	–	–	–	–
Fatty acids content[5]								
Myristic (%)	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05
Linolenic (%)	≤ 1.0	≤ 1.0	≤ 1.0	≤ 1.0	≤ 1.0	≤ 1.0	≤ 1.0	≤ 1.0
Arachidic (%)	≤ 0.6	≤ 0.6	≤ 0.6	≤ 0.6	≤ 0.6	≤ 0.6	≤ 0.6	≤ 0.6
Eicosenoic (%)	≤ 0.4	≤ 0.4	≤ 0.4	≤ 0.4	≤ 0.4	≤ 0.4	≤ 0.4	≤ 0.4
Behenic (%)	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.3	≤ 0.3	≤ 0.3
Lignoceric (%)	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.2
Sum of transoleic isomers (%)	≤ 0.05	≤ 0.05	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.20	≤ 0.40	≤ 0.40
Sum of translinoleic and translinolenic isomers (%)	≤ 0.05	≤ 0.05	≤ 0.10	≤ 0.30	≤ 0.30	≤ 0.10	≤ 0.35	≤ 0.35

Continued

Table 2.1. *Continued*

Characteristic	Category (Oil type)							
	Extra virgin olive oil	Virgin olive oil	Lampante olive oil	Refined olive oil	Olive oil (virgin + refined)	Crude olive-pomace oil	Refined olive-pomace oil	Olive-pomace oil
Sterol composition								
Cholesterol (%)	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5
Brassicasterol (%)	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.2	≤ 0.2	≤ 0.2
Campesterol (%)	≤ 4.0	≤ 4.0	≤ 4.0	≤ 4.0	≤ 4.0	≤ 4.0	≤ 4.0	≤ 4.0
Stigmasterol (%)	< Camp.	< Camp.	–	< Camp.	< Camp.	–	< Camp.	< Camp.
β-Sitosterol (%)[6]	≥ 93.0	≥ 93.0	≥ 93.0	≥ 93.0	≥ 93.0	≥ 93.0	≥ 93.0	≥ 93.0
δ-7-Stigmasterol (%)	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5
Total sterols (mg/kg)	≥ 1 000	≥ 1 000	≥ 1 000	≥ 1 000	≥ 1 000	≥ 2 500	≥ 1 800	≥ 1 600
Erythrodiol and uvaol (%)**	≤ 4.5	≤ 4.5	≤ 4.5[3]	≤ 4.5	≤ 4.5	> 4.5[4]	> 4.5	> 4.5

[1] Sum of isomers that could (or could not) be separated by capillary column.
[2] Or if the median of defects is less than or equal to 2.5 and the median of fruity is 0.
[3] Oils with a wax content of between 300 mg/kg are considered to be lampante olive oil if the total aliphatic alcohol content is less than or equal to 350 mg/kg or if the erythrodiol and uvaol content is less than or equal to 3.5%.
[4] Oils with a wax content of between 300 mg/kg and 350 mg/kg are considered to be crude olive-pomace oil if the total aliphatic alcohol content is above 350 mg/kg and if the erythrodiol and uvaol content is greater than 3.5%.
[5] Other fatty acids present (%): palmitic: 7.5 to 20.0; palmitoleic: 0.3 to 3.5; heptadecanoic: ≤ 0.3; stearic: 0.5 to 5.0; oleic: 55.0 to 83.0; linoleic: 3.5 to 21.0.
[6] Sum of: δ-5-23-stigmastadienol + cholesterol + β-sitosterol + sitostanol + δ-5-avenasterol + δ-5-24-stigmastadienol.

Notes:
(a) The results of the analyses must be expressed to the same number of decimal places as used for each charasteristic. The last digit must be increased by one unit if the following digit is greater than 4.
(b) If just a single characteristic does not match the values stated, the category of an oil can be changed or the oil declared impure for the purposes of the Regulation.
(c) If a characteristic is marked with an asterisk (*), referring to the quality of the oil, this means the following:
– for lampante olive oil, it is possible for both the relevant limits to be different from the stated values at the same time.
– for virgin olive oils, if at least one of these limits is different from the stated values, the category of the oil will be changed, although they will still be classified in one of the categories of virgin olive oil.
(d) If a characteristic is marked with two asterisks (**) this means that for all types of olive-pomace oil, it is possible for both the relevant limits to be stated values at the same time.

2.4. Crude olive-pomace oil

Oil obtained from olive pomace by treatment with solvents or by physical means or oil corresponding to lampante olive oil, except for certain specified characteristic, excluding oil obtained by means of re-esterification and mixtures with other types of oils, and the other characteristic of which comply with those laid down for this category.

2.5. Refined olive-pomace oil

Oil obtained by refining crude olive-pomace oil having a free acid content expressed as oleic acid of not more than 0.5 g per100 g and the other characteristics which comply with those laid down for this category.

2.6. Olive-pomace oil

Oil obtained by blending refined olive-pomace oil and virgin olive oil other than lampante oil, having a free acid content expressed as oleic acid of not more than 1.5 g per 100 g and the other characteristics which comply with those laid down for this category.

3. Chemical Composition

Overall, olive oil can be divided from the point of view of chemical composition into major and minor fractions. The major components, which include glycerols, represent more than 98% of total oil weight. Minor components, which are present in very low amounts (about 2% of oil weight), include more than 230 chemical compounds such as aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds and antioxidants (carotenoids and phenolic compounds) (Servili *et al.*, 2004). Microscopic bits of olive can also be found in the oil. Because characteristics of minor components, these molecules are present almost exclusively in virgin olive oil since the processes (mainly refining) involved in the production of these oils remove them. Although usually when mentioning minor components it is normal to refer to minor components from virgin olive oil, we will refer here to minor components of olive oil since some species from that fraction may be present in types of olive oil other than virgin olive oil.

3.1. Major components

This fraction is also known as the saponifiable fraction or glyceride fraction. It constitutes about 98–99% of the oil weight and is composed mainly of triacylglycerols (Gunstone *et al.*, 1994). The fatty acids profile of a typical virgin olive, compared with other edible oils, is shown in Table 2.2.

Table 2.2. Typical fatty-acid profile (g/100 g) of different oils and fats.

Fatty acid	Palm oil	Butter	Margarine	Virgin olive oil	Canola oil	Sunflower oil	Corn oil	Soybean oil	Fish oil
14:0	1.5	6.77	–	–	–	–	–	0.1	11
16:0	45.1	11.94	7.83	8.7	5.4	6.35	12.6	10.6	26.7
16:1*n*-7	–	1.64	0	1.1	0.3	0.2	0.2	0.1	13.6
18:0	4.8	10.44	5.81	1.9	1.6	4.5	1.9	3.8	4.1
18:1*n*-9	36.8	30.7*	44.59**	78.7	56.3	32.1	24.1	23	12.6
18:2*n*-6	10.2	19.67	30.5	8.3	25	55.92	60.1	52.4	1.7
18:3*n*-3	0.5	1.82	3.29	0.9	8.4	0.1	1	8.9	–
20:5*n*-3	–	–	–	0.03	–	0.03	–	–	13.8
22:6*n*-3	–	–	–	0.05	–	0.12	–	–	6.8
Total saturated	51.9	45.9	18	10.9	8.2	12.9	14.6	15.6	41.8
Total monounsaturated	37	29	29.2	79.8	58.4	32.3	24.3	23.1	27.2
Total polyunsaturated	10.7	25.5	33.8	9.3	33.4	54.8	61.1	61.3	31

*Of which 2.84 are trans. ** Of which 15.64 are trans.

Oleic acid (a monounsaturated fatty acid) is represented in much higher concentration (68–81.5%) than the other acids (linoleic, palmitic and stearic acids). Structures of the major fatty acids present in virgin olive oil are shown in Fig. 2.1. Oleic acid (18:1*n*-9) and palmitoleic acid (16:1*n*-7) have one double bond in their structure, linoleic acid (18:2*n*-6) two double bonds and linolenic acid (18:3*n*-3) three double bonds. More of these fatty acids are present as triglycerides (triacylglycerols). The triglyceride structure is formed by one molecule of glycerol and three fatty acids (Fig. 2.2) and the distribution of fatty acids in olive oil triglycerides is summarized in Table 2.3. Free fatty acids, monoglycerides and diglycerides are also present in olive oil although triglycerides are the most abundant (Table 2.4). Finally, it is very interesting to describe the different molecular species of triglycerides in olive oil classified

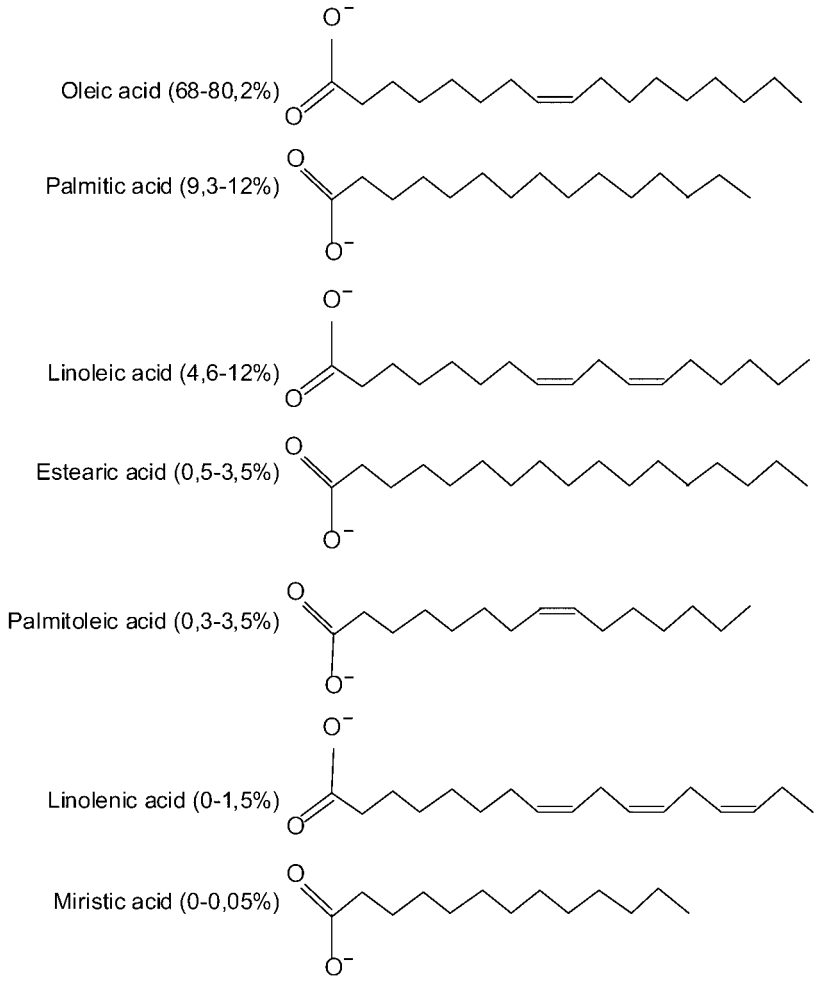


Fig. 2.1. Main fatty acids present in olive oil showing their concentration range. Oleic acid (18:1*n*-9), palmitic acid (16:0), linoleic acid (18:2*n*-6), estearic acid (18:0), palmitoleic acid (16:1*n*-9), linolenic acid (18:3*n*-3), miristic acid (14:0).

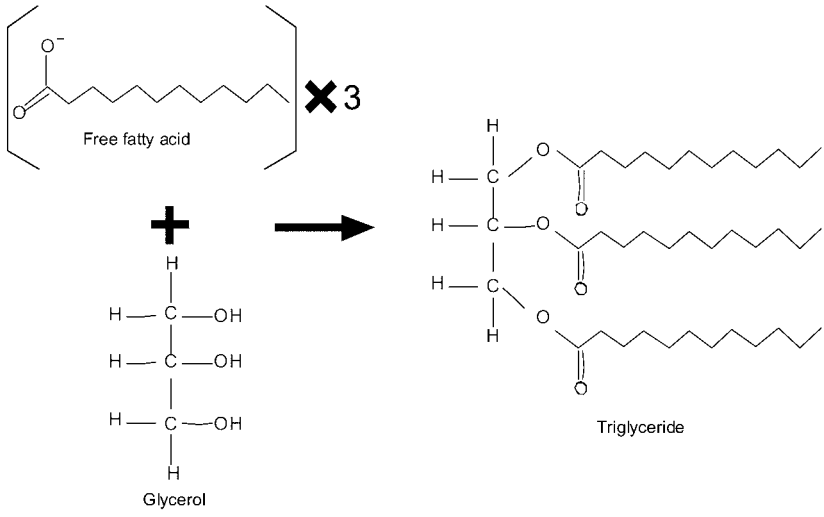


Fig. 2.2. Schematic representation of glycerol and fatty acid structure and triglyceride formation.

Table 2.3. Positional distribution of fatty acids in triglycerides of olive oil.

	Fatty acids (mol %)					
Position	16:0	16:1	18:0	18:1	18:2	18:3
1	13.1	0.9	2.6	71.8	9.8	0.6
2	1.4	0.7	–	82.9	14.0	0.8
3	16.9	0.8	4.2	73.9	5.1	1.3

Adapted from Gunstone *et al.*, 1994.

Table 2.4. Free fatty acids, mono, di- and triglycerides of olive oil according to carbon number.

Fatty acid	0.1	Monoglyceride	0.2	Diglyceride	5.5	Triglyceride	93.3	Others	0.9
C16	–	C18	0.2	C32	0.1	C46	0.1		
C18	0.1			C34	1.4	C48	0.3		
				C36	4	C50	6.2		
						C52	33.2		
						C54	51.1		
						C56	2.1		
						C58	0.3		

Values are expressed as g/100 g. Adapted from Gunstone *et al.*, 1994.

according to the number of double bonds (Table 2.5). The main molecular specie is with three double bonds (oleate-oleate-oleate; 39.2 mol/100 mol) following by two double bonds (palmitate-oleate-oleate and stearate-oleate-oleate; 26 mol/100 mol) and more than three double bonds (linoleate-oleate-oleate; 13.7 mol /100 mol).

Table 2.5. Molecular species of tryglycerides in olive oil.

No double bonds		One double bond		Two double bonds		Three double bonds		More than three double bonds	
PPPo	0.01	MOP	4.3	POO	26	OOO	39.2	LOO	13.7
		MOS		SOO		POL	5	OLO	4.6
		POP		OPO	0.3	SOL		Others	3
		POE		PLP		MLO	–		
		SOS		SLP	0.5	PLO	3		
		PPO	0.2	Others		SLO			
		SPO				Others	0.6		
		PSO							
		SSO							
		Others							

Values are expressed as mol/100 mol. P: palmitate; Po: palmitoleate; O: oleate; S: stearate; M: miristate; L: linoleate; Ln: linolenate. Adapted from Gunstone *et al.*, 1994.

3.2. Minor components

Minor components in olive oil represent about 2% of oil weight and include more than 230 chemical compounds.

3.2.1. Non-glyceride esters

Most of the fatty acids from virgin olive oil are esterified with glycerol. However, small amounts of these fatty acids form esters with different alcoholic compounds including methanol, ethanol and some long-chain alcohols; also with sterols like β-Sitosterol, capesterol and stigmasterol and with triterpene alcohols like trycycloartenol and 24-methyl-cycloartenol (Kiritsakis and Markakis, 1987). Total amounts of non-glyceride esters are in the range of 100–250 mg/kg.

Waxes are esters of long chain aliphatic alcohols (C_{27}–C_{32}). There are waxes containing up to 58 carbon atoms, which affects their physical properties like high molecular weight, melting point higher than 70°C, and others. Waxes are present in the skin of the olive and prevent water loss. They are abundant in pomace oils and lampante oils. Waxes are quickly increased in acid oils because of esterification of aliphatic alcohols with free fatty acids (Jiménez *et al.*, 2001).

3.2.2. Aliphatic alcohols

This is a fraction mainly composed of long-chain saturated alcohols (C_{18}–C_{30}). The total amount of these compounds is about 60 to 200 mg/kg (Ranalli *et al.*, 1999).

3.2.3. Triterpene alcohols

Triterpene alcohols are present in a range of 500 to 3000 mg/kg. Two of the most important are two dihydroxy triterpenes named erythrodiol and uvaol (Fig. 2.3). Quantification of these two components is very important since it provides a basis for recognizing impure oils (Kiritsakis and Markakis, 1987).

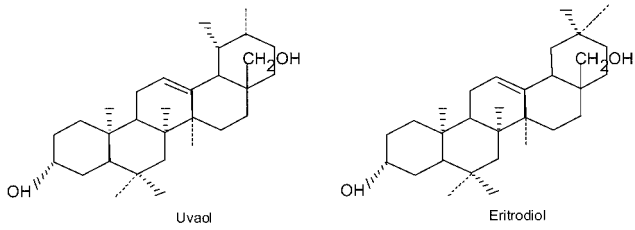


Fig. 2.3. Structures of uvaol and eritrodiol.

3.2.4. Sterols

Sterols are tetracyclic compounds biosynthesized from squalene. In olive oil sterols are present in the range of 1800 to 4939 mg/kg (Mulinacci *et al.*, 2005). The amount of sterols in a particular oil may be used to identify its origin and also its purity. Decreased level of sterols during oil storage has been associated with increases in the peroxide value. Fig. 2.4 shows the most abundant sterols present in olive oil.

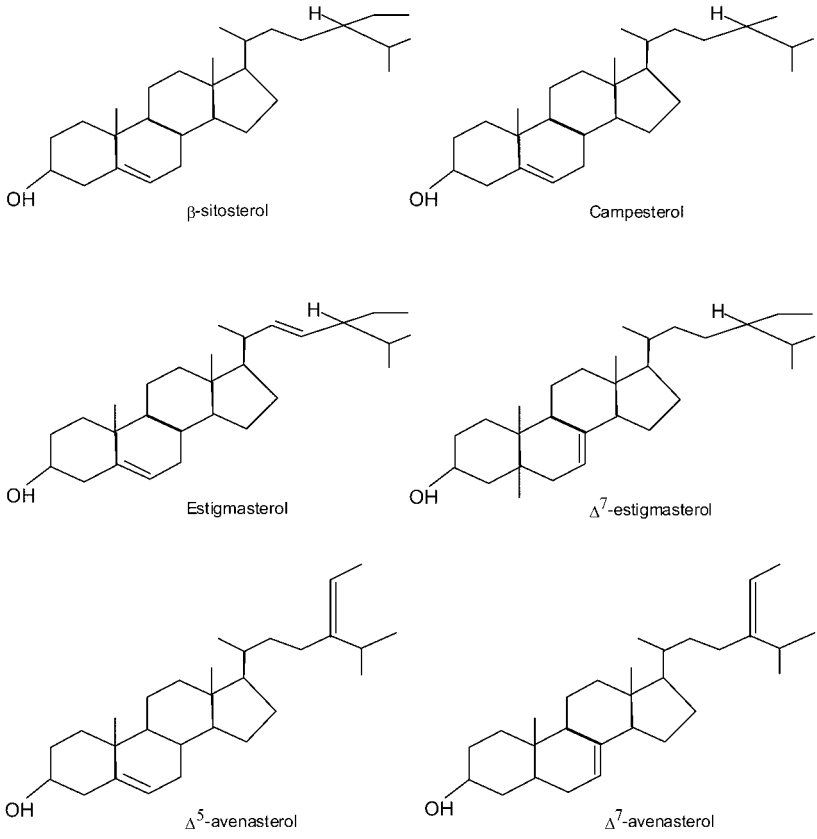


Fig. 2.4. Structures of β-sitosterol, campesterol, estigmasterol, Δ^7-estigmasterol, Δ^5-avenasterol and Δ^7-avenasterol.

3.2.5. Hydrocarbons

Squalene (Fig. 2.5) is the main hydrocarbon of olive oil (1250–7500 mg/kg). Total hydrocarbons are in the range of 1500–8000 mg/kg. Other hydrocarbons present as volatile in olive oil are phenanthrene, pyrene, fluoranthrene, 1,2-benzanthracene, chrysene, and perilene (Fedeli, 1977). Carotenoids are also hydrocarbons present in olive oil minor fraction. The most important carotenoids present in olive oil are β-carotene and lycopene. They are also responsible of olive oil colour. In virgin olive oils produced from mature olives, the concentrations of β-carotene have been reported to vary from 0.33 to 3.69 mg/kg (Rahmani and Saari Csallany, 1991). Depending on some aspects, levels up to 10 mg/kg may be found (Ranalli *et al.*, 1999).

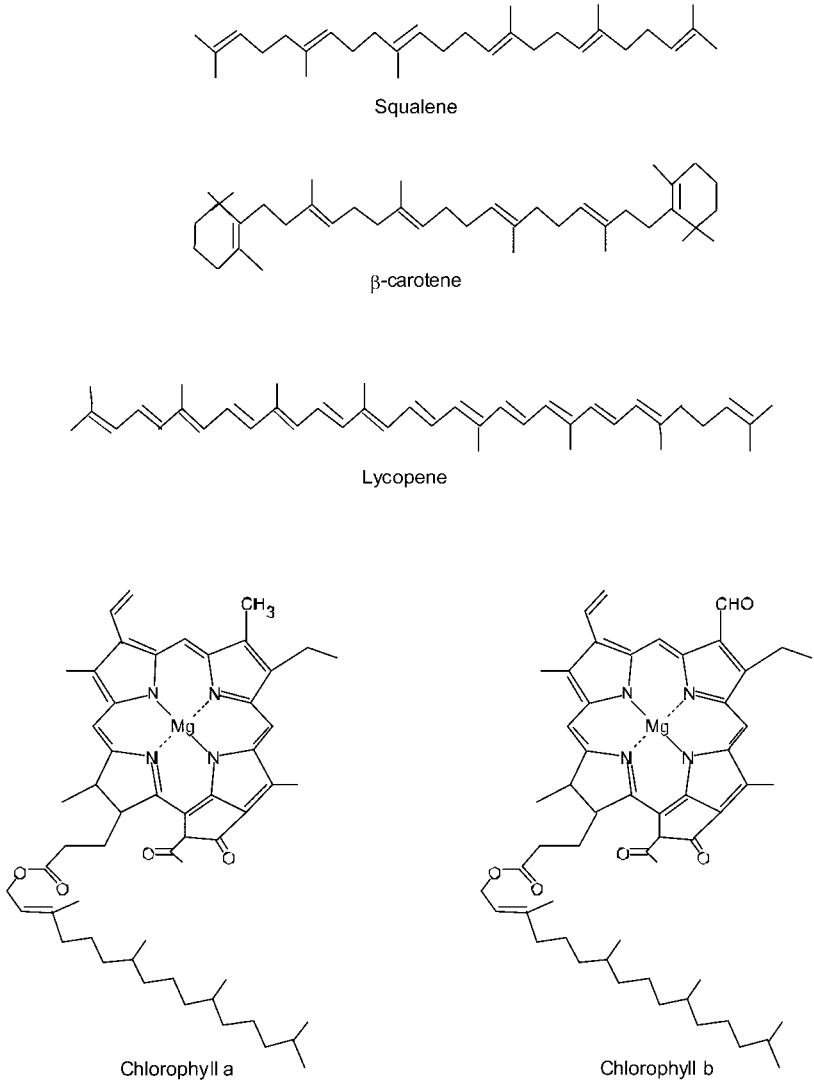


Fig. 2.5. Structures of squalene, β-carotene, lycopene, chlorophyll a and chlorophyll b.

3.2.6. Pigments

Several components are responsible for olive oil colour. Among them, chlorophylls (Fig. 2.5) are the most important. Chlorophylls a and b and their oxidation products, pheophytins a and b, are naturally present in olive oil and are responsible for the greenish colour of the oils. The quantity of chlorophylls in olive oil depends on a number of factors such as the variety, the degree of maturity of the olives, method of oil extraction, and some other biological and technical factors (Fedeli, 1977). In virgin olive oil from mature olives the levels of chlorophyll are about 1 to 10 mg/kg and those of pheophytins are about 0.2 to 24 mg/kg (Rahmani and Saari Csallany, 1991).

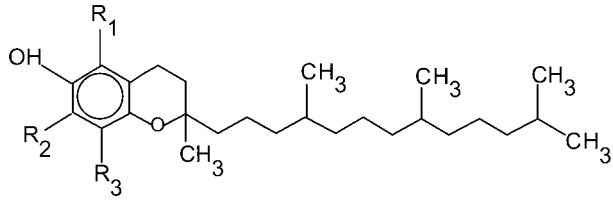
3.2.7. Lipophilic phenolics

The most important lipophilic phenols present in olive oil are tocopherols and tocotrienols (Fig. 2.6). The most abundant is α-tocopherol, which may be present in a range of 12 to 400 mg/kg.

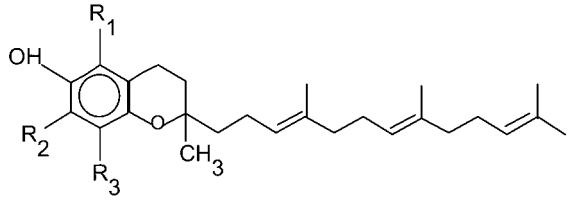
3.2.8. Hydrophilic phenolics

Hydrophilic phenolics have been identified as being responsible for most of the antioxidant properties of virgin olive oil. These compounds are peculiar to virgin olive oil; they are not present in any other vegetable oil (Boskou, 1996). Hydrophilic phenols (40–1000 mg/kg) may be divided into different classes such as phenolic acids, phenolic alcohols, secoiridoids, lignans and flavones (Servili *et al.*, 2004). Phenolic acids (Fig. 2.7) were the first group of phenolic compounds described in virgin olive oil. There are two series of these acids, the benzoic series (benzoic acid, *p*-hydroxybenzoic acid, protocatechuic acid, gallic acid, vanillic acid and syringic acid) and the cinnamic series (cinnamic acid, *p*-coumaric acid, *o*-coumaric acid, caffeic acid, ferulic acid and sinapic acid). These substances are present in small amounts. Phenolic alcohols (Fig. 2.8) are (3,4-dihydrozyphenyl)ethanol, also called hydroxytyrosol; (*p*-hydroxyphenyl)ethanol, also named tyrosol; and (3,4-dihidroxyphenyl)ethanol-glucoside (Montedoro *et al.*, 1992; Bianco *et al.*, 1998). Owen *et al.* (2000a) reported mean concentrations of 14.4 mg/kg and 27.45 mg/kg of hydroxytyrosol and tyrosol, respectively in a batch of 18 virgin olive oil samples. Servilli *et al.* (2004) studied 210 virgin olive oil samples, extracted by industrial plants from different areas of Mediterranean countries, and reported median values of 1.8 (lower quintile 1; upper quintile 3.6) and 1.9 (lower quintile 0.6; upper quintile 5.0) mg/kg of hydroxytyrosol and tyrosol, respectively.

Secoiridoids are characterized by the presence of either elenolic acid or elenolic acid derivatives in their molecular structure. The most important secoiridoids present in virgin olive oil are the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol, the dialdehydic form of decarboxymehyl elenolic acid linked to tyrosol, oleuropein aglycon, ligstroside aglycon, oleuropein, the dialdehydic form of oleuropein aglycon and the dialdehydic form of ligstroside aglycon (Fig. 2.8). Secoiridoids, together with lignans, are the most



R_1	R_2	R_2	
CH_3	CH_3	CH_3	α-tocopherol
CH_3	H	CH_3	β-tocopherol
H	CH_3	CH_3	γ-tocopherol
H	H	CH_3	δ-tocopherol



R_1	R_2	R_2	
CH_3	CH_3	CH_3	α-tocotrienol
CH_3	H	CH_3	β-tocotrienol
H	CH_3	CH_3	γ-tocotrienol
H	H	CH_3	δ-tocotrienol

Fig. 2.6. Structures of tocopherols and tocotrienols.

abundant phenolics present in virgin olive oil. Owen *et al.* (2000a) reported 27.72 mg/kg of total secoiridoids in virgin olive oil. Concerning lignans (Fig. 2.9), these compounds were first detected in virgin olive oil by Owen *et al.* (2000b). These authors found in virgin olive oil a mean of 41.53 mg/kg of total lignans, with samples reaching up to 100 mg/kg. Two compounds from the lignan family, named (+)-1-pinoresinol and (+)-1-acetoxypinoresinol, were specifically identified. Brenes reported that Spanish olive oils contain (+)-1-pinoresinol in a range of 20 to 45 mg/kg and (+)-1-acetoxypinoresinol was found in a range of 2 to 95 mg/kg (Brenes *et al.*, 1999). Finally, among hydrophilic phenolics of olive oil, can be found different flavones such as apigenin or luteolin (Servili *et al.*, 2004), which structures are shown in Fig. 2.9.

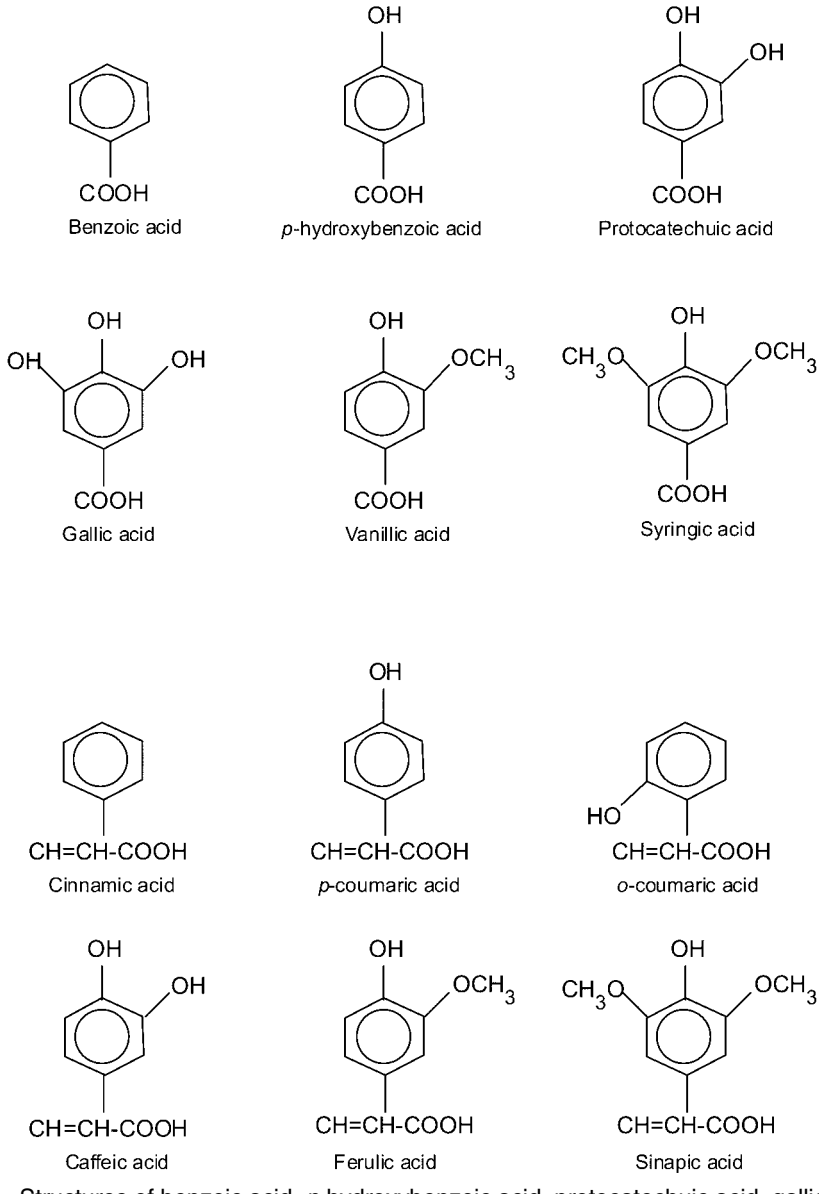


Fig. 2.7. Structures of benzoic acid, *p*-hydroxybenzoic acid, protocatechuic acid, gallic acid, vanillic acid, syringic acid, cinnamic acid, *p*-coumaric acid, *o*-coumaric acid, caffeic acid, ferulic acid and sinapic acid.

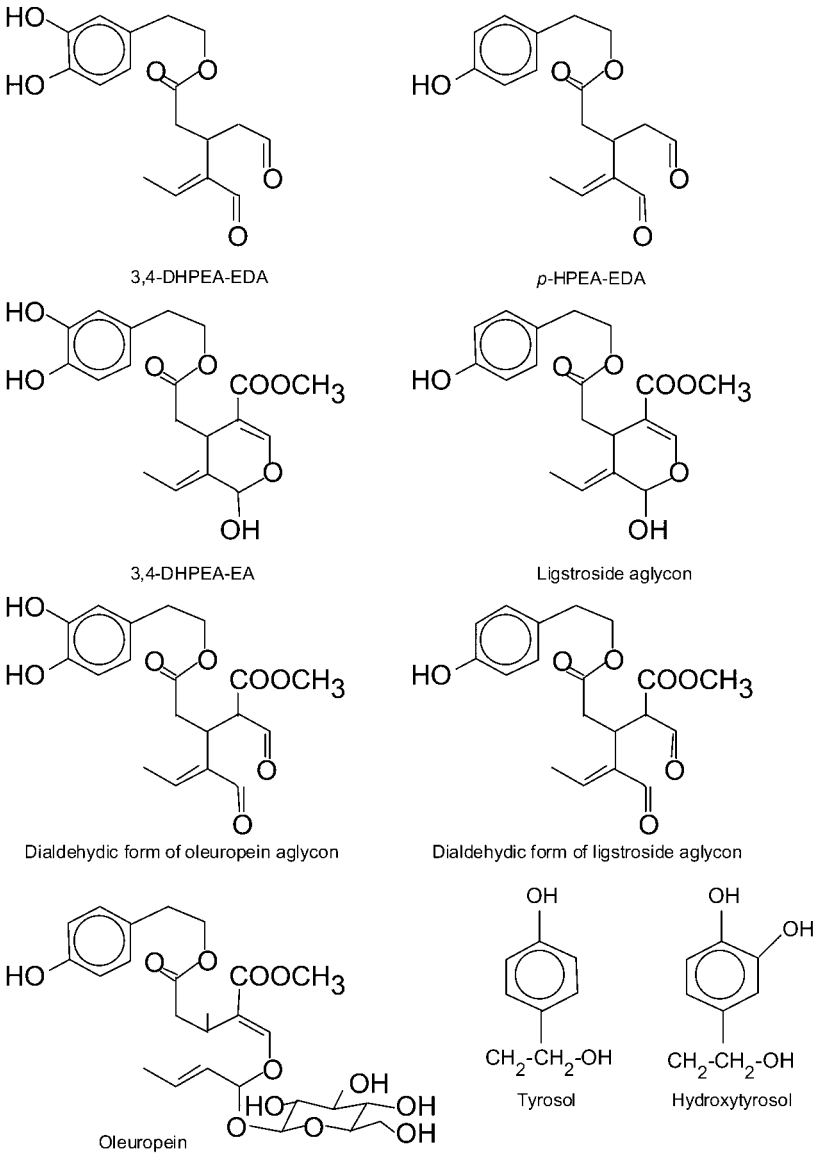


Fig. 2.8. Structures of dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA); the dialdehydic form of decarboxymethyl elenolic acid linked to tyrosol (*p*-DHPEA-EDA); oleuropein aglycon (3,4-DHPEA-EA); ligstroside aglycon; oleuropein; the dialdehydic form of oleuropein aglycon; the dialdehydic form of ligstroside aglycon; hydroxytyrosol ((3,4-dihydrosyphenyl)ethanol or 3,4 DHPEA); and tyrosol ((*p*-hydroxyphenyl)ethanol or *p*-HPE).

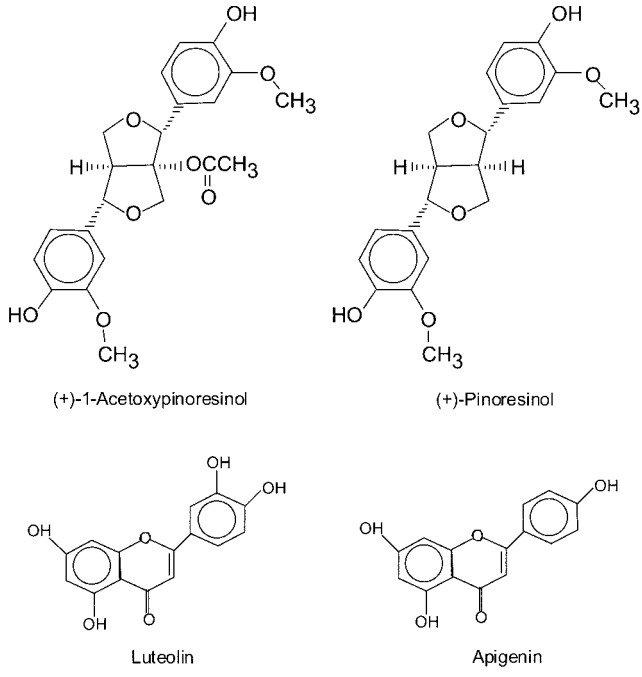


Fig. 2.9. Structures of (+)-1-acetoxypinoresinol, (+)-1-pinoresinol, luteolin and apigenin.

3.2.9. Volatile compounds

Volatile compounds are retained by virgin olive oils during their mechanical extraction process. Volatile compounds, stimulating the olfactive receptors, are responsible for the whole aroma of the virgin olive oil (Angerosa *et al.*, 2004). Many compounds, mainly carbonyl compounds, alcohols, esters and hydrocarbons, have been identified in the volatile fraction of virgin olive oil (Fedeli, 1977; Kiritsakis and Markakis, 1987). A summary of some of these compounds is presented in Table 2.6.

Acknowledgements

Dr Carmen Ramírez-Tortosa and Dr José L. Quiles are supported by a 'Ramón y Cajal' contract from the Spanish Ministry of Education and Science and the University of Granada. Sergio Granados is a recipient of the programme 'Becas de formación de Doctores' from the University of Granada.

Table 2.6. Some of the volatile compounds found in virgin olive oil.

Aldehydes	Ketones	Esters
Acetaldehyde	Pentan-3-one	Methyl acetate
Propanal	1-Penten-3-one	Butyl acetate
2-Methyl-propanal	1-Octen-3-one	Ethyl acetate
Hexanal		Ethyl propanoate
Heptanal	Alcohols	Ethyl butyrate
Octanal		Ethyl isobutyrate
Nonanal	Ethanol	Ethyl 2-methylbutyrate
Decanal	Pentan-1-ol	Ethyl 3-methylbutyrate
2-Methyl butanal	Hexan-1-ol	*cis*-3-Hexenyl acetate
3-Methyl butanal	2-Methyl-propan-1-ol	Hexyl acetate
2-Methyl-2-butenal	2-Methylbutan-1-ol	3-Methylbutyl acetate
trans-2-Pentenal	*cis*-2-Penten-1-ol	Methyl 2-methylbutyrate
cis-2-Pentenal	*trans*-3-Hexen-1-ol	Methyl decanoate
trans-2-Hexenal	*cis*-3-Hexen	Methyl nonanoate
cis-2-Hexenal	*trans*-2-Hexen-1-ol	
trans-3-Hexenal	*cis*-2-Hexen-1-ol	Others
cis-3-Hexenal	1-Penten-3-ol	
2-Octenal		Methylbenzene
cis-2-Nonenal	Acids	Ethylbenzene
trans-2-Nonenal		Ethylfuran
2-Decenal	Acetic acid	Dimethyl sulphide
2,4-Hexadienal	Propanoic acid	Dipropyl disulphide
2,4-Heptadienal	Butanoic acid	Cyclopropane
2,4-Nonadienal	Pentanoic acid	
2,6-Nonadienal	Hexanoic acid	
2,4-Decadienal	3-Methylbutyric	
Benzaldehyde	2-Methylbutyric	
Phenylacetaldehyde		

4. References

Angerosa, F., Servili, M., Selvaggini, R., Taticchi, A., Esposto, S. and Montedoro, G. (2004) Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. *Journal of Chromatography A* 1054, 17–31.

Bianco, A.D., Muzzalupo, L., Romeo, G., Scarpati, M.L., Soriero, A. and Acella, N. (1998) Microcomponents of olive oil—III. Glucosides of 2(3,4-dihydroxy-phenyl)ethanol. *Food Chemistry* 63, 461–464.

Boskou, D. (1996) Olive oil chemistry and technology, AOCS Press, Champaign, IL, USA, pp. 52–83.

Brenes, M., García, A., García, P., Rios, J.J. and Garrido, A. (1999) Phenolic compounds in Spanish olive oils. *Journal of Agricultural and Food Chemistry* 47, 3535–3540.

EC Regulation no. 133/66 (1966) *Official Journal of the European Communities* L172, 3025–3066.

EC Regulation no. 1513/2001 (2001) *Official Journal of the European Communities* L201, 4.

EC Regulation no. 163/98 (1998) *Official Journal of the European Communities* L210, 32.

EC Regulation no. 1989/2003 (2003) *Official Journal of the European Communities* L295, 57.

EC Regulation no. 2568/91 (1991) *Official Journal of the European Communities* L248, 1.

EC Regulation no. 356/92 (1992) *Official Journal of the European Communities* L39, 1.

Fedeli, E. (1977) Lipids of olives. *Progress in Chemistry of Fats and Other Lipids*, 57–74.

Gunstone, F.D., Harwood, J.L. and Padley, F.B. (1994) *The Lipid Handbook*, 2nd edn. Chapman & Hall, London, UK, pp. 53–117.

Jiménez, J., Rondón, D., Martínez, L. and Mataix, J. (2001) Chemical composition of olive oils. In: Mataix, J. (ed.) *Aceite de oliva virgen: nuestro patrimonio alimentario.* Universidad de Granada and PULEVA Food, Granada, Spain, pp. 115–136.

Kiritsakis, A. and Markakis, P. (1987) Olive oil: a review. *Advances in Food Research* 31, 453–483.

Montedoro, G., Servilli, M., Baldioli, M. and Miniati, E. (1992) Simple and hydrolysable phenolic compounds in virgin olive oil 1. Their extraction, separation and quantitative and semiquantitative evaluation by HPLC. *Journal of Agricultural and Food Chemistry* 40, 1571–1576.

Mulinacci, N., Giaccherini, C., Innocenti, M., Romani, A., Vincieri, F.F., Marotta, F. and Mattei, A. (2005) Analysis of extra virgin olive oils from stoned olives. *Journal of the Science of Food and Agriculture* 85, 662–670

Owen, R.W., Giacosa, A., Hull, W.E., Haubner, R., Spiegelhalder, B. and Bartsch, H. (2000a) The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. *European Journal of Cancer* 36, 1235–1247.

Owen, R.W., Mier, W., Giacosa, A., Hull, W.E., Spiegelhalder, B. and Bartsch, H. (2000b) Identification of lignans as major components in the phenolic fraction of olive oil. *Clinical Chemistry* 45, 976–988.

Rahmani, M. and Saari Csallany, A. (1991) Chlorophyll and β-carotene pigments in Moroccan virgin olive oils measured by high-performance liquid chromatography. *Journal of the American Oil Chemists Society* 68, 672–674.

Ranalli, A., Ferrante, M.L., De Mattia, G. and Costantini, N. (1999) Analytical evaluation of virgin olive oil of first and second extraction. *Journal of Agriculture and Food Chemistry* 47, 417–424.

Ruiz-Gutierrez, V., de la Puerta, R. and Perona, J. (2000) Beneficial effects of virgin olive oil on health. *Recent Research and Development in Nutrition* 3, 173–197.

Servili, M., Selvaggini, R., Esposto, S., Taticchi, A., Montedoro, G. and Morozzi, G. (2004) Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and thecnological aspects of production that affect their occurrence in the oil. *Journal of Chromatography A* 1054, 113–127.

3 Total Antioxidant Capacity of Olive Oils

NICOLETTA PELLEGRINI[1] AND MAURIZIO BATTINO[2]

[1]*Department of Public Health, University of Parma, Italy*; [2]*Institute of Biochemistry, Faculty of Medicine, Marche Polytechnic University, Ancona, Italy*

1. Introduction

Reactive oxygen species (ROS) are continuously generated in the body during normal metabolic functioning or as part of the body defence against microorganisms (Battino *et al*., 1999). Excessive production of ROS can cause oxidative damage to biological macromolecules such as DNA, lipids, carbohydrates and proteins (Halliwell and Gutteridge, 1999). Damage to these molecules has in turn been associated with increased risk of chronic diseases such as cancer, cardiovascular disease, eye disease and a number of immune and neuro-degenerative disorders (Halliwell and Gutteridge, 1999). The human body has evolved complex strategies to utilize oxygen and to minimize the deleterious effects of ROS; among such strategies, antioxidants are believed to be crucial in health maintenance through the modulation of oxidative processes in the body. The antioxidants within cells, cell membranes and extracellular fluids can be upregulated and mobilized to neutralize excessive ROS formation avoiding oxidative damage (Quiles *et al.*, 1994, 1999). Moreover, diet contains a large array of compounds that may function as antioxidants, including vitamins E and C, carotenoids, flavonoids and other phenolic compounds (Halliwell and Gutteridge, 1999).

In recent years, epidemiological studies have demonstrated an inverse association between the intake of antioxidants from fruits and vegetables and the morbidity and mortality from coronary heart diseases (Cook and Samman, 1996; Stephens *et al.*, 1996) and cancer (Cohen *et al.*, 2000; La Vecchia *et al.*, 2001; Terry *et al.*, 2001). Nevertheless, many clinical studies have not shown direct beneficial effects of individual antioxidant molecules, such as vitamin E and β-carotene, on various chronic diseases (Hennekens *et al.*, 1996; Omenn *et al.*, 1996; Heinonen *et al.*, 1998), suggesting that the functionality of dietary antioxidants might be strongly linked to cooperative mechanisms among different antioxidant molecules present in the matrix. Based on these observations, a number of assays have been introduced in the past decade for determining the

 Olive Oil and Health
(eds J.L. Quiles, M.C. Ramírez-Tortosa and P. Yaqoob)

total antioxidant capacity (TAC) of food extracts and beverages (Cao *et al.*, 1993; Ghiselli *et al.*, 1995; Benzie and Strain, 1996; Pellegrini *et al.*, 2003a). This parameter considers the cumulative action and the synergistic interaction of all the antioxidants present in food giving the cumulative capacity of food to scavenge free radicals. These assays diverge in that they relate to the generation of different radicals, often acting through different mechanisms and/or target molecules, and the measures are made on a range of different end-points (Re *et al.*, 1999).

In general, two types of approach have been used: (i) inhibition assays, for which the extent of the scavenging of a pre-formed free radical by hydrogen- or electron-donation is the marker of antioxidant activity; (ii) assays involving the presence of antioxidant systems during the generation of the radical, for which the activity is measured on the rate of oxidation of a target molecule. When applying these methods, it is crucial to know exactly what the different assays are measuring and what kind of information can be obtained by adopting one technique instead of another.

2. Antioxidant Composition of Olive Oil

Olive oil, obtained from whole fruits (drupes) of *Olea europea* L., in addition to its high proportion of monounsaturated fatty acids, i.e. oleic acid, and the modest presence of polyunsaturated fatty acids, contains natural antioxidants such as tocopherols, carotenoids, sterols, ubiquinols and phenolic compounds (Boskou, 1996). Among the phenolic compounds found in olive oils, gallic, caffeic, vanillic, *p*-coumaric, syringic, ferulic, homovanillic, *p*-hydroxybenzoic and protocatecuic acids, tyrosol and hydroxytyrosol, oleuropein and its aglycones and ligstroside and its aglycones are the most representative (Montedoro *et al.*, 1992; Mannino *et al.*, 1993; Boskou, 1996). The absolute concentration and the relative proportions of these minor compounds in olive oils are the result of different manufacturing processes; extra virgin olive oil (EVOO), obtained by the first physical cold pressure of the olive paste, is much richer in phenolic compounds than refined oils, which are obtained by refining process from oils that exceed the limits of acidity and are virtually devoid of phenolics (Visioli and Galli, 1995). Commercial olive oil (OO), probably the most commonly consumed olive oil in the non-Mediterranean countries, is a vaguely defined mixture of refined olive oil and EVOO in which the amount of EVOO may vary from 33 to 95% (Andrikopoulos *et al.*, 1989). The level of phenolic compounds is an important parameter in the evaluation of olive oil quality, since phenolics are strictly related to both oil resistance to oxidation, because of their antioxidant properties, and to the typical bitter taste of the olives (Boskou, 1999). Moreover, they contribute, with other antioxidant compounds, to the TAC of olive oil.

3. Total Antioxidant Capacity of Oils

A number of assays have been published for the evaluation of TAC of oil using colorimetric, spectrophotometric, spectrofluorimetric, electrochemical or chemiluminescent techniques (Tubaro *et al.*, 1996; Mannino *et al.*, 1999; Ninfali *et al.*, 2001;

Pellegrini *et al.*, 2001; Papadoupolos *et al.*, 2003). As already mentioned, each assay for measuring the total antioxidant capacity of food has its own characteristics and differences exist in the free radical-generating system, molecular target, endpoint, kinetic, biological matrix, residence in lipo- and hydrophilic compartment, and physiological relevance. In evaluating the TAC of oils, two analytical approaches are usually employed: (i) polar compounds, mainly constituted by phenolics, are extracted before the analysis; (ii) the TAC is directly determined in the oil diluted in an appropriate solvent. This latter approach is not frequently applied, owing to the hydrophobic moiety, which does not allow the use of oil in most TAC assays, where an aqueous mixture is generally used as reaction medium.

Conversely, the approach in which the TAC is measured in extracted polar compounds is widely employed. In this case, extraction is usually carried out by using aqueous methanol, which permits the extraction of a large portion of water-soluble simple phenolics of lower molecular weight and other phenolic compounds of higher molecular weight. According to this approach, the oxygen radical absorbance capacity (ORAC) of vegetable oils was investigated using a TAC spectrofluorimetric method which measures the protection of the phenolic substances of the oil on the β-phycoerythrin fluorescence decay in comparison with the standard Trolox, a water-soluble analogue of vitamin E (Ninfali *et al.*, 2001). The TAC values ranged from 1.78 to 5.08 μmol Trolox equivalent/g and 4.15 to 6.20 μmol Trolox equivalent/g for minor brand EVOOs, produced by local Italian small-scale producers, and name brand EVOOs, produced and distributed nationally by major producers, respectively (Ninfali *et al.*, 2001). Moreover, the same authors (Ninfali *et al.*, 2001) found that the TAC value of refined olive and peanut oils was lower (1 and 1.5 μmol Trolox equivalent/g, respectively) than that of less efficient analysed EVOO. In addition, a significant correlation between the TAC values of olive oils and the total amount of phenolics, evaluated by the Folin-Ciocalteu method, was found (Ninfali *et al.*, 2001).

Similar results were reported analysing acetone extracts of five different Spanish olive oils (i.e., olive oils, EVOO and lampante) by four different radical scavenging tests; EVOO possessed the highest content of the antioxidant compounds and the highest antioxidant capacity, whereas the lampante oil was the lowest in all the assays (Gorinstein *et al.*, 2003). Moreover, all the assays for measuring the TAC of oils were correlated to the content of total phenolics, even if the method based on β-carotene linoleate model system was the best correlated. The TAC assays of different EVOO extracts appeared also to be related to the degradation level of oils (Lavelli, 2002). In fact, EVOOs with a low degradation degree, measured by oxidation and hydrolysis indices indicated by the EU Regulation on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis (Regulation 2568/91, 1991), were three to five times more efficient as scavengers of the synthetic 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and two times more efficient as inhibitors of the xanthine oxidase-catalysed reaction than oils with intermediate and advanced degradation levels. On the contrary, in the same study, another applied TAC assay, able to generate ROS relevant to cell damage *in vivo*, was inhibited by EVOOs having low or intermediate degradation levels, but not by the most degraded oils. Whatever the measuring system, the correlation between phenolic compounds and TAC values was once again confirmed.

Papadopoulos *et al.* (2003) proposed a sensitive and simple procedure for measuring the TAC of olive oil and seed oil aqueous extracts using the chemiluminiscence of lucigenin. The authors found that olive oil aqueous extracts showed two to ten times higher TAC (range from 37.1 to 67.5%) than seed oils (range from 7.3 to 20.2%). Moreover, due to the high correlation between the fluorimetric and spectrophotometric measurements of oil extracts, used to evaluate the total phenolics, they suggested that the proposed method could be used for the quantification of total phenolics in aqueous oil extracts. Similar results were presented by Mosca *et al.* (2000), who analysed the methanolic extracts of six EVOOs obtained from various locations in Italy by using two TAC assays. Applying both the assays (i.e. DPPH assay and thiobarbituric acid-reacting substances production in oxidized LDL), the authors demonstrated that the TAC of olive oil extracts was proportional to their phenolic concentration, as determined by both the Folin-Ciocalteu assay and a new proposed enzymatic method based on the oxidation of phenolic compounds by tyrosinase. Moreover, they showed that the proposed method offers some advantages over the classic methods employed to assess olive oil phenolics such as simplicity, reproducibility, specificity and sensitivity of analysis.

However, it is noteworthy that phenolic compounds are not the only substances that contribute to the antioxidant capacity of oil. It was proved that the antioxidant capacity of the unsaponifiable fraction of different EVOOs could also be related to the content of α-tocopherol and squalene (Finotti *et al.*, 1998). Furthermore, Espin *et al.* (2000), analysing 57 edible oils, including 24 olive oils from different origins, demonstrated that the lipidic fraction, that is the remaining fraction obtained after methanolic extraction of oil, possessed antioxidant capacity measured by the DPPH assay. According to the authors, such antioxidant capacity of different oils is mainly due to their different concentration and type of tocopherols. In fact, a higher antioxidant capacity was observed for soybean oil rich in δ-tocopherol, which is the most efficient antioxidant compound among the tocopherols, and the lowest for olive oil, which contains a low concentration of tocopherols, with the major compound being α-tocopherol, the least efficient as antioxidant. Moreover, the authors reported that the TAC of each oil, dissolved in ethyl acetate without any fractionation, was higher than the arithmetic sum of the experimental recordings corresponding to the methanolic and lipidic fractions. They state that this could be due to the synergistic effect of the different antioxidants present in both fractions. Taking this into account, it is unlikely that the antioxidant capacity of oil is accounted for by polar compounds only, because it ignores the contribution of lipophilic compounds as well as the synergistic effects among the antioxidants present in olive oils. However, to date there is still little information about the direct evaluation of oil TAC.

The antioxidant capacity of olive oils has been measured by a kinetic analysis of crocin bleaching inhibition (Tubaro *et al.*, 1996). By this procedure, the ability of oils, diluted in dimethyl formamide, to quench peroxyl radicals is measured in terms of α-tocopherol equivalents, by analysing the kinetic competition of a parallel reaction where peroxyl radicals bleach the carotenoid crocin. The method is accurate, although, because the results are expressed as kinetic competition equations, comparisons with other methods and the interpretation of data are difficult.

Another method for measuring directly the oil TAC, based on the electrochemical properties of antioxidants present in olive oil, has been developed by Mannino *et al.* (1999). In this procedure, the oil sample is diluted in the mobile phase and directly injected in a flow injection apparatus with an electrochemical detector operating at a potential of 0.5 V (*vs* Ag/AgCl). This method gives particularly interesting results for its rapidity of analysis (90 samples/h) and a good agreement has been found between the results obtained by such electrochemical method and those obtained by the 2,2′-azinobis-(3-thylbenz-thiazoline-6-sulphonic) radical cation ($ABTS^{\bullet+}$) decolorization assay (Pellegrini *et al.*, 1999). The latter is based on the ability of antioxidants to quench the long-lived $ABTS^{\bullet+}$ in relation to that of Trolox. This simple and effective method is able to discriminate among different olive oils on the basis of their antioxidant composition. In fact, as reported in Table 3.1, the differences in antioxidant composition between olive oils and EVOO influence the TAC values. Thus, the TAC ranged from 0.72 mmol of Trolox equivalent/kg of oil to 1.06 in olive oil, and from 1.53 mmol of Trolox equivalent/kg of oil to 2.69 in EVOO. However, in a recent publication by the same group, it has been demonstrated that EVOO was not the most efficient oil to quench the $ABTS^{\bullet+}$; in fact, probably due to the high content of tocopherols, soybean oil had higher TAC value (Pellegrini *et al.*, 2003b).

The above TAC values for EVOOs have been recently confirmed by a preliminary screening carried out on EVOOs from selected native olive cultivars of the Marche Region (centre-east of Italy): these EVOOs showed TAC up to 2.2 mmol of Trolox equivalent/kg of oil with a good correspondence between TAC value and the cultivar employed (unpublished data). A low TAC value was obtained for EVOO when the chain-breaking antioxidant capacity was evaluated using egg lecithin as oxidizable substrate subjected to the peroxyl radical attack by a

Table 3.1. Content of α-tocopherol and phenolic compounds and total antioxidant capacity (TAC) of commercial oils. Adapted from Pellegrini *et al.* (2001).

Oil	α-tocopherol (mg/kg)	Total phenolics (GAE[a], mg/kg)	TAC (mmol Trolox/kg)
Olive oils			
OOBE	194	24	1.06
OOSA	146	30	0.94
OOCA	121	14	0.72
Extra virgin olive oils			
EVOOPR	254	265	2.69
EVOOCD	312	171	2.19
EVOOSA	251	231	2.16
EVOOBE	314	133	1.94
EVOOCA	288	117	1.76
EVOORG	369	73	1.53
Refined olive oil			
ROO	138	4	0.61

[a] Values are expressed as mg of gallic acid equivalents per kg of oils.

lipophilic azo compound (Cabrini *et al.*, 2001). In this assay, the TAC value of edible oils was determined by measuring the length of time during which oxygen uptake was inhibited by the presence of oil antioxidants. When using this approach the TAC increased in the order EVOO < peanut < soybean < corn < sunflower. This pattern was approximately in accordance with the unsaturation index of fatty acid residues of oil triglycerides.

4. The Utilization of TAC for Measuring the Stability of Oil

The analytical approach for determining the TAC of oils and oil extracts has recently been applied to the study of the effect of heating and frying (Espin *et al.*, 2000; Pellegrini *et al.*, 2001; Quiles *et al.*, 2002a,b), as well as the influence of different storage conditions (Brighenti *et al.*, 1999; Keceli and Gordon, 2001).

The decrease in quality of cooking oils and fats during frying and heating has been correlated with chemical and physical changes of the oil and fat constituents (Andrikopoulos *et al.*, 2002). All chemical changes of fats and oils at elevated temperatures originate from hydrolysis, oxidation, isomerization, polymerization or cyclization reactions. All these reactions may be promoted by oxygen, moisture, traces of metal and free radicals (Fedeli, 1988). These processes may reduce the amount of antioxidants in the oil, decrease its stability and produce new compounds which are responsible for loss of nutritional value and quality of the oil. There is agreement among authors that the heating of oils produces a decrease of their TAC (Espin *et al.*, 2000; Pellegrini *et al.*, 2001; Quiles *et al.*, 2002a), although the behaviour of different edible oils is different. Among edible oils, EVOO possesses the highest resistance compared with olive and sunflower oils to heating, measured as TAC, and this is mainly due to its content of phenolics and tocopherols (Quiles *et al.*, 2002a,b). However, to date, it has not been completely elucidated which antioxidant is the main contributor to oil stability during heating. Espin *et al.* (2000) showed that the lipophilic fraction of olive oil possessed higher resistance to heating at 180°C than the polar one; as stated by the authors, this behaviour could be explained by the relatively high resistance of tocopherols to temperature. Conversely, when refined olive oils, containing a fixed amount of α-tocopherol and increasing amounts of phenolics extracted from EVOO, underwent heating at different temperatures and for different lengths of time, a sparing effect of phenolics on α-tocopherol content was evident (Pellegrini *et al.*, 2001). In fact, the residual α-tocopherol content in oil was significantly related to phenol content ($F = 24.86$; $p < 0.0001$) and heating time ($F = 4.32$; $p = 0.015$), but not with heating temperature ($F = 1.32$; $p = 0.29$) (Fig. 3.1). Similarly, the residual TAC was significantly related with phenol content ($F = 533.16$; $p < 0.0001$) and heating time ($F = 5.16$; $p = 0.007$), but not with heating temperature ($F = 1.82$; $p = 0.174$) (Fig. 3.2). The sparing effect of phenolics on the content of α-tocopherol during the frying process was also confirmed by Quiles *et al.* (2002b).

The authors concluded that the frying process (i.e., four cycles of 15 min at 180°C with 2-h rest periods between each frying cycle) of EVOO and sun-

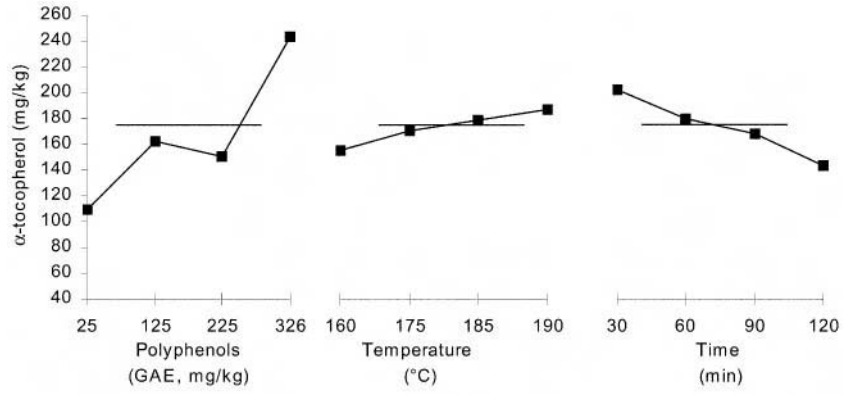


Fig. 3.1. Changes in α-tocopherol content of experimental oils according to the level of the factors. Adapted from Pellegrini *et al.* (2001). Statistical analysis: effect of phenol content ($F = 24.86$; $p < 0.0001$), heating temperature ($F = 1.32$; $p = 0.29$) and heating time ($F = 4.32$; $p = 0.015$) on α-tocopherol content. Continuous line represents the average α-tocopherol content.

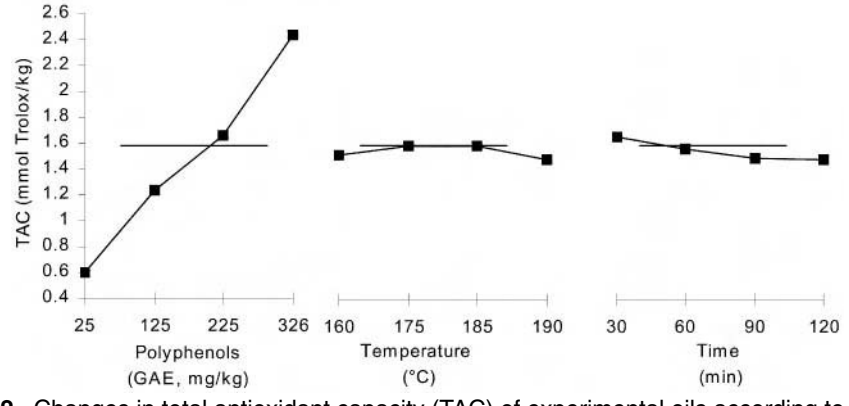


Fig. 3.2. Changes in total antioxidant capacity (TAC) of experimental oils according to the level of the factors. Adapted from Pellegrini *et al.* (2001). Statistical analysis: effect of phenol content ($F = 533.16$; $p < 0.0001$), heating temperature ($F = 1.82$; $p = 0.174$) and heating time ($F = 5.16$; $p = 0.007$) on the total antioxidant capacity (TAC). Continuous line represents the average TAC.

flower oils affected the antioxidant content of both oils, but in a different way. Thus, although both oils lost tocopherol after frying, the loss from sunflower oil started at 15 min and from EVOO after 45 min of frying. This difference, together with the fact that sunflower oil lost, in absolute terms, a greater amount of α-tocopherol, suggests that phenolic compounds contribute to the stability of EVOO. In addition, the same authors demonstrated, during a similar frying process of an EVOO, a smaller decrease in α-tocopherol with respect to phenolic compounds (Battino *et al.*, 2002).

Among phenolic compounds present in EVOO, dihydrophenolics, such as hydroxytyrosol and its sercoiridoid derivates, are the most efficient as antioxidants; in fact, their concentration decreased rapidly with the number of frying

operations (Gomez-Alonso *et al.*, 2003). By the end of the first process (10 min at 180°C), the above mentioned components had decreased relative to their original concentration by 40–50%, and after six frying operations less than 10% of the original content of these components remained. The observed trend is consistent with the high antioxidant activity of hydroxytyrosol. On the contrary, another important family of compounds within the phenolic fraction is tyrosol and its sercoiridoid derivates, which showed a completely different response to the 12 frying processes studied. Actually, the reduction was much smaller than that observed for the dihydrophenol family and presented an almost linear relationship with the number of frying operations.

The influence of different storage conditions on the TAC of EVOO has also been studied (Brighenti *et al.*, 1999; Lavelli, 2002). Lavelli (2002) demonstrated that EVOOs, stored in clear glass bottles at 30°C for 30 days were less efficient as scavengers of the DPPH radical and as inhibitors of the xanthine oxidase-catalysed reaction, which generates superoxide radical and hydrogen peroxide, than newly produced EVOOs. The influence of storage conditions on olive oil TAC was reported also in samples of EVOO stored for 5 weeks in transparent-glass volumetric flasks at room temperature under different conditions (N_2 or O_2 atmosphere, dark or daylight) (Brighenti *et al.*, 1999). The auto-oxidation process was followed by evaluating the total antioxidant capacity, total phenol and vitamin E content at regular intervals. In all samples, the loss of vitamin E content was higher than that of total phenol content, especially in the presence of daylight and O_2, demonstrating in this case no apparent effect of total phenolics in sparing vitamin E from degradation during storage in unfavourable conditions (Table 3.2). Conversely, Keceli and Gordon (2001) showed that methanolic extracts from olive oil were significantly more effective than α-tocopherol in stabilizing stripped olive oil during 6 days of storage at 60°C. Furthermore, when ferric chloride was added to stripped olive oil, the phenolics continued to act as antioxidants despite their ability to reduce metal ions, which in the case of Fe^{3+} could lead to the more active pro-oxidant Fe^{2+}.

Table 3.2. Effect of storage at room temperature in different conditions (N_2 or O_2 atmosphere, dark or daylight) on extra virgin olive oil. Adapted from Brighenti *et al.* (1999).

Time (week)	TAC[a] (mmol Trolox/kg)				Vitamin E (mg/kg)				Phenolics (GAE,[b] mg/kg)			
	O_2		N_2		O_2		N_2		O_2		N_2	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
0	1.62	1.62	1.62	1.62	197	197	197	197	215	215	215	215
1	1.45	1.61	1.56	1.61	75	194	77	196	186	213	202	210
2	1.24	1.58	1.48	1.61	24	187	62	191	163	202	185	196
4	1.29	1.67	1.53	1.61	–	180	38	193	144	199	196	216
5	1.26	1.64	1.38	1.61	–	190	27	197	158	210	182	209

[a] Total antioxidant capacity; [b] values are expressed as mg of gallic acid equivalents per kg of oils.

5. Conclusions

In conclusion, the measuring of oil TAC seems to be a suitable approach to effectively evaluate the nutritional quality of oils with different compositions, since the loss of organoleptic quality in oils is secondary to the loss of their antioxidant capacity. Moreover, this parameter could be introduced beside the oxidation and hydrolysis indices already indicated by the EU Regulation to further differentiate among different quality EVOOs. Using this approach, olive oil, and particularly its highest quality (i.e., EVOO), shows high TAC values mainly due to its antioxidant content. Moreover, since this parameter is affected by heating and storage conditions, it could be useful to predict oil shelf life and behaviour during industrial and domestic processes. However, due to the huge quantity of assays developed for measuring the TAC, it would be advisable to further standardize these methods.

Acknowledgements

This work was supported in part by Marche Polytechnic University and A.M.P.O., Ancona. The helpful assistance of M. Glebocki in editing the manuscript is gratefully acknowledged.

6. References

Andrikopoulos, N.K., Hassapidou, M.N. and Manoukas, A.G. (1989) The tocopherol content of Greek olive oils. *Journal of the Science of Food and Agriculture* 46, 503–509.

Andrikopoulos, N.K., Dedoussis, G.V.Z., Falirea, A., Kalogeropoulos, N. and Hatzinikola, H.S. (2002) Deterioration of natural antioxidant species of vegetable edible oils during the domestic deep-frying and pan-frying of potatoes. *International Journal of Food Sciences and Nutrition* 53, 351–363.

Battino, M., Bullon, P., Wilson, M. and Newman, H. (1999) Oxidative injury and inflammatory periodontal diseases: the challenge of anti-oxidants to free radicals and reactive oxygen species. *Critical Reviews in Oral Biology & Medicine* 10, 458–476.

Battino, M., Quiles, J.L., Huertas, J.R., Ramirez-Tortosa, M.C., Cassinello, M., Manas, M., Lopez-Frias, M. and Mataix, J. (2002) Feeding fried oil changes antioxidant and fatty acid pattern of rat and affects rat liver mitochondrial respiratory chain components. *Journal of Bioenergetics and Biomembranes* 34, 127–134.

Benzie, I.F.F. and Strain, J.J. (1996) The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Analytical Biochemistry* 239, 70–76.

Boskou, D. (1996) Olive oil composition. In: Boskou, D. (ed.) *Olive oil: chemistry and technology.* AOCS Press, Champaign, Illinois, pp. 52–83.

Boskou, D. (1999) Non-nutrient antioxidants and stability of frying oils. In: Boskou, D. and Elmadfa, I. (eds) *Frying of Food.* Technomic Publishing Co, Lancaster, UK, pp. 183–204.

Brighenti, F., Cammarata, T. and Pellegrini, N. (1999) Direct evaluation of antioxidant activity of extra-virgin olive oil. In: Lasztity, R., Pfannhauser, W., Simon-Sarkadi, L. and Tomoskozi, S. (eds) *Functional Foods – A NewCchallenge for the Food Chemist*. Publishing Company of TUB, Budapest, pp. 464–470.

Cabrini, L., Barzanti, V., Cipollone, M., Fiorentini, D., Grossi, G., Tolomelli, B., Zambonin, L. and Landi, L. (2001) Antioxidants and total peroxyl radical-trapping ability of olive and seed oils. *Journal of Agricultural and Food Chemistry* 49, 6026–6032.

Cao, G., Alessio, H.M. and Cutler, R.G. (1993) Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology & Medicine* 14, 303–311.

Cohen, J.H., Kristal, A.R. and Stanford, J.L. (2000) Fruit and vegetable intakes and prostate cancer risk. *Journal of National Cancer Institute* 92, 61–68.

Cook, N.C. and Samman, S. (1996) Flavonoids – Chemistry, metabolism, cardioprotective effects, and dietary sources. *The Journal of Nutritional Biochemistry* 7, 66–76.

Espin, J.C., Soler-Rivas, C. and Wichers, H.J. (2000) Characterization of the total free radical scavenger capacity of vegetable oils and oil fraction using 2,2-diphenyl-1-picrylhydrazyl radical. *Journal of Agricultural and Food Chemistry* 48, 648–656.

Fedeli, E. (1988) The behaviour of olive oil during cooking and frying. In: Varela, G., Bender, A.E. and Morton, I.D. (eds) *Frying of Food, Principles, Changes, New Approaches*. Ellis Horwood Press, Chichester, England, pp. 52–81.

Finotti, E., Paoletti, F., Bertone, A., Galassi, P. and Quaglia, G. (1998) Antioxidant capacity determination of extra virgin olive oils unsaponifiable fraction by crocin bleaching inhibition method. *Nahrung* 42, 324–325.

Ghiselli, A., Serafini, M., Maiani, G., Azzini, E. and Ferro-Luzzi, A. (1995) A fluorescence based method for measuring total plasma antioxidant capability. *Free Radical Biology & Medicine* 18, 29–36.

Gomez-Alonso, S., Fregapane, G., Desamparados, S. and Gordon, M.H. (2003) Changes in phenolic composition and antioxidant activity of virgin olive oil during frying. *Journal of Agricultural and Food Chemistry* 51, 667–672.

Gorinstein, S., Martin-Belloso, O., Katrich, E., Lojek, A., Ciz, M., Gligelmo-Miguel, N., Haruenkit, R., Park, Y-S., Jung, S-T. and Trakhtenberg, S. (2003) Comparison of the contents of the main biochemical compounds and the antioxidant activity of some Spanish olive oils as determined by four different radical scavenging tests. *The Journal of Nutritional Biochemistry* 14, 154–159.

Halliwell, B. and Gutteridge, J.M.C. (1999) *Free Radicals in Biology and Medicine*, 3rd edn. Clarendon Press, Oxford, England.

Heinonen, O.P., Albanes, D., Virtamo, J., Taylor, P.R., Huttunen, J.K., Hartman, A.M., Haapakoski, J., Malila, N., Rautalahti, M., Ripatti, S., Maenpaa, H., Teerenhovi, L., Koss, L., Virolainen, M. and Edwards, B.K. (1998) Prostate cancer and supplementation with alpha-tocopherol and beta-carotene: incidence and mortality in a controlled trial. *Journal of National Cancer Institute* 6, 440–446.

Hennekens, C.H., Buring, J.E., Manson, J.E., Stampfer, M., Rosner, B., Cook, N.R., Belanger, C., LaMotte, F., Gaziano, J.M., Ridker, P.M., Willett, W. and Peto, R. (1996) Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *The New England Journal of Medicine* 2, 1145–1149.

Keceli, T. and Gordon, M.H. (2001) The antioxidant activity and stability of the phenolic fraction of green olives and extra virgin olive oil. *Journal of the Science of Food and Agriculture* 81, 1391–1396.

La Vecchia, C., Altieri, A. and Tavani, A. (2001) Vegetables, fruit, antioxidants and cancer: a review of Italian studies. *European Journal of Nutrition* 40, 261–267.

Lavelli, V. (2002) Comparison of the antioxidant activities of extra virgin olive oils. *Journal of Agricultural and Food Chemistry* 50, 7704–7708.

Mannino, S., Cosio, M.S. and Bertuccioli, M. (1993) High performance liquid chromatography of phenolic compounds in virgin olive oils using amperometric detection. *Italian Journal of Food Science* 4, 363–370.

Mannino, S., Buratti, S., Cosio, M.S. and Pellegrini, N. (1999) Evaluation of the 'antioxidant power' of olive oils based on a FIA system with amperometric detection. *The Analyst* 124, 1115–1118.

Montedoro, G., Servili, M., Baldioli, M. and Miniati, E. (1992) Simple and hydrolyzable phenolic compounds in virgin olive oil. 1. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. *Journal of Agricultural and Food Chemistry* 40, 1571–1576.

Mosca, L., De Marco, C., Visioli, F. and Cannella, C. (2000) Enzymatic assay for the determination of olive oil phenol content: assay conditions and validation of the method. *Journal of Agricultural and Food Chemistry* 48, 297–301.

Ninfali, P., Aluigi, G., Bacchiocca, M. and Magnani, M. (2001) Antioxidant capacity of extra-virgin olive oils. *Journal of the American Oil Chemists' Society* 78, 243–247.

Omenn, G.S., Goodman, G.E., Thornquist, M.D., Balmes, J., Cullen, M.R., Glass, A., Keogh, J.P., Meyskens, F.L., Jr., Valanis, B., Williams, J.H., Jr., Barnhart, S., Cherniack, M.G., Brodkin, C.A. and Hammar, S. (1996) Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. *Journal of National Cancer Institute* 88, 1550–1559.

Papadopoulos, K., Triantis, T., Yannakopoulou, E., Nikokavoura, A. and Dimotikali, D. (2003) Comparative studies on the antioxidant activity of aqueous extracts of olive oils and seed oils using chemiluminescence. *Analytica Chimica Acta* 494, 41–47.

Pellegrini, N., Re, R., Yang, M. and Rice-Evans, C.A. (1999) Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying the 2,2′-azobis(3-ethylenebenzothiazoline-6-sulfonic) acid radical cation decolorization assay. *Methods in Enzymology* 299, 379–389.

Pellegrini, N., Visioli, F., Buratti, S. and Brighenti, F. (2001) Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. *Journal of Agricultural and Food Chemistry* 49, 2532–2538.

Pellegrini, N., Del Rio, D., Colombi, B., Bianchi, M. and Brighenti, F. (2003a) Application of the 2,2′-azobis(3-ethylenebenzothiazoline-6-sulfonic acid) radical cation assay to a flow injection system for the evaluation of antioxidant activity of some pure compounds and beverages. *Journal of Agricultural and Food Chemistry* 51, 260–264.

Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M. and Brighenti, F. (2003b) Total antioxidant capacity of plant foods, beverages, and oils consumed in Italy assessed by three different in vitro assays. *Journal of Nutrition* 133, 2812–2819.

Quiles, J.L., Huertas, J.R., Mañas, M., Battino, M., Cassinello, M., Littarru, G.P., Lenaz, G. and Mataix, F.J. (1994) Peroxidative extent and coenzyme Q levels in the rat: influence of physical training and dietary fats. *Molecular Aspects of Medicine* 15, s89–s95.

Quiles, J.L., Huertas, J.R., Mañas, M., Ochoa, J.J., Battino, M. and Mataix, J. (1999). Oxidative stress induced by exercise and dietary fat modulates the coenzyme Q and vitamin A balance between plasma and mitochondria. *International Journal of Vitamin and Nutrition Research* 69, 243–249.

Quiles, J.L., Ramirez-Tortosa, M.C., Gomez, J.A., Huertas, J.R. and Mataix, J. (2002a) Role of vitamin E and phenolic compounds in the antioxidant capacity, measured by ESR, of virgin olive, olive and sunflower oils after frying. *Food Chemistry* 76, 461–468.

Quiles, J.L., Huertas, J.R., Battino, M., Ramirez-Tortosa, M.C., Cassinello, M., Mataix, J., Lopez-Frias, M. and Manas, M. (2002b) The intake of fried virgin olive oil or sunflower oils differentially induces oxidative stress in rat liver microsomes. *British Journal of Nutrition* 88, 57–65.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C.A. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine* 26, 1231–1237.

Regulation 2568/91 (1991, July 11) *Official Journal of the European Community*, 1–83.

Stephens, N.G., Parsons, A., Schofield, P.M., Kelly, F., Cheeseman, K. and Mitchinson, M.J. (1996) Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet* 347, 781–786.

Terry, P., Terry, J.B. and Wolk, A. (2001) Fruit and vegetable consumption in the prevention of cancer: an update. *Journal of Internal Medicine* 250, 280–290.

Tubaro, F., Micossi, E. and Ursini, F. (1996) The antioxidant capacity of complex mixture by kinetic analysis of crocin bleaching inhibition. *Journal of the American Oil Chemists' Society* 73(2), 173–179.

Visioli, F. and Galli, C. (1995) Natural antioxidants and prevention of coronary heart disease: the potential role of olive oil and its minor constituents. *Nutrition Metabolism and Cardiovascular Diseases* 5, 306–314.

4 Effect of Frying and Thermal Oxidation on Olive Oil and Food Quality

FRANCISCO J. SÁNCHEZ-MUNIZ AND SARA BASTIDA

Departamento de Nutrición, Facultad de Farmacia, Universidad Complutense de Madrid, Spain

1. Introduction

Frying is a very old method of cooking food. According to oral tradition, the frying technique was discovered more than 6000 years ago and extensively used as early as 1600 BC by the ancient Egyptians. Cultivation of olive trees first began in Mesopotamia around 4000 BC and subsequently spread throughout the Mediterranean, reaching Crete and Egypt by 2000 BC, Spain by 1500 BC and the Italian peninsula by 500 BC References to frying date from the earliest period in which the use of pots and pans is recorded. According to Banks (1996) *sic*:

> The Latin and Greek words for frying stem from those used for roasting, suggesting that frying may have developed from roasting. The early Egyptians roasted food in pots made of soapstone. Roasting in a pot that holds heat for an extended period of time is only a short step away from frying, particularly if the pot should be left unattended. Fat and cooking juice would be rendered from the meat, the moisture would boil away, and the result would be pan-fried meat. Frying may very well have been discovered by roasting a fatty piece of meat in a soapstone pot over a hot fire.

The Royal Academy of the Spanish Language (Real Academia Española, 1992) defines the verb 'to fry' as: 'to succeed in making a raw food edible by keeping it in boiling oil or fat for a sufficient period of time.' Thus, the basic process for deep-fat frying recorded by the Romans remained essentially unchanged until recent times. Deep-fat frying was traditionally performed in a kettle of oil heated on a stove or over an open fire. Individual servings or small batches of food were immersed in hot oil until, from experience, the cook determined that the food was ready to eat.

Frying is now extensively used throughout the world and new systems have been developed to increase economic benefits and production efficiency. In 1929, the J.D. Ferry Company (Banks, 1996) introduced the first technological advance in frying: continuous potato chip cookers, which increased production, reduced

 Olive Oil and Health (eds J.L. Quiles, M.C. Ramírez-Tortosa and P. Yaqoob)

cost and improved finished product quality. The development of both industrial and food service frying equipment led to an increase of fried foods in the American diet. Growth of the market was spurred by customer demand. At present, frying should be considered a select technology associated with very sophisticated devices that continuously produce large amounts of processed food. However, the quality of the fried food depends to a large extent on frying conditions, for which reason some authors believe that frying should be considered an art (Blumenthal, 1991; Monferrer and Villalta, 1993).

Moreover, although the frying process seems rather simple – dehydration accompanied by entry of hot oil into the food and superficial browning (Gupta, 1993), the study of this culinary technique from a scientific point of view is rather complex and requires multidisciplinary collaboration. Frying is not merely a form of cooking; it reflects a lifestyle and dietary customs strongly rooted in the use of olive oil and influenced by climate and various socio-cultural factors (Bastida and Sánchez-Muniz, 2001).

2. Frying Methods

Two principal frying techniques exist: shallow and deep-fat frying. Shallow frying is performed in relatively flat pots or pans containing little oil in which the product is not completely immersed. The food in direct contact with the hot oil is fried. In deep-fat frying all the food is immersed in oil and the cooking process takes place throughout the entire product simultaneously. This kind of frying is performed in domestic and industrial fryers and in deep pans containing large amounts of oil (Monferrer and Villalta, 1993). According to Bognár (1998), oil uptake in meat and fish fried using the deep-fat frying method is lower than that which occurs in the superficial modality.

Frying can be done in a continuous or discontinuous manner, depending on whether or not the oil is left to cool (sometimes to room temperature) between fryings or sets of fryings. Jorge *et al.* (1996) report that a laboratory model of continuous potato frying displayed a lower degree of oil alteration than did a discontinuous one. Frying can also be classified as being characterized by frequent, slow or null replenishment of fresh oil. As will be discussed in this chapter, frequent addition of fresh oil minimizes oil alteration (Cuesta and Sánchez-Muniz, 1998). The many factors that influence the quality and alteration of frying oil are summarized in Fig. 4.1.

3. Changes that Occur During Heating and Frying

Frying modifies the physicochemical and organoleptic properties of foods (Varela, 1988), producing, for example, the crispy texture and rich flavours and aromas that make foods a pleasure to eat.

Thermal alteration is also responsible for the formation of cyclic compounds such as triacylglycerol monomers showing intermolecular cyclization or triacylglycerol monomers showing intramolecular cyclization.

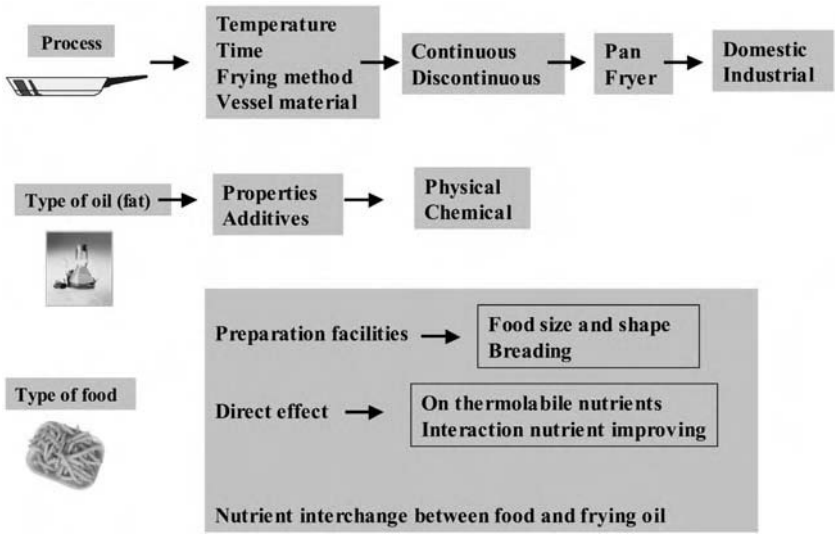


Fig. 4.1. Factors influencing the alteration of frying fats.

Three main groups of reactions take place during frying: hydrolysis, oxidation and polymerisation (Cuesta *et al.*, 1993; Sánchez-Muniz *et al.*, 1993b; Dobarganes *et al.*, 1999; Cuesta and Sánchez-Muniz, 2001) (Fig. 4.2). The main causes of culinary fat alteration and the compounds produced in the three main alteration pathways are summarized in Table 4.1

It is well known that fat undergoes autoxidation, which, according to Porter *et al.* (1995), is a free radical chain process consisting of three steps: initiation, propagation and termination (Fig. 4.3).

3.1. Initiation

The formation of a lipid radical ($R^{\bullet}$) from an unsaturated fatty acid (RH) is the key event in the initiation step. This can occur by thermal or photochemical homolytic cleavage of an RH double bond or by hydrogen atom abstraction from RH by a free radical initiator.

3.2. Propagation

Propagation normally begins with the addition of molecular oxygen to $R^{\bullet}$ but the rate-limiting reaction consists of abstraction of a hydrogen atom from RH by peroxyl radical ($ROO^{\bullet}$) to form hydroperoxides (ROOH) and another radical $R^{\bullet}$ (Sánchez-Muniz and Sánchez-Montero, 1999). Propagation observed in oxidation (Fig. 4.3) may be more complicated than the simple transfer and addition (Porter *et al.*, 1995). The primary products formed from the peroxidation of lipids generally include oxygen coupling to radical, hydrogen atom or group transfer from substrate to the chain carrying the peroxyl radical, fragmentation

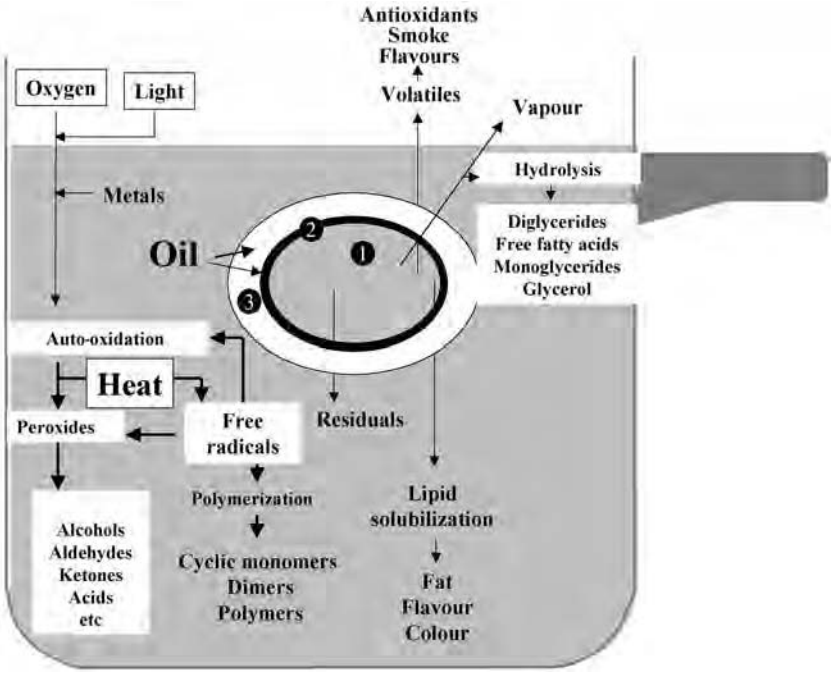


Fig. 4.2. The three major frying pathways are summarized. (1) Interior of food. Cooking is performed at 100°C due to vapour production. (2) Crust. The fat content increases and the water content decreases. A well-defined crust prevents dehydration and excessive penetration of grease in food. (3) Surface. Total dehydration occurs. Food becomes crispy and with typical odours. Browning and caramelizing take place.

Table 4.1. Main causes of alteration compound formation in culinary fats.

Type of alteration	Causal agent	Resultant compounds
Oxidative alteration	Air	Oxidized monomers Oxidized dimers and polymers Short fatty acids Volatiles (hydrocarbons, aldehydes, ketones, alcohols, acids)
Thermal alteration	Temperature	Cyclic monomers Dimers and polymers Geometric isomers
Hydrolytic alteration	Moisture	Free fatty acids Monoglycerides Diglycerides Glycerol

of the chain containing the peroxyl radical to supply oxygen and a lipid radical, rearrangement of the chain containing the peroxyl radical, and cyclization of the peroxyl radicals, essentially by intramolecular radical addition (Frankel, 1991; Sánchez-Muniz and Sánchez-Montero, 1999).

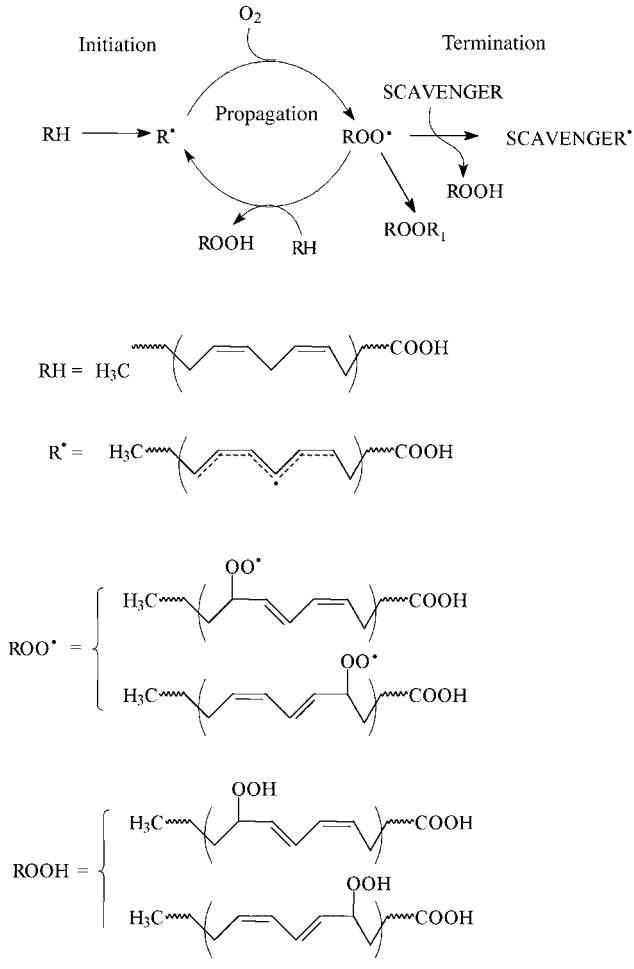


Fig. 4.3. Major steps in oxidation. RH, a lipid containing a polyunsaturated fatty acid (e.g., linoleic acid); R•, carbon-centred lipid radical; ROO•, lipid peroxyl radical; SCAVENGER•, peroxide radical scavenger. Adapted from Sánchez-Muniz and Sánchez-Montero (1999).

Reaction product mixtures of autoxidized lipids are usually very complicated because many new compounds can be formed as a result of these few important propagation reactions. In addition, the oxidation products, hydroperoxides and cyclic peroxides are themselves very reactive species, and secondary products can be formed due to the decomposition of these unstable primary oxidation products. These secondary compounds contain oxygenated groups such as hydroxy, hydroperoxy, keto and epoxy (Márquez-Ruiz and Dobarganes, 1996) as well as products of decomposition including hydrocarbons, aldehydes and ketones (Frankel, 1991).

On the other hand, free radicals assure the 'vicious circle' of autooxidation contributing to cycling and polymerizing reactions (Figs 4.2 and 4.3).

The heating of oil (in the absence of food) increases the speed of autoxidation (Nawar, 1984). Thus, when the temperature of the reaction medium is rela-

tively low (below 100°C), the formation rate of hydroperoxides is higher than that of their decomposition. Therefore, the compounds formed are mainly monomers of triacylglycerols with one or more acyl chains at any possible stage of oxidation. All these compounds are classified as oxidized triacylglycerol monomers.

At higher temperatures, all autooxidation reactions speed up, and the amount of altered compounds formed depends on the heating time applied to the oil or fat. At temperatures close to 200°C, decomposition and other reactions of hydroperoxides are faster than their formation. In this case, the major compounds generated are dimers and polymers of triacylglycerols (Nawar, 1984) (Figs 4.2 and 4.4). Thermal alteration is also responsible for the formation of cyclic compounds such as triacylglycerol monomers showing intermolecular cyclization (Fig. 4.4) or triacylglycerol monomers showing intramolecular cyclization (Fig. 4.5).

Frying clearly differs from heating because the presence of food in the oil bath makes the situation rather more complex. In frying, autooxidation is also accelerated due to the relatively high temperature of the process. Moisture from foods induces hydrolytic alteration of oxidized and non-oxidized triacylglycerols, generating diacylglycerols, monoacylglycerols and free fatty acids that are commonly classified as hydrolytic products (Table 4.1 and Fig. 4.2). Moreover, the presence of some compounds from the foods (e.g. antioxidants such as phenolic compounds and vitamins and prooxidants such as transition metals), as well as light, can substantially modify the thermal oxidation reactions due to the heat process itself (Fig. 4.2).

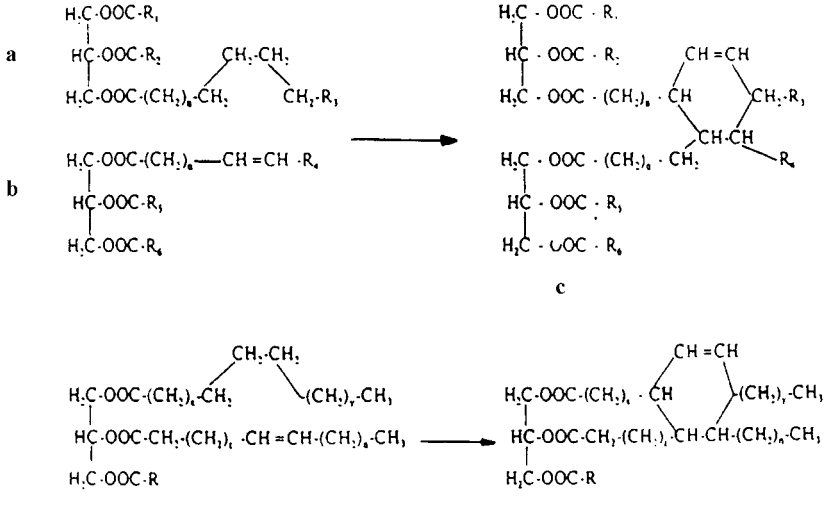


Fig. 4.4. Formation of triglyceride dimers.

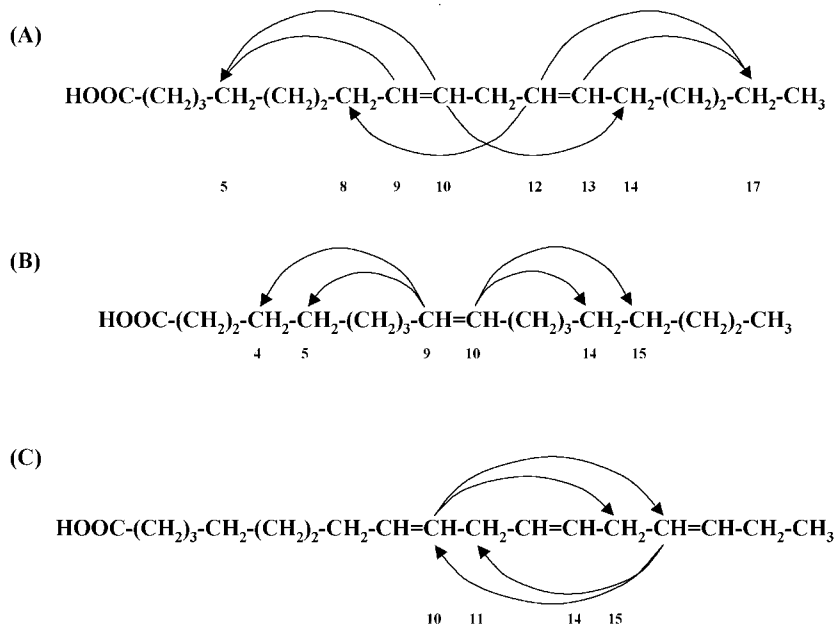


Fig. 4.5. Cyclization of (A) linoleic acid, (B) oleic acid, and (C) linolenic acid. Rows indicate the possible losses of two hydrogens and formation of new bonds.

All these thermally oxidized and hydrolytic compounds are present in different quantities in overused fat as well as in moderately thermally oxidized oils and non-used oils. As part of the fat source of our diet, they are subject to different physiological processes such as digestion, absorption, metabolism and excretion.

3.3. Termination

In the termination phase, the peroxidation chain reaction ends when peroxyl radicals combine with a radical scavenger such as vitamin E (Porter *et al.*, 1995).

4. Frying Changes Assessment – Present Legislation

Consumption of relatively high amounts of altered oil can be harmful to health (López-Varela *et al.*, 1995; Márquez-Ruiz and Dobarganes, 1996). The debate on deep-fat frying is primarily focused on the point at which any oil used for frying should be discarded. Polar material and polymer content determinations constitute the basis of the pertinent legislation in some European countries (Firestone, 1996; Sánchez-Muniz and Bastida, 2003). Polar material is determined by silica column chromatography using a mix of hexane/diethyl ether for fresh and used oils (Waltking and Wessels, 1981; Ministerio de Relaciones con las Cortes y de Secretaría del Gobierno, 1989; Arroyo *et al.*, 1992) (Fig. 4.6a). Triacylglycerol

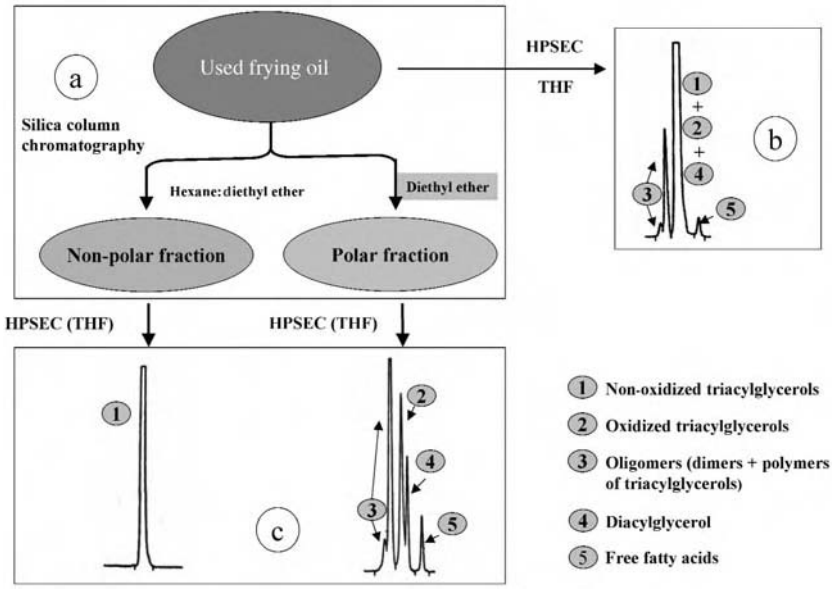


Fig. 4.6. Analytical procedure for determination of altered oxidized and polymerized compounds. (a) Separation of non-polar and polar fractions from used frying oils by silica gel chromatography (SGC); (b) direct high performance size exclusion chromatography (HPSEC) of used frying oils: unaltered and oxidized triacylglycerols and diacylglycerols elute together; (c) combination of SGC and HPSEC permits identification of compounds in the non-polar and polar fractions, and the overlapping of non-altered triacylglycerols with oxidised triacylglycerols and diacylglycerols. Modified from Dobarganes *et al.* (1999) and Sánchez-Muniz and Bastida (2003).

polymers can be directly determined using high performance size exclusion chromatography (HPSEC) (IUPAC, 1992) (Fig. 4.6b), but the HPSEC analysis of polar fractions previously obtained by column chromatography (Fig. 4.6c) is preferable, due to the methodological limitations in samples containing less than 3% in polymers. Moreover, this combination of column chromatography and HPSEC permits the separation and quantification of non-oxidized triacylglycerols, oxidized triacylglycerols and diacylglycerols (Fig. 4.6c). Although polar material determination has been recommended due to its simplicity and accuracy, some compounds considered polar material (e.g. diacylglycerols, free fatty acids) are not necessarily altered (Bastida and Sánchez-Muniz, 2002). For this reason, polymer determination offers more precise information about the alteration of the oil, and therefore of its potential toxicity to the consumer, than polar material determination.

Many European countries have established legislation making it obligatory to discard a frying oil when its altered part (polar material: thermally oxidized plus hydrolysed compounds) surpasses 25% of total oil mass (Firestone, 1996; Sánchez-Muniz and Bastida, 2003). Other countries have selected the level of 10% polymers as a cut-off point, after which oil must be discarded (Firestone, 1996; Sánchez-Muniz and Bastida, 2003). Our group (Sánchez-Muniz and Bastida, 2003) found that various oils with the critical level of 25% in polar material

displayed very different polymer levels (Fig. 4.7). For instance, the 25 % polar material content in palm olein corresponds with a polymer level of about 10%, while the same polar material level in sunflower oil corresponds with a 15% polymer content. Thus, taking into account the increased use of polyunsaturated oils for frying purposes, we suggest that oil should be discarded when it contains a polymer content of 10% or, in any case, a total polar material content of 20%, rather than the 25% polar material limit presently permitted. The need to unify the criteria of different countries with regard to when to discard frying oil is evident.

Nonetheless, alterations in oil can be minimized with adequate control of the frying variables, and frying should be considered a suitable and healthy food-processing technique. The numerous advantages and potential disadvantages of this culinary procedure will be described in the following section and a summary is found in Table 4.2.

5. Advantages of Frying

5.1. Very common culinary procedure

As previously commented, fried foods are extensively consumed throughout the world. The Mediterranean diet relies heavily on frying and eating habits can be modified by changing the frying oil used and the foodstuffs to be fried. Recommendations to decrease the consumption of fried foods could change dietary habits, and specifically the energy and nutrient intakes, of persons accustomed to eating these foods, for which reason such advice would not be followed in many cases. The truth is that the gastronomy and economy of such communities are very sensitive to changes in the production and consumption of fried foods (Bastida and Sánchez-Muniz, 2001).

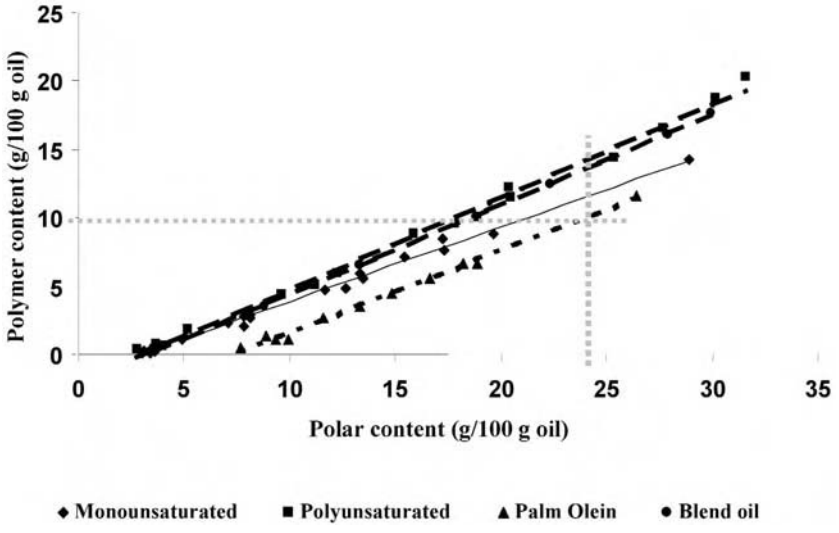


Fig. 4.7. Relationship between the polar and polymer contents of different oils. Modified from Sánchez-Muniz and Bastida (2003).

Table 4.2. Advantages and disadvantages of frying.

Advantages
* The most common culinary process in the Mediterranean countries. Cultural importance. * Little cooking time required. * Improves food palatability (texture, taste, flavour). * Improves food composition. Food is enriched in fat-soluble vitamins and bioactive compounds from oils (e.g. carotenoids, tocopherols). It can produce more balanced and cardio-healthy foods with regard to their fatty acid composition. * No greater damage to food quality than that produced by other culinary techniques.
Disadvantages
* Often related to poor frying performance (e.g. null or low addition of fresh oil during a large number of frying operations). * Decreased palatability related to poor quality fats and inadequate frying conditions. * Energy enrichment of food, not recommended in overweight and obese individuals. * Changes in food composition. Liberation of important fatty acids, such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, from foodstuffs into the fryer media. Losses by lixiviation of hydrosoluble compounds. Possible nutritional value losses (essential fatty acids, antioxidants, thermal-sensible vitamins). * Food adsorption of alteration compounds from frying fats (oxidized and polymerized triacylglycerols) could induce toxicity. However, a proper frying technique does not give rise to a concentration of altered compounds above the level established by the present legislation (>25% polar material).

5.2. Reduced cooking time

Normally less than 10 minutes are needed to fry any foodstuff. In recent studies, our group found that the time needed to fry various kinds of foods (mostly frozen pre-fried foods (Cuesta and Sánchez-Muniz, 2001; Bastida *et al.*, 2003), under domestic conditions was between 2 and 8 minutes, much less than that required by other culinary techniques.

Time-saving cooking methods are very important in fast-paced modern societies in which individuals want to have tasty foods available in the shortest time possible. For this reason, interest in this technique has greatly increased worldwide.

5.3. Increased palatability

Frying notably increases food palatability. The term 'palatability' refers to the quality of being agreeable to the palate and is associated with several sensations produced by the interaction of food with the mouth, tongue and palate receptors. Fat, sugar and salt are the palatable agents par excellence. Frying directly influences many food qualities, but the influence of frying on flavour is subtle. The flavour and aroma of fried products are a result of the auto-oxidation, decomposition and hydrolysis of amino acids, proteins and carbohydrates that take place at frying temperatures (Perkins, 1996; Nawar, 1998).

Diacylglycerols produced by hydrolysis can be further hydrolysed, giving rise to free fatty acids (Fig. 4.2). These compounds are very reactive and produce new compounds that can be fragrant. Nonetheless, a large percentage of these fatty acids are volatile and disappear with the vapour generated during frying.

Oxidation produces many primary and secondary compounds. Frying with oils rich in linoleic or linolenic acids produces more of these oxidation products than when olive oil is used. The decomposition products formed belong to the series of alkanes, alkenes, ketones, methylketones with a variable number of carbon atoms. A list of some of the compounds present in the oils used for frying and in fried potatoes is found in Table 4.3.

Some volatiles have been used to define the quality of oils and to describe their flavour. Thus acrolein has been associated with 'acrid', cooked soybean odour with 'beany', olive oil with 'fruity', hexanal with 'grassy' and raw soybean with 'green' (Perkins, 1996; Nawar, 1998). However, in the words of Nawar (1998), 'it is obvious

Table 4.3. Volatile compounds found in frying oil and fried potatoes.

Oil	French Fries
Butanal	Hexanal
Hexane	Metyl pirazine
1-Butanol	2-Hexenal
Pentanol	2-Heptanone
Heptane	Nonane
Hexanal	Heptanal
2-Hexenal	2,5-Dimetylpyrazine
2-Heptanone	2-Heptenal
Nonane	2-Pentylfuran
Heptanal	Octanal
2-Heptenal	*t*2, *t*4-Heptadienal
2-Pentylfuran	2-Octenal
Octanal	Nonanal
*t*2, *t*4-Heptadienal	2-Nonenal
2*t* 4-Heptadienal	Decanal
2-Octenal	2-Decenal
Nonanal	2, *c* 4-Decenal
2-Nonenal	2, *c* 4-Decadienal
Decanal	Undecanal
1-Decene	2, *t*4-Decadienal
3-Octanone	
2-Decenal	
2, *c* 4-Decadienal	
Undecanal	
2, *t*4-Decadienal	
2-Octen-1-ol	
2-Undecenal	
Dodecanal	

In order of elution on a non-polar capillary column.
Source: Adapted from Perkins (1996).

that a single analysis of the volatiles cannot be sufficient. Certain volatiles commonly used as key markers of oxidation may be markedly reduced by facile interaction with other components from the food. Their absence may thus be misleading.' Negative flavour contribution increases with product age but this alteration can be 'modulated' by inert gas or vacuum packaging (Banks, 1996).

A crisp outer texture, which adds immensely to eating pleasure, is a definitive characteristic of most fried foods. This crisp texture develops through rapid surface dehydration during frying. Crispiness or crunchiness can be controlled by adjusting a number of variables, including product preparation, a butter-based breadcrumb coating, frying procedures and post-frying conditions. Using simple polarographic methods, Varela *et al.* (quoted by Varela, 1988) proved that monounsaturated oils produce a more defined crust than polyunsaturated ones. Many fried product advertisements portray the delicate crispiness or the crackle and crunch of the products. The organoleptic properties of the oil deeply affect the taste and acceptance of the fried food. The taste and flavour of the oil is very important, as oil partially substitutes water in the fried food and greatly contributes to the desired odour, flavour and taste of the final product.

According to Bognár (1998), results of sensory analyses show that good to very good sensory quality can be achieved when potatoes, breaded meat, poultry and fish are deep-fried or pan-fried. Only medium quality is achieved when food is cooked in a conventional oven, even when the food surface was sprayed with fat. This was due to inconsistent surface browning and crust formation and to a less pronounced final odour and taste. According to Moreiras-Varela *et al.* (1988) and Varela (1988), fat used 20 times did not significantly influence the sensory quality of the fried potatoes. Romero *et al.* (2001) demonstrated that frozen pre-fried croquettes fried in virgin olive oil, high oleic sunflower oil and sunflower oil previously used to fry several frozen foods, received an average score of 6.8, on a scale of 1 to 10, in a sensory evaluation. No significant differences were found in croquettes fried in the different oils. However, the frequent addition of fresh oil to maintain the oil level in the fryer improved the sensory quality of the croquettes.

According to the Blumenthal curve (quoted by Quaglia and Bucarelli, 2001), fried food quality depends on the oil degradation process (Fig. 4.8), which differentiates between five stages of oil. These stages (break-in oil, fresh oil, optimum oil, degrading oil and runaway oil) suggest that oil with a certain degree of alteration performs better in frying than fresh oil. The goal of the process engineer is therefore to maintain a balance between the amounts of undamaged and altered oils in the fryer. Such a blend avoids the browning problems typical of frying with new oil. Programming partial discarding properly avoids bringing the oil to a peak of alteration and having to discard a large amount of oil (Friedman, 1991).

5.4. Changes in food composition

5.4.1. Changes in fatty acid profile

The fatty acid composition of some foods can thoroughly change during frying, becoming similar to that of the oil used (Gall *et al.*, 1983; Sebedio *et al.*, 1990; Sánchez-Muniz *et al.*, 1992a; Capita *et al.*, 2003; García-Arias *et al.*, 2003).

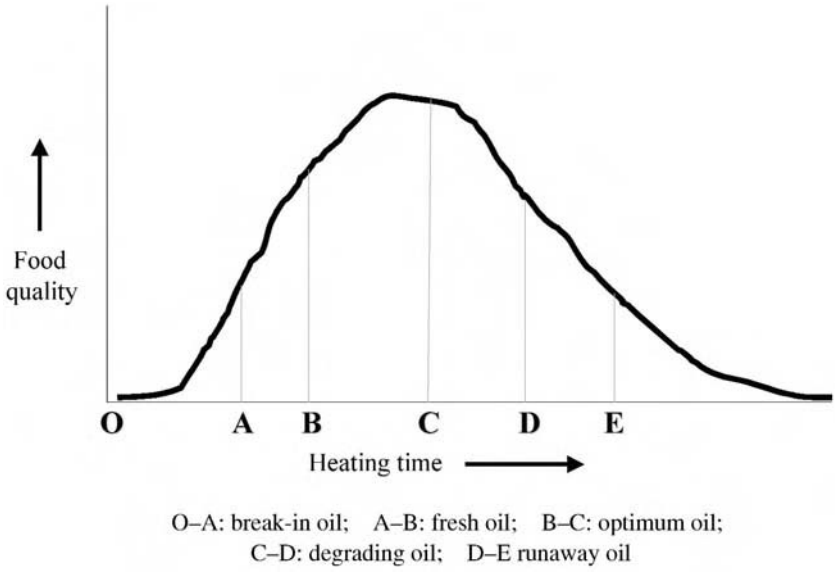


Fig. 4.8. Relationship between the frying oil alteration phases and the fried food quality.

Frying implies a mass transfer involving a partial exchange between the oil in the fryer and the water in the interior of the food (Fig. 4.2). Furthermore, minor fat-soluble compounds may exit the food during frying and the major fatty acids of the oil may enrich the fried product by means of a fatty acid-gradient concentration. The fatty acid profile of the fried food may thus reflect a more suitable final saturated:monounsaturated:polyunsaturated fatty acid ratio (Sánchez-Muniz *et al.*, 1992a; Sánchez-Muniz and Bastida, 1997; García-Arias *et al.*, 2003). To this effect, sardines fried in olive oil became greatly oleic acid-enriched, while their n-6:n-3 ratio varied moderately from that of their raw counterparts. Sardines fried in sunflower oil, on the other hand, displayed a significant increase in their linoleic acid content and their n-6:n-3 ratio varied greatly (Fig. 4.9) (Sánchez-Muniz *et al.*, 1992a). Capita *et al.* (2003), studying the changes produced by deep-fat frying in the proximate and fatty acid composition of chicken meat and chicken-based products, found that frying with olive oil and sunflower oil increased the percentage of oleic acid and linoleic acid, respectively, in chicken meat. These increases were higher in chicken burgers than in chicken sausages. According to this study, the final fat and fatty acid content of fried chicken and fried chicken-based products depends on: (i) the fat content of the raw product; (ii) the presence of an edible coating which limits the penetration of fat during frying; (iii) the composition of the oil used.

These findings are of great nutritional importance because, according to Varela and Ruiz-Roso (1998), a large percentage (more than 50–60%) of the fat consumed in Mediterranean countries is of culinary origin. Cooking performed with olive oil or another oleic acid-enriched oil enriches the diet in oleic acid, whereas frying with corn, sunflower, safflower or soybean oils produces linoleic acid-enriched diets. In the same way, culinary use of oils such as palmolein, employed extensively in the sweet roll or pre-fried food industry (Pantzaris, 1999) implies a palmitic acid-enrichment of the diet (Cuesta *et al.*, 1998).

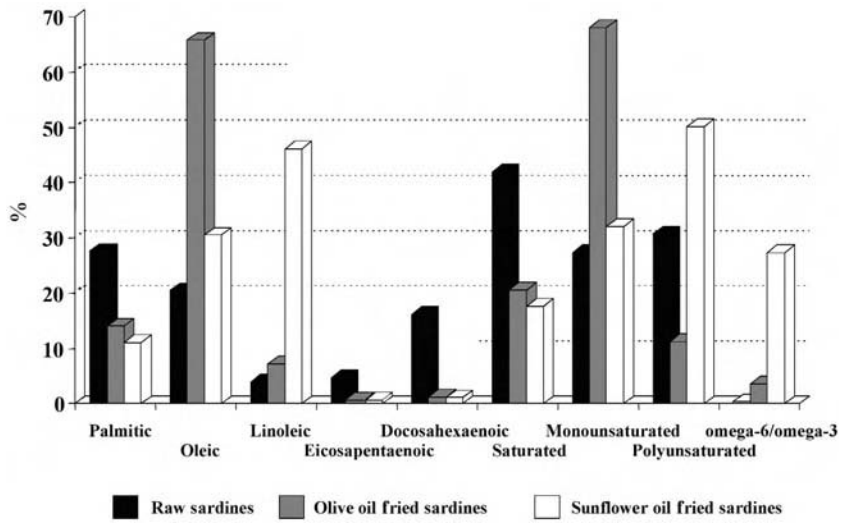


Fig. 4.9. Fatty acid composition of raw sardines and those fried in olive and sunflower oils. Adapted from Sánchez-Muniz *et al.* (1992a).

These findings are very important because lipoprotein metabolism, and therefore the cholesterol and low density lipoprotein (LDL) level, can be modulated by the type of fatty acid available in the liver, which in turn depends on the predominant dietary fatty acids (Dietschy, 1998). According to Dietschy (1998), unsaturated fatty acids (oleic acid more than linoleic acid) increase gene expression of LDL receptors, maintaining the amount and activity of these receptors high and thus decreasing the concentration of serum LDL, while palmitic acid in the liver maintains gene expression of LDL-receptors low and serum LDL concentration high.

Moreover, lipoprotein composition is related to fatty acid consumption. Thus, LDL of individuals who consume olive oil appear to be oleic-acid enriched and are less susceptible to oxidation (Mata *et al.*, 2002) and promote less monocyte chemotaxis (Tsimikas *et al.*, 1999). These facts are important with regard to the prevention of cardiovascular disease.

As frying thoroughly changes the fatty acid composition of food, Sánchez-Muniz *et al.* (1992b, 1996) studied whether the hypolipidemic properties of fatty fish remain intact after frying. To this end, sardines were fried in different oils and used as the only source of protein and fat in cholesterol-enriched diets of growing Wistar rats. As shown in Table 4.4, the hypercholesterolaemic effects of casein diets (containing olive oil or sunflower oil) were greater than those observed in rats fed fried sardines. Although the n-3 fatty acid level in sardines decreased after frying (Fig. 4.9), the hypotriglyceridemic effect of fried sardine diets was outstanding. Moreover, Sánchez-Muniz *et al.* (1992b, 1996) determined the level of some cell-damage markers and found that rats fed fried sardines displayed much lower levels of these markers than their respective basal counterparts (Table 4.5). This effect should be attributed to a more balanced n-6:n-3 ratio in cells of rats given the fried sardine diet.

Table 4.4. Changes in serum cholesterol and triglycerides in rats fed different diets containing fried sardines.

	Cholesterol (mmol/l)		Triglycerides (mmol/l)	
	Initial	Final	Initial	Final
Diet 1 Casein + olive oil	2.36 (0.18)	16.25 (2.93)^{b}	0.77 (0.21)	0.97 (0.09)^{a}
Diet 2 Sardines from the 1st and 2nd fryings in olive oil	2.43 (0.34)	3.39 (0.23)**^{a}	1.00 (0.20)	0.42 (0.08)*^{a}
Diet 3 Sardines from the 8th to 10th fryings in olive oil	2.40 (0.09)	3.36 (0.28)**^{a}	0.90 (0.18)	0.41 (0.05)*^{a}
Diet 4 Casein + sunflower oil	2.41 (0.24)	20.64 (2.94)^{b}	0.98 (0.15)	1.25 (0.15)^{a}
Diet 5 Sardines from the 1st and 2nd fryings in sunflower oil	2.28 (0.32)	2.71 (0.19)$^{++}$	1.16 (0.25)	0.47 (0.02)$^{+a}$
Diet 6 Sardines from the 8th to 10th fryings in sunflower oil	2.65 (0.35)	3.07 (0.39)$^{++}$	1.05 (0.19)	0.50 (0.10)$^{+a}$

Results are mean (SE). Asterisks show significant differences between diet 1 and diets 2 or 3 (* $p < 0.05$; ** $p < 0.01$). Crosses show significant differences between diet 4 and diets 5 or 6 ($^{+}$ $p < 0.05$; $^{++}p < 0.01$). Values bearing a letter were significantly different from their respective initial values (a: $p < 0.05$; b: $p < 0.01$). All diets contained the same amount of cholesterol and bile salt as hypercholesterolaemic agents. Adapted from Sánchez-Muniz *et al.* (1992b) and Sánchez-Muniz *et al.* (1996).

Our group has recently found that growing Wistar rats recover more quickly from hypercholesterolaemia when whole olive oil-fried sardines are the only dietary source of protein and fat, instead of casein plus fat extracted from olive oil-fried sardines or casein plus olive oil (Sánchez-Muniz *et al.*, 2003).

5.4.2. Changes in other food compounds

The fact that frying can enrich foods in compounds present in oil, such as fat-soluble vitamins and other minor compounds including carotenoids, phytosterols, phytoestrogens and phenolic compounds, is of major nutritional importance.

Phenolic compounds may also play a role in the beneficial effects of the Mediterranean diet (Herrera *et al.*, 2001; Martínez-Dominguez *et al.*, 2001; Masella *et al.*, 2001). The antioxidant activity of phenolic compounds is thought to be responsible for reducing the susceptibility of LDL to oxidation (Masella *et al.*, 2001), and these compounds have protective vascular effects in rats (Herrera *et al.*, 2001).

Addition of antioxidants to oil extends its frying life (Papadopoulos and Boskou, 1991) but high tocopherol levels (e.g. >100 mg/kg) can promote oxidation. Thus, the addition of large amounts of tocopherols should be avoided, and the addition of permitted antioxidants is recommended only to recover the antioxidant lost during frying. In this respect, frying performed with a fre-

Table 4.5. Activities (UI/l at 30°C) of some liver damage marker enzymes.

	Lactate dehydrogenase (LDH)	α-Hydroxybutirate dehydrogenase (α-HBDH)	Aspartate aminotransferase (AST)	Alanine aminotransferase (ALT)	Alcaline phosphatase (ALP)	γ-glutamyl transferase (γ -GT)
Diet 1 Casein + olive oil	779.5 (155.9)	172.8 (31.0)	78.5 (10.7)	43.0 (4.6)	734.5 (36.1)	2.7 (0.4)
Diet 2 Sardines from the 1st and 2nd fryings in olive oil	595.8 (116.0)	124.7 (24.2)	73.7 (9.6)	33.3 (7.2)*	617.8 (49.5)	1.3 (0.5)
Diet 3 Sardines from the 8th to 10th fryings in olive oil	591.6 (61.3)	116.0 (10.5)	104.0 (9.3)	27.2 (7.1)*	530.4 (26.6)	14.4 (1.0)**
Diet 4 Casein + sunflower oil	3165.2 (586.8)	1153.8 (138.7)	214.8 (24.7)	ND	ND	0.2 (0.2)
Diet 5 Sardines from the 1st and 2nd fryings in sunflower oil	561.5 (100.5)$^{++}$	113.7 (20.)$^{++}$	95.5 (13.1)	25.8 (3.3)	ND	4.8 (0.8)$^{+}$
Diet 6 Sardines from the 8th to 10th fryings in sunflower oil	535.0 (75.7)$^{++}$	109.7 (16.7)$^{++}$	99.3 (6.7)	4.8 (0.8)$^{+}$	ND	25.3 (1.7)$^{++}$

Results are mean (SEM). Asterisks show significant differences between diet 1 and diets 2 and 3 (*$p < 0.05$; **$p < 0.01$).
Crosses show significant differences between diet 4 and diets 5 and 6 ($^{+}p < 0.05$; $^{++}p < 0.01$). All diets contained same amounts cholesterol and bile salt as hypercholesterolaemic agents. ND: Not determined. Adapted from Sánchez-Muniz *et al.* (1992b, 1996).

quent addition of fresh oil helps to maintain the level of antioxidants in the oil and to increase or maintain the antioxidant content of the fried food as well. Thus, frying can actually improve the nutritional quality of food. Lecithin, a phospholipid naturally present in many oils, may help avoid oxidation due to its synergism with tocopherols. However, the lecithin concentration must be maintained below 100 mg/kg oil in order to limit foam formation.

Fig. 4.10 indicates the antioxidant activity of some phenolic compounds present in virgin olive oil. Of these compounds, hydroxytyrosol displays the greatest antioxidant protection when added to refined olive oil.

5.5. Thermal damage no greater than that produced by other culinary techniques

Frying normally lasts less than 10 minutes (Fig. 4.11), for which reason the thermal damage presumably induced in the oil is relatively low (Bognár, 1998). Moreover, almost the whole process is performed at lower temperatures than might be expected (Arroyo *et al.*, 1992; Sánchez-Muniz *et al.*, 1993b; Romero *et al.*, 1999; Bastida and Sánchez Muniz, 2001; Cuesta and Sánchez-Muniz, 2001). Figure 4.11 shows the temperature changes observed when frying three different types of food. The temperature drops quickly during the

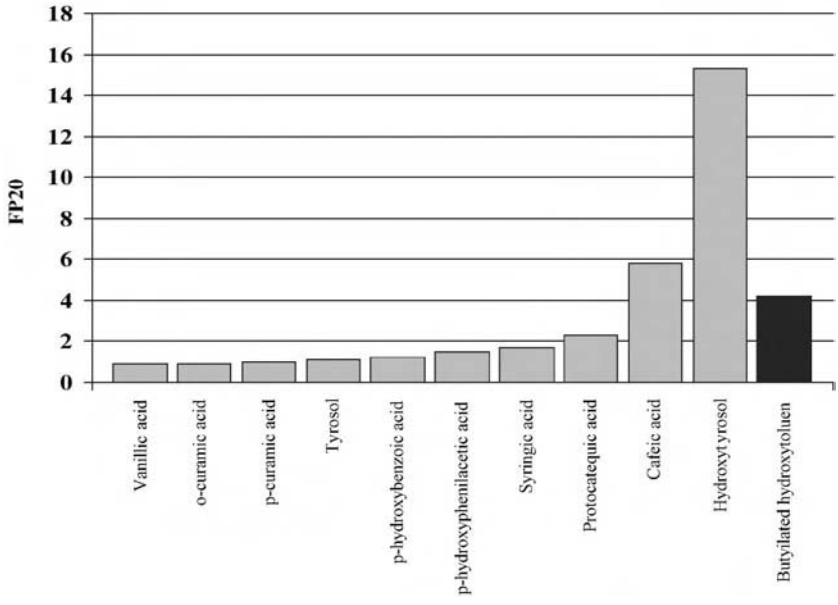


Fig. 4.10. Antioxidant activities of some phenols present in extra virgin olive oil when added to a refined olive oil. Protection factors (FP20) were calculated according to the time needed for the peroxide value of refined olive oil to surpass the level of 20 mequivalents O_2/kg. Butylated hydroxytoluene was used as reference. Adapted from Papadopoulos and Boskou (1991) and Cuesta and Sánchez-Muniz (2001).

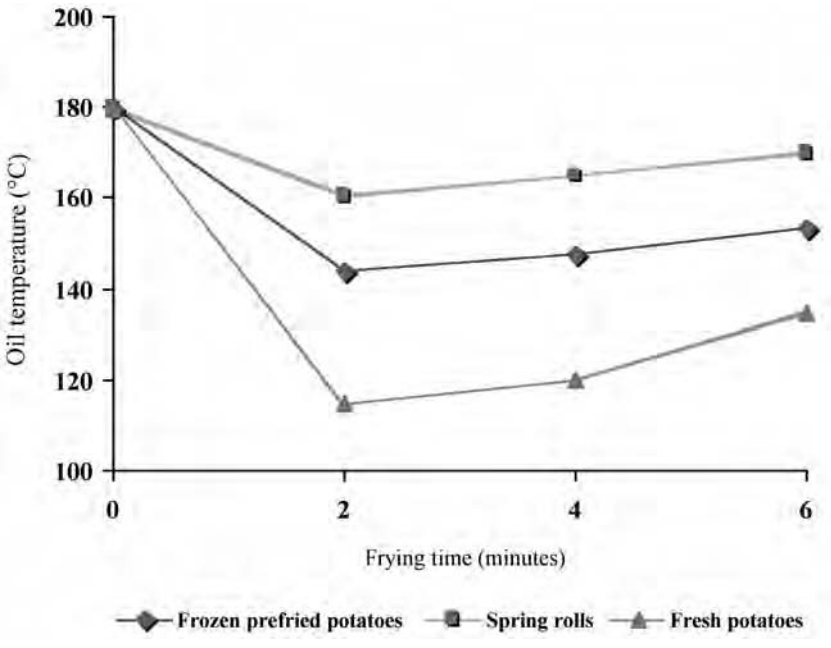


Fig. 4.11. Changes in the temperature of the oil during the frying of fresh potatoes, frozen pre-fried potatoes and spring rolls.

first two minutes, coinciding with water loss and vapour formation. Subsequently, oil temperature tends to increase due to the entry of hot oil into the food and reduced vapour formation. Varela (1988) and Varela and Ruiz-Roso (1998) report that oil does not penetrate food until a large portion of the water in the food has evaporated. The interior of the food thus remains below 100°C and is protected from thermal damage. Pravasani and Calvelo (1986) proposed the existence of a 103°C mobile front located between the crust and the centre.

On the other hand, cooked foods are an important source of dietary vitamins. As vitamins B_1 and C are very susceptible to thermal damage and lixiviation, a lot of research has been done on their loss during cooking. Lower losses of vitamin C take place during deep-fat frying (5–35%) than during boiling (30%) and stewing (76%). Nonetheless, vitamin C retention depends on the absence of peroxidative enzyme systems in the raw food. Thus, vitamin C retention in chips and frozen croquettes was close to 100% after frying, due to the absence of peroxidase activity in the uncooked foodstuffs (Bognár, 1998).

According to Bognár (1988), vitamin B_1 losses during cooking range between 0 and 10% in potatoes and vegetables and 10 and 70% in meat and fish. Boiling and stewing are responsible for great vitamin loss in both meat and fish, whereas losses are significantly lower in deep-fried and pan-fried unbreaded meat and breaded meat and fish. Similarly, retention of vitamins B_2 and B_6 is higher in fried foods than in their boiled, steamed and stewed counterparts.

These data suggest that the digestive and metabolic utilization of nutrients from fried and raw foods may be quite similar. Moreiras-Varela *et al.* (1988) report that protein digestibility of fried hake, beef, pork and swordfish is the same as that of their raw equivalents. Likewise, these authors observed that protein metabolic utilization (measured as biological value and net protein utilization) was unaffected by frying and only displayed a slight decrease when carbohydrates were added in meatball preparation.

Moreover, after frying, food retains a high proportion of its initial protein, mineral and carbohydrate content, while other culinary processes, such as boiling and steaming, reduce mineral content by 25–50% (Bognár, 1998).

6. Disadvantages of Frying

The possible disadvantages and undesirable effects of frying, mostly due to an incorrect frying technique, are summarized in Table 4.2. These undesirable effects take place when frying is done with a low oil turnover (Friedman, 1991; Sánchez-Muniz *et al.*, 1993a; Cuesta and Sánchez-Muniz, 1998; Romero *et al.*, 1998) or when oil is preheated at high temperatures for long periods before frying.

When fresh oil is frequently added to replace that which is absorbed by the food, less alteration of the oil occurs, producing fried food of higher quality (Sánchez-Muniz *et al.*, 1993a; Cuesta and Sánchez-Muniz, 1998; Romero *et al.*, 1998, 2000).

6.1. Decreased food palatability and acceptability

Acceptability of fried food is evaluated by affective testing based on hedonic scales with descriptive terms denoting likeability of products or product characteristics. Acceptability depends on the type of food, the oil used and frying conditions (Melton, 1996). The German Society for Fat Research (1973) indicates that oil is deteriorated when a panel considers that its taste and odour are unacceptable. Fat oxidation produces rancidity and thus foods that are poorly accepted (Melton, 1996).

According to Bender (1978), alteration of the fat affects its palatability more than its nutritive value. Oxidation and hydrolysis spoil the taste of a given food although it may contain only a small amount of fat, and this alteration in palatability takes place before any significant change occurs in its nutritive value.

Billek (1985) analysed more than 400 frying fats and compared their polar material with the sensory analysis data from a panel. This author found that the flavour and taste of fats whose polar material content ranged between 25 and 30% were still acceptable, but that the flavour and taste of those whose polar content surpassed 30% were unacceptable, indicating their deterioration. In short, as sensory tests detect oil alteration before fried foods are digested and absorbed, frying appears to be a safe cooking technique. Nonetheless, Sánchez-Muniz *et al.* (1998) and Garrido-Polonio *et al.* (2004) show that diets whose fat contained between 18 and 27% polar material were well accepted by growing

rats and did not significantly affect food intake. The deleterious effect of altered fat consumption will be discussed in section 6.4.

6.2. Loss of foodstuff weight and energy enrichment

As previously mentioned, dehydration occurs during frying. Gall *et al.* (1983) and García-Arias *et al.* (2003) found that fish loses more weight as a result of frying than due to other cooking methods. Thus, in terms of efficiency (amount of cooked food obtained from raw food) this weight loss is disadvantageous. Certain variants of this culinary method, however, minimize this drawback; breading, in fact, actually prevents food dehydration.

Weight loss diets restrict consumption of fried foods due to their theoretically high energy density (Bastida and Sánchez-Muniz, 2001). Nonetheless, according to Ruiz-Roso and Varela (2001) the amount of fat in fried foods is lower than that of the same foods prepared using other techniques. On the other hand, repeated frying use modifies the physicochemical characteristics of the oil, so that altered oils fatten foods more than fresh oils, decreasing frying oil efficiency (Garrido-Polonio *et al.*, 1994; Sánchez-Muniz *et al.*, 1994).

6.3. Changes in fatty acid composition and minor compounds

As previously commented, frying can improve the fatty acid profile of food. However, repeated use of frying oil results in the loss of some important compounds. Thus, frying reduces the percentage of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in sardines (Fig. 4.9). This is primarily a result of the dilution of fatty acid concentration in the fish caused by the entrance of oil and the exit of fatty acids from the sardines during frying. In fact, the amount of such n-3 fatty acids in frying oil increases after repeated use (Sánchez-Muniz *et al.*, 1992a). Nonetheless, these fatty acids are easily peroxidized. Oil used 15 times to fry potatoes and the fat extracted from the potatoes fried in this oil displayed a 10% loss in essential fatty acids (Sánchez-Muniz *et al.*, 1994).

Food water losses imply lixiviation of water-soluble compounds such as minerals, vitamins, phenolic compounds, etc. Nonetheless, these losses are not always high (Vaquero, 1998) or greater than losses caused by other culinary methods (Bognár, 1998).

It is clear from the data given in the previous sections that frying affects both major and minor oil compounds. Special mention must be made of frying's possible detrimental effect on α-tocopherol and other phenolic compounds. Quiles *et al.* (1999) report that four 15-minute frying uses can affect phenolic compounds in virgin olive oil, sunflower oil, refined olive oil and refined olive oil supplemented with 200 mg vitamin E/kg oil (Figs 4.12 and 4.13). This study investigated the formation of polar material and loss of resistance against oxidation, evaluated by electron spin resonance (ESR), of the different oils. Study results showed that:

1. The α-tocopherol levels (Fig. 4.12) decreased in all oils, but a remnant, related to the initial values, was always present. Total phenols (Fig. 4.12) were

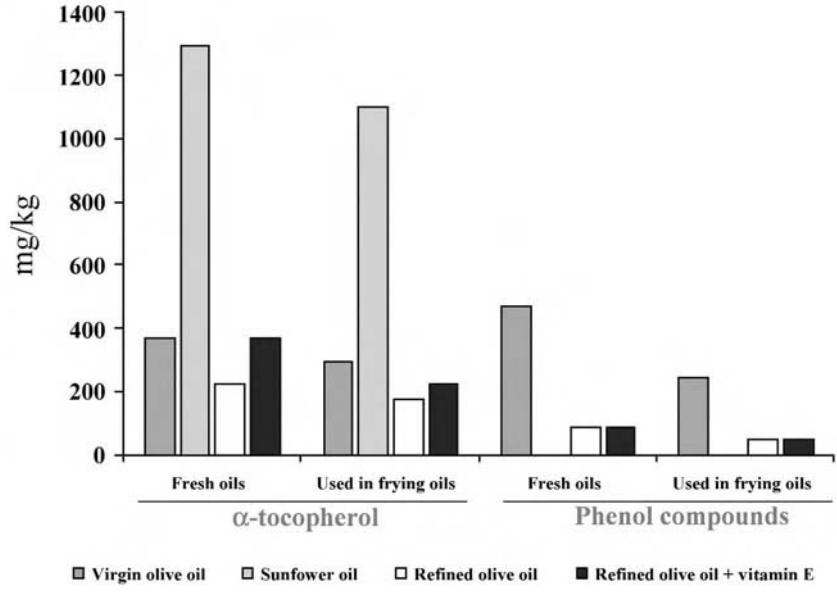


Fig. 4.12. Stability of frying oils. Changes in α-tocoferol (mg/kg) and phenol compounds (mg/kg) in virgin olive oil, sunflower oil, refined olive oil and refined olive oil supplemented with 200 mg/kg vitamin E. Source: Quiles *et al*. (1999), adapted from Cuesta *et al*. (2001).

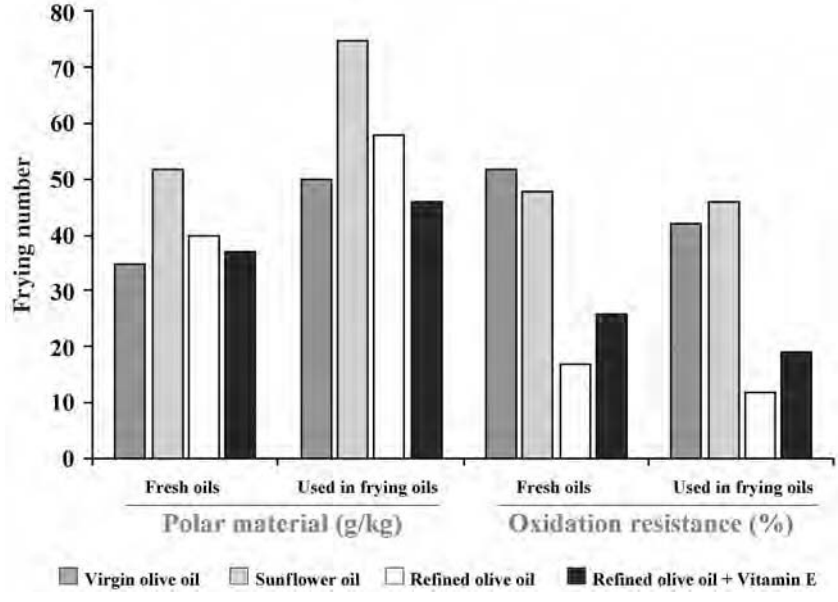


Fig. 4.13. Stability of frying oils. Changes in polar compounds (g/kg) and resistance to oxidation (%, determined by electronic-spin resonance) in virgin olive oil, sunflower oil, refined olive oil and refined olive oil supplemented with 200 mg/kg vitamin E. Source: Quiles *et al*. (1999), adapted from Cuesta *et al*. (2001).

higher in virgin olive oil than in the other olive oils but were not present in sunflower oil. This culinary technique also decreased these compounds, although 50% were still present in virgin olive oil after frying use.

2. Initial polar material (g/kg) was similar in all oils except sunflower oil. Frying increased polar material in all oils, but mainly in refined olive oil and sunflower oil (Fig. 4.13). The antioxidant capacity determined by ESR (Fig. 4.13) was more evident in fresh virgin olive and sunflower oils and those same oils used in frying. The culinary procedure, however, decreased this capacity in all oils.

According to Romero *et al.* (2003), the total phenol content of virgin olive oil decreased after the oil was used to fry fresh potatoes, with frequent addition of fresh oil, 75 times. Nonetheless, more than one quarter of phenolic compounds present in the fresh oil remained in the oil after these fryings.

In conclusion, frying diminishes the level of some nutrients and antioxidants present in oil, altering its stability. However, these losses are not necessarily complete, as oil used in non-abusive frying displays acceptable levels of these antioxidants and nutrients.

6.4. Absorption of altered compounds

During frying, food can absorb variable amounts of fat and, therefore, the altered compounds present in it (Fig. 4.2). This can change both food digestibility and nutrient utilisation (Cuesta *et al.*, 1988; Márquez-Ruiz and Dobarganes, 1996; González-Muñoz *et al.*, 1998, 2003). Moreover, the altered compounds undergo intestinal absorption and thus present potential toxicity. The absorption of altered compounds has been assessed by our group and others (Márquez-Ruiz and Dobarganes, 1996; González-Muñoz *et al.*, 1998, 2003).

Some authors (Garrido-Polonio *et al.*, 1994; Sánchez-Muniz *et al.*, 1994; Pokorny, 1998; Romero *et al.*, 2000, 2003) have found higher concentrations of these altered compounds in food than in the oil media (Table 4.6). However, others do not mention such differences (Pérez-Camino *et al.*, 1990; Sebedio *et al.*,

Table 4.6. Alteration in frying oil and in the fat extracted from fried food.

				Polar materials (mg/100 mg oil)		Trans-fatty acids (mg/100 mg oil or fat)	
Oil/fat	Food	Frying	Oil addition	Frying 8	Frying 20	Frying 8	Frying 20
Extra virgin olive oil (EVO)	Frozen	20	Yes	7.13	13.5*	0	0.24*
Extra virgin olive oil (EVO)	Frozen	20	No	8.08	17.3	0.22	0.42
Fat from potatoes fried in EVO	Frozen	20	Yes	8.14	14.1	0.19	0.37
Fat from potatoes fried in EVO	Frozen	20	No	9.23	19.3	0.22	0.49

* The comparisons between linear adjustments for concentrations in oils and foods or addition systems were significantly different (ANCOVA). Mathematical adjustments are not shown. Adapted from Romero *et al.* (2000, 2003).

1990). Our own experience indicates that the altered compound content of food and oil remains relatively low when repeated frying is performed with frequent fresh oil addition (Romero *et al.*, 1998, 1999, 2000).

Frankel *et al.* (1984) indicate that 5% of the fat consumed by Americans is oxidized, a situation that may prove harmful, especially should this percentage increase. Nonetheless, López-Varela and Sánchez-Muniz (1997) and López-Varela *et al.* (1998) report that the inclusion in pregnant rats' diet of mildly altered olive oil (9% polar material), as the only fat source, did not negatively affect pregnancy body weight gain, or foetus size, number or morphology. Mothers' serum cholesterol and phospholipids were not affected, either. However, inclusion in the diet (15% w/w) of sunflower oils used in frying (19–27% polar material, 11–16.6% triglyceride polymers), as the only fat source, slowed growth in young rats and increased the amount of peroxides (measured as TBARS) in liver, plasma and lipoproteins (Sánchez-Muniz *et al.*, 1998; Garrido-Polonio *et al.*, 2004). Moreover, some indications of liver damage were found (López-Varela *et al.*, 1995). Billek (1985) reports that fats heated under domestic or appropriate industrial conditions contained approximately 10 to 20% polar material and their consumption was not harmful to laboratory animals.

7. Frying in the Culinary Process Chain

Nowadays, new culinary practices have become more popular due to the demand for faster preparation times. Modern culinary procedures imply the sequential use of cooking and freezing and later, just before consumption, reheating of the processed food. Cooking-freezing-reheating (CFR) has become an alternative system of handling foods in catering, where the prepared food is either frozen or chilled before the reheating procedure is carried out shortly prior to eating (Skjöldebrand *et al.*, 1984). The production of the food will take place either in the food production premises or in specially designed production kitchens related to catering. Moreover, when it comes to finding ways to save time, CFR is used more at home nowadays (Creed, 2001). Heat (boiling, baking, roasting, frying or grilling) is applied to food in different ways to improve its hygienic quality by inactivation of pathogenic microorganisms and to enhance its flavour and taste, and increase shelf life (Bornár, 1998; Pokorny, 1999). The reactions involved are often interrelated, depending on time/temperature treatment and water activity (Skjöldebrand, 1984). On the other hand, the use of the microwave oven for defrosting or cooking has increased considerably during the past few decades (Sumnu, 2001). Microwave ovens change regular electricity into high frequency microwaves that water, fat and sugar can absorb, causing food particle vibration, and thus the heating of the foodstuff. García-Arias *et al.* (2003) studied the effect of CFR on proximate and fatty acid composition of sardine fillets using three different ways of cooking (frying, oven-baking and grilling) and two reheating systems (conventional and microwave ovens).

Both cooking and freezing-reheating significantly affected the proximate composition. Frying produced the highest water loss and fat gain, followed by

grilling and then by oven-baking. Microwave oven-reheating (MR) induced higher dehydration than conventional oven reheating (OR), with grilled-frozen-MR samples also losing fat and ash. Frying significantly affects the fatty acid composition of sardine, increasing oleic acid and linoleic acid content. Freezing-reheating significantly affected the fatty acid composition of sardine, increasing oleic and linoleic acids and decreasing eicosapentaenoic and docosahexaenoic acids. Oven-baking and grilling minimally affected the fatty acid content. Freezing reheating significantly affected the fatty acid composition with the content of oleic acid increasing and those of the n-3 fatty acids decreasing more in MR than in OR. Thus, according to the positive effect attributed to n-3 fatty acids, cooked samples with no further treatment would be preferred to their respective CFR counterparts. However, OR should be used instead of MR when the CFR system is performed.

8. Social Aspects of Oil Consumption

The principal oils used in the restaurant trade are olive, sunflower and virgin olive oils. Most eating establishments in Spain use olive oil, and to a lesser degree sunflower and virgin olive oils (Parras, 2001). Proprietors of restaurants claim to know the differences between these oils. However, cafeterias and modest restaurants predominantly use sunflower oil for frying, while the frying oil of choice in the better establishments is olive oil. For other culinary purposes (salad dressings, sautéing, etc.) olive oil is that which is most used, making it the most versatile oil on the Spanish market. Olive oil is almost the only oil used for culinary purposes in the best restaurants (Parras and Torres, 1995).

Taste appears to be the attribute which most greatly influences the decision of which oil to buy for any use, although this is especially so when the oil is to be used raw. Secondary considerations, above all for frying purposes, are efficiency and price. Qualities such as 'natural', 'absence of chemicals', 'healthful' and 'non-adulterated' appear to be of minor importance when shopping for oil (Bastida and Sánchez-Muniz, 2001; Parras, 2001).

9. Advantages of Frying With Olive Oils

Frying with olive oil presents important advantages, which are discussed in detail in the present section and summarized in Table 4.7.

9.1 Oil composition

From a nutritional point of view, olive oil is unique. Its fatty acid composition (a high proportion of monounsaturated fatty acids and a moderate percentage of saturated and polyunsaturated ones) has been suggested to have a beneficial effect in the prevention of certain diseases (Mataix, 2001). In addition, olive oil

Table 4.7. Advantages of frying food with olive oil (extra virgin olive oil, in particular) as compared with other edible oils.

a. Oil composition High percentage of monounsaturated fatty acids (oleic acid) with well-established health properties. Modest percentage of n-6 polyunsaturated fatty acids (linoleic acid), whose percentage in the diet should not be too high. High amount of phenolic compounds and phytosterols that display antioxidant attributes and other properties beneficial to health. Acceptable levels of tocopherols, which are antioxidants.
b. Low formation of polar material and thermal oxidized compounds during frying due to the fatty acid profile and minor compound content of olive oil. Low hydroperoxide production. The hydroperoxides formed break down at frying temperatures originating volatiles contributing to improve food palatability. Low production of secondary alteration compounds (e.g. polymers, dimers and cyclic monomers).
c. Olive oil high stability implies a long, frying life, and the oil can be used a high number of occasions for frying.
d. Formation of a well-defined crust, with good texture and palatability, that prevents excess penetration of fat.
e. Obtention of more cardio-healthy foods Foods fried in olive oils have a more balanced fatty acid composition (more appropriate saturated:monounsaturated:n-6 polyunsaturated:n-3 polyunsaturated fatty acid ratio). Food enrichment in antioxidants and bioactive compounds from the frying oil. Food with a low alteration-compound content, and therefore a low potential toxicity.

displays great stability when used for frying and other culinary purposes (Romero *et al.*, 1999; Cuesta and Sánchez-Muniz, 2001). Apart from its fatty acid composition, olive oil has an interesting content of minor compounds. Thus, olive oil presents a certain amount of tocopherols, large amounts of Δ^5-avenasterol (with antioxidant and antipolymerizing properties) and its phenolic compounds content is unique with respect to other oils. With regard to phenolic compounds, some authors suggest that these compounds display greater antioxidant activity than tocopherols.

The positive effect of olive oil consumption (fatty acid and minor compound content) has been commented on in the 'Advantages of Frying' section above. More information on the health effects of biophenols present in olive oil and olives is available in Boskou and Visioli (2003) and in other chapters in this book, some of which suggest that the biological activities of these compounds extend beyond their antioxidant capacity. In fact, hydroxytyrosol, tested for its effect on platelet function, was found to inhibit chemically-induced platelet aggregation, accumulation of the pro-aggregation agent thromboxane in human serum, production of pro-inflammatory leukotrienes by activated human leukocytes and arachidonate lipoxygenase activity. The potent (EC50s in the 10^{-5} M range) inhibitory effect of hydroxytyrosol on all these parameters

discloses unpredicted biological activities of olive oil phenolic compounds and confirms their potential cardioprotective attributes.

9.2. Resistance to thermal oxidation of olive oil during frying

The notable stability of virgin olive oil at frying temperatures has been demonstrated during the preparation of potatoes and other foods (Andrikopoulus and Demopoulus, 1989; Aggelousis and Lalas, 1997; Fedeli, 1998; Quiles *et al.*, 1999; Romero *et al.*, 1999).

In comparison with other vegetable oils such as sunflower, cotton, corn and soybean oils, olive oil presents a lower degree of alteration, as demonstrated by measuring viscosity, polar material and tocopherol losses. A comparative study of the frying behaviour of extra virgin olive oil, high oleic acid sunflower oil and 'conventional' sunflower oil used repeatedly to fry potatoes is presented in Fig. 4.14, in which extra virgin olive oil is seen to perform better than the other two oils. A plausible explanation for the resistance to rapid deterioration of extra virgin olive oil at high temperatures may be its fatty acid composition. The possibility of producing hydroperoxides during frying is lower with oleic acid, the major fatty acid in olive oil, than with diunsaturated fatty acids such as linoleic acid. This lower hydroperoxide formation reduces the possibility of generating potentially toxic thermal oxidation compounds in the frying oil. That, in turn, implies a lower uptake of such compounds by the food during frying, and thus lower toxicity and healthier foods. Moreover, hydroperoxides decompose at high temperatures, producing volatiles that increase the palatability of fried food.

In addition to this excellent fatty acid composition, olive oil presents a large amount of minor compounds with powerful antioxidant capacity in the unsaponifiable fraction. These antioxidants are mainly α-tocopherol, squalene and some phytosterols, such as Δ^5-avenasterol (Boskou and Morton, 1976; Gordon and Magos, 1983). Virgin olive oil is high in phenolic compounds, diphenols, phenolic acid and hydroxytyrosol, which, in particular, displays a great antioxidant capacity (Boskou, 1999) (Fig. 4.10).

The specific protection of α-tocopherol and other phenolic compounds against degradation induced by frying has been demonstrated by Beltran Maza *et al.* (1998). Tocopherols undergo gradual degradation during frying or storage. Therefore, supplementation with phenolic compounds has been recommended in order to preserve the original level of tocopherols in the oil (Kajimoto *et al.*, 1985; Cuesta and Sánchez-Muniz, 2001).

As oils lose minor compounds during the refining process, the addition of antioxidants lengthens their frying life. Nonetheless, current legislation permits addition of antioxidants only to refined olive oil, and not to virgin olive oil.

9.3. Long frying life

Olive oil and virgin olive oil are more stable than other oils, as previously commented in point 9.2 above. This implies that they can be used more often for fry-

ing before reaching the 25% polar material cut-off point (Ministerio de Relaciones con las Cortes y de Secretaría del Gobierno, 1989; Sánchez-Muniz and Bastida, 2003) and thus have a longer frying life than other extensively used oils (Figs 4.13 and 4.14).

Using the 10% polymer limit as the criterion to discard frying oil, olive oil had a longer frying life than sunflower oil when both were used to fry frozen pre-fried foods (Fig. 4.15). Moreover, the blend of olive oil and sunflower oil gives values that are in-between those of the separate oils. Data shown in Fig. 4.15 clearly show the lower degree of polymer formation in olive oil than in other oils during frying and therefore suggest the potentially lower toxicity of products fried in the former oil.

The comparison between Figs 4.14 and 4.15 also suggests that frying life depends on the frequency of oil replenishment. The alteration of different oils (measured according to their polymer and cyclic monomer contents) used to fry fresh potatoes and frozen pre-fried foods is summarized in Table 4.8. Frying fresh potatoes produces less oil alteration. Moreover, oils replenished frequently displayed less alteration than oils to which fresh oil was not added. Furthermore, the fat extracted from the fried food displayed a greater degree of alteration (measured in polar material and *trans* fatty acids) than that observed in the oil (Table 4.6). In conclusion, the use of extra virgin olive oil for frying and the practice of frequent oil replenishment reduce oil and food alteration and therefore produce quality foods.

9.4. Crust formation

As previously commented, olive oil forms a more defined crust in fried food than other more unsaturated oils. Such a crust prevents food from absorbing fat and dehydrating and improves its palatability. Sánchez-Muniz *et al.* (1990) found less fat in sardines fried with olive oil than in those fried with sunflower oil or lard.

Table 4.8. Alteration in frying of fresh and frozen pre-fried foods.

Oil	Food	Frying number	Oil addition	Oligomers (mg/100 mg oil)		Cyclic monomers (mg/kg oil)	
				Initial	Final	Initial	Final
Extra virgin olive oil	Potatoes	75	Yes	0.03	2.55*	0	195*
High oleic sunflower oil	Potatoes	75	Yes	0.21	3.36	64	334
Extra virgin olive oil	Frozen	20	Yes	0.08	5.41	0	574
Extra virgin olive oil	Frozen	20	No	0.08	7.59	0	684
High oleic sunflower oil	Frozen	20	Yes	0.27	5.91	64	608
High oleic sunflower oil	Frozen	20	No	0.27	7.15	64	706
Sunflower oil	Frozen	20	Yes	1.40	8.58	71	697
Sunflower oil	Frozen	20	No	1.40	11.40	71	855

*Comparisons between linear adjustments for concentrations in types of oils or addition systems were significantly different (ANCOVA). Adapted from Romero *et al.* (1999, 2003).

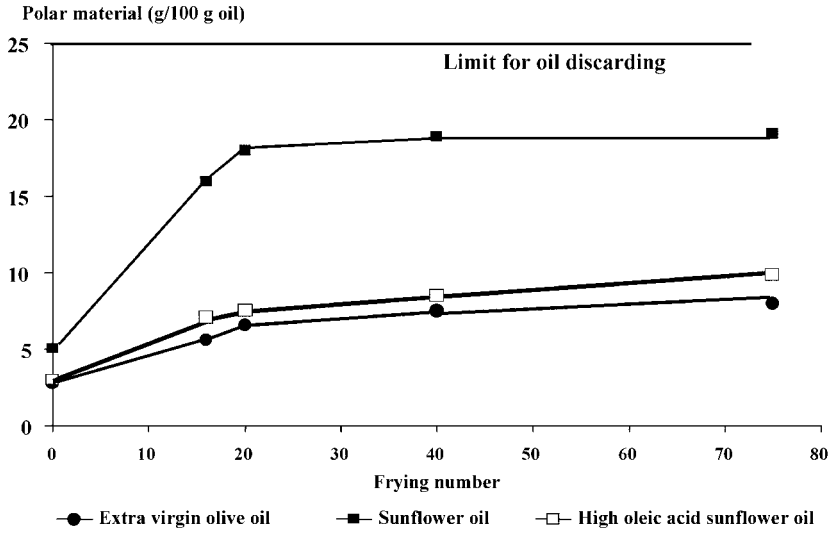


Fig. 4.14. Extra virgin olive oil, high oleic acid sunflower oil and sunflower oil behaviour in 75 frying of fresh potatoes performed with frequent oil turnover. Polar material content (g/100 g oil) change.

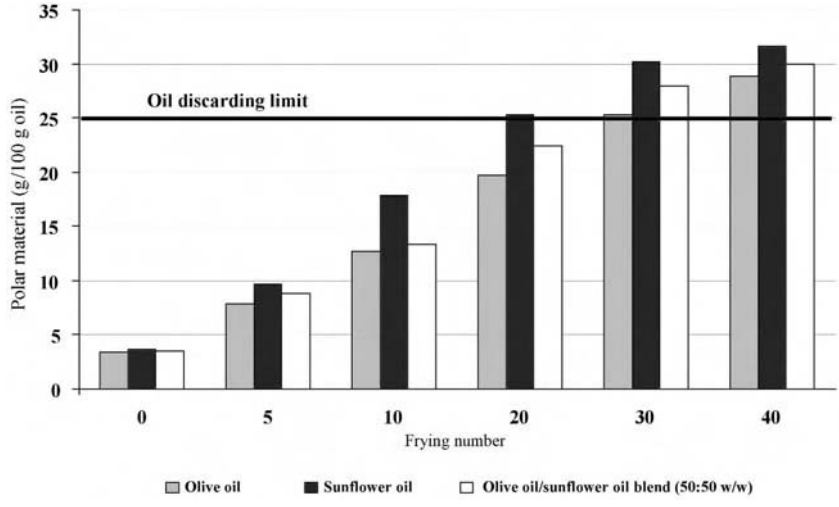


Fig. 4.15. Changes in polar material (g/100 g oil) of olive oil, sunflower oil and a blend of both oils during the frying of different fresh and frozen-pre-fried foods performed with slow oil turnover. Line at 25 g/100 g oil reflects the cut-off point selected for oil disposal in many countries. Adapted from Bastida and Sánchez-Muniz (2002).

The lower degree of alteration in olive oil during sardine frying contributes to forming a more defined crust and permits frying at relatively low temperatures, decreasing oil penetration. In the words of Friedman (1991), 'frying at lower temperatures shows that foods will be as crisp and crusty, or even better, if they are fried at only 325°F and 335°F'.

9.5. Cardio-healthier food production

Fried food consumption can be useful in correcting certain unbalances between saturated:monounsaturated:n-6 polyunsaturated:n-3 polyunsaturated fatty acid ratios in the present diet (Sánchez-Muniz and Bastida, 1997, 1999). Furthermore, frying also enriches foods with some bioactive compounds found in the frying oil.

10. Conclusions

Frying is an increasingly popular culinary technique consisting of both heat and material transfer, which shortens food preparation time and produces highly palatable products. Good oils improve flavour and taste and produce food with a high nutritional value. Correct frying performance helps to lengthen oil frying life. Olive oil, and in particular virgin olive oil, is the most suitable frying oil due to its resistance to thermal oxidation and its contribution to the production of nutritionally cardio-healthy products.

Acknowledgements

This review was supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica, Project AGL 2001-2398-C03-03.

11. References

Aggelousis, G. and Lalas, S. (1997) Quality changes of selected vegetable oils during frying of doughnuts. *Rivista Italiana Sostance Grasse* 74, 559–562.

Andrikopoulos, N.K. and Demopoulos, C.A. (1989) Deterioration of some vegetable oils. II. After two years storage of fried and non-fried samples. *Revue Française du Corps Gras*, 36, 213–215.

Arroyo, R., Cuesta, C., Garrido-Polonio, C., López-Varela, S. and Sánchez-Muniz, F.J. (1992) High-performance size-exclusion chromatographic studies on polar compounds formed in sunflower oil used for fry-

ing. *Journal of American Oil Chemists' Society* 69, 557–563.

Banks, D. (1996) Introduction. In: Perkins, E.G. and Erickson, M.D. (eds) *Deep Frying. Chemistry, Nutrition, and Practical Applications*. AOCS Press, Champaign, Illinois, pp. 1–3

Bastida, S. and Sánchez-Muniz, F.J. (1999) Nutrición y Obesidad. *Revista de Nutrición Práctica* 3, 49–58.

Bastida, S. and Sánchez-Muniz, F.J. (2001) Selección del aceite en la fritura de los alimentos. Desde el aceite de oliva a los nuevos aceites. *Revista de Nutrición Práctica* 5, 67–79.

Bastida, S. and Sánchez-Muniz, F.J. (2002) Polar content vs. TAG oligomer content in the frying-life assessment of monounsaturated and polyunsaturated oils used in deep-frying. *Journal of American Oil Chemists' Society* 79, 447–451.

Bastida, S., Sánchez-Muniz, F.J. and Trigueros, G. (2003) Aplicación de un test colorimétrico al estudio del rendimiento y vida útil en fritura de alimentos precocinados y frescos en aceite de oliva, aceite de girasol y su mezcla. *Grasas y Aceites* 54, 32–40.

Beltran Maza, G., Jiménez Márquez, A., García Mesa, J.A. and Friaz Ruiz, L. (1998) Evolution of extra virgin olive oil natural oxidants during continuous frying. *Grasas y Aceites* 49, 372 (Abstract).

Bender, A. (1978) Lipids. In: *Food Processing and Nutrition*. Academic Press, London, pp. 82–83.

Billek, G. (1985) Heated fats in the diet. In: Padley, F.B. and Podmore, U. (eds) *The Role of Fats in Human Nutrition.* Ellis Horwood Ltd., Chichester, England, pp. 163–171.

Blumenthal, M.M. (1991) A new look at the chemistry and physics of deep-fat frying. *Food Technology* 45, 68–71 and 94.

Bognár, A. (1998) Comparative study of frying to other cooking techniques. Influence on the nutritive value. *Grasas y Aceites* 49, 250–260.

Boskou, D. (1999) Non-nutrient antioxidants and stability of frying oils. In: Boskou, D. and Elmadfa, I. (eds) *Frying of Food. Oxidation, Nutrient and Non-nutrient Antioxidants, Biologically Active Compounds and High Temperatures*. Technomic Publishing Co Inc., Lancaster, Pennsylvania, USA, pp. 183–204.

Boskou, D. and Morton, I.D. (1976) Effect of plant sterols on the rate of deterioration of heated oils. *Journal of the Science of Food and Agriculture* 27, 928–932.

Boskou, D. and Visioli, F. (2003) Biophenols in olive oil and olives. In: Vaquero, M.P., García-Arias, T., Carbajal, A. and Sánchez-Muniz, F.J. (eds) *Bioavailability of Micronutrients and Minor Dietary Compounds. Metabolic and Technological Aspects.* Research Singpost, Kerala, India, pp. 161–169.

Capita, R., García-Linares, M.C., Tomé, M., García-Fernández, M.C., García-Arias, M.T. and Sánchez-Muniz, F.J. (2003) Deep-frying of chicken meat and chicken-based product. Changes in the proximate and fatty acid compositions. *Italian Journal of Food Science* 15, 225–240.

Creed, P.G. (2001) The potential of foodservice systems for satisfying consumer needs. *Innovative Food Science and Emerging Technology* 2, 219–227.

Cuesta, C. and Sánchez-Muniz, F.J. (1998) Quality control during repeated frying. *Grasas y Aceites* 49, 310–318.

Cuesta, C. and Sánchez-Muniz, F.J. (2001) La fritura de los alimentos. Fritura en aceite de oliva y aceite de oliva virgen extra. In: Mataix, J. (ed.) *Aceite de oliva virgen: nuestro patrimonio alimentario*. Vol 1. Universidad de Granada and Puleva Food (Instituto Omega 3), Granada, pp. 173–209.

Cuesta, C., Sánchez-Muniz, F.J. and Varela, G. (1988) Nutritive value of frying fats. In: Varela, G., Bender, A.E. and Morton, I.D. (eds), *Frying of Foods. Principles, Changes, New Approaches*. Ellis Horwood Ltd., Chichester, England, pp. 112–118.

Cuesta, C., Sánchez-Muniz, F.J., Garrido-Polonio, M.C., López-Varela, S. and Arroyo, R. (1993) Thermoxidative and hydrolytic changes in sunflower oil used in frying with a fast turnover of fresh oil. *Journal of American Oil Chemists' Society* 70, 1069–1073.

Cuesta, C., Ródenas, S., Merinero, M.C., Rodríguez-Gil, S. and Sánchez-Muniz, F.J.

(1998) Lipoprotein profile and serum peroxide levels of aged women consuming palmolein or oleic acid-rich sunflower oil diets. *European Journal of Clinical Nutrition* 52, 675–683.

Dietschy, J.M. (1998) Dietary fatty acids and the regulation of plasma low density lipoprotein cholesterol concentrations. *Journal of Nutrition* 128, 444S–448S.

Dobarganes, M.C., Márquez-Ruiz, G., Berdeux, O. and Velasco, J. (1999) Determination of oxidation compounds and oligomers by chromatographic techniques. In: Boskou, D. and Elmadfa, I. (eds), *Frying of Food. Oxidation, Nutrient and Non-nutrient Antioxidants, Biologically Active Compounds and High Temperatures*, Technomic Publishing Co Inc., Lancaster, Pennsylvania, USA (see Boskou, 1999), pp. 143–161.

Fedeli, E. (1988) The behaviour of olive oil during cooking and frying. In: Varela, G., Bender, A.E. and Morton, I.D. (eds), *Frying of Foods. Principles, Changes, New Approaches,* Ellis Horwood Ltd., Chichester, England, pp. 52–81.

Firestone, D. (1996) Regulation of frying fat and oil. In: Perkins, E.G. and Erickson, M.D. (eds), *Deep Frying. Chemistry, Nutrition, and Practical Applications*, AOCS Press, Champaign, Illinois, pp. 323–334.

Frankel, E.N. (1991) Review. Recent advances in lipid oxidation. *Journal of the Science of Food and Agriculture* 54, 495–511.

Frankel, E.N., Smith, L.M., Hamblin, C.L., Creveling, R.K. and Cliford, A.J. (1984) Occurrence of cyclic fatty acid monomers in frying oils used for fast foods. *Journal of American Oil Chemists' Society* 61, 87–90.

Friedman, B. (1991) New control of frying process provides major reduction of oil in food. In: Haberstroh, C. and Morris, C.E. (eds), *Fat and Cholesterol Reduced Foods. Technologies and Strategies. Advances in Applied Biotechnology Series.* Vol. 12, Portfolio Publishing Company, The Woodlands, Texas, pp. 141–152.

Gall, K.L., Otweel, J.A., Koburger, J.A. and Eppledorf, H. (1983) Effects of four cooking methods on the proximate mineral and fatty acid composition of fish fillets. *Journal of Food Science* 48, 1068–1074.

García-Arias, M.T., Álvarez Pontes, E., García-Linares, M.C., García-Fernández, M.C. and Sánchez-Muniz, F.J. (2003) Cooking–freezing–reheating (CFR) of sardine (*Sardina pilchardus*) fillets. Effect of different cooking and reheating procedures on the proximate and fatty acid compositions. *Food Chemistry* 83, 349–356.

Garrido-Polonio, M.C., Sánchez-Muniz, F.J., Arroyo, R. and Cuesta, C. (1994) Small scale frying of potatoes in sunflower oil: Thermoxidative alteration of the fat content in the fried product. *Zeitschrift für Ernährungswissenschaft* 33, 267–272.

Garrido-Polonio, M.C., García-Linares, M.C., García-Arias, M.T., López-Varela, S. and Sánchez-Muniz, F.J. (2004) Thermal oxidized sunflower oil increases liver and serum peroxides and modifies lipoprotein composition in rats. *British Journal of Nutrition* 92: 257–265.

German Society for Fat Research (1973) Symposium und Rundtischgespräch, Bratfette un Siedefette, Münster, Westphalia.

González-Muñoz, M.J., Bastida, S. and Sánchez-Muniz, F.J. (1998) Short-term *in vivo* digestibility of triglyceride polymers, dimers and monomers of thermoxidized palm olein used in deep-frying. *Journal of Agricultural and Food Chemistry* 46, 5188–5193.

González-Muñoz, M.J., Bastida, S. and Sánchez-Muniz, F.J. (2003) Short term in vivo digestibility of a highly oxidised and polymerised sunflower oil. *Journal of the Science of Food and Agriculture* 83, 413–418.

Gordon, M.H. and Magos, P. (1983) The effect of sterols on the oxidation of edible oils. *Food Chemistry* 10, 141–147.

Guillaumin, R. (1988) Kinetics of fat penetration in food. In: Varela, G., Bender, A.E. and Morton, I.D. (eds) *Frying of Food. Principles, Changes, New Approaches.* Ellis Horwood Ltd, Chichester, England, pp. 82–90.

Gupta, M.K. (1993) Designing frying fat. In: Applewhite, T.H. (ed.) *Proceedings of the World Conference on Oilseed and Technology and Utilization.* AOCS Press, Champaign, Illinois, pp. 204–208.

Herrera, M.D., Perez-Herrero, C., Marhuenda, E. and Ruiz-Gutierrez, M.V. (2001) Effects of dietary oleic rich oils (virgin olive and high

oleic acid sunflower) on vascular reactivity in Wistar-Kyoto and spontaneously hypertensive rats. *British Journal of Nutrition* 86, 349–357.

IUPAC (1992) Standard method 2.508: Determination of polymerized triglycerides in oils and fats by high performance liquid chromatography. In: International Union of Pure and Applied Chemistry (ed.), *Standard Method for the Analysis of Oils, Fats and Derivatives, 1st supplement to the 7th edition*, Blackwell, Oxford, England.

Jorge, N., Márquez-Ruiz, G., Martín-Polvillo, M., Ruiz-Méndez, M.V. and Dobarganes, M.C. (1996) Influence of dimethylpolysiloxane addition to frying oils: performance of sunflower oil in discontinuous and continuous laboratory frying. *Grasas y Aceites* 47, 20–25.

Kajimoto, G., Yoshida, H. and Shibahara, A. (1985) A role of tocopherol on the heat stability of vegetable oils. *Nippon Eiyo Shokuryo Gakkaishi* 38, 301–307.

López-Varela, S. and Sánchez-Muniz, F.J. (1997) Lipemia and liver composition in pregnant rats consuming olive oil and olive oil used for frying. *Zeitschrift für Ernährungswissenschaft* 36, 205–213.

López-Varela, S., Sánchez-Muniz, F.J. and Cuesta, C. (1995) Decreased food efficiency ratio, growth retardation and changes in liver composition in rats consuming thermoxidized and polymerized sunflower oil used for frying. *Food and Chemical Toxicology* 33, 181–189.

López-Varela, S., Sánchez-Muniz, F.J., Granados, A.M. and Cuesta, C. (1998) Maternal body weight gain and fetus development of rats fed a moderately altered olive oil. *Journal of Physiology and Biochemistry* 54, 23–32.

Márquez-Ruiz, G. and Dobarganes, M.C. (1996) Nutritional and physiological effect of used frying fats. In: Perkins, E.G. and Erickson, M.D. (eds), *Deep Frying. Chemistry, Nutrition and Practical Applications.* AOCS Press, Champaign, Illinois, pp. 160–182.

Martinez-Dominguez, E., de la Puerta, R. and Ruiz-Gutierrez, V. (2001) Protective effects upon experimental inflammation models of a polyphenol-supplemented virgin olive oil diet. *Inflammation Research* 50, 102–106.

Masella, R., Giovanni, C., Vari, R., Di Benedetto, R., Coni, E., Volpe, R., Fraone, N. and Bucci, A. (2001) Effects of dietary virgin olive oil phenols on low density lipoprotein oxidation in hyperlipidemic patients. *Lipids* 36, 1195–1202.

Mata, P., Alonso, R. and Mata, N. (2002) Los omega-3 y omega-9 en la enfermedad cardiovascular. In: Mataix, J. and Gil, A. (eds) *Libro blanco de los omega-3. Los ácidos grasos poliinsaturados omega 3 y monoinsaturados tipo oleico y su papel en la salud.* Instituto Omega 3, Puleva Food, Madrid, pp. 50–63.

Mataix, J. (ed.) (2001) *Aceite de oliva virgen: nuestro patrimonio alimentario.* Vol. 2, Universidad de Granada and Puleva Food (Instituto Omega 3), Granada, pp. 5–272.

Melton, S.L. (1996) Sensory evaluation of frying fat and deep-fried products. In: Perkins, E.G. and Erickson, M.D. (eds) *Deep Frying. Chemistry, Nutrition, and Practical Applications*, AOCS Press, Champaign, Illinois, pp. 311–322.

Ministerio de Relaciones con las Cortes y de Secretaría del Gobierno (1989) Orden de 26 de Enero de 1989 (B.O.E. número 26 del 3 de Enero de 1989). Aceites y Grasas. Normas de calidad para los calentados.

Monferrer, A. and Villalta, J. (1993) La fritura desde un punto de vista práctico. I. *Alimentación equipos y Tecnología* abril, 87–91.

Moreiras-Varela, O., Ruiz-Roso, B. and Varela, G. (1988) Effects of frying on the nutritive value of food. In: Varela, G., Bender, A.E. and Morton, I.D. (eds) *Frying of Food. Principles, Changes, New Approaches.* Ellis Horwood Ltd, Chichester, England, pp. 93–102.

Nawar, W.W. (1984) Chemical changes in lipids produced by thermal processing. *Journal of Chemical Education* 61, 299–302.

Nawar, W.W. (1998) Volatile components of frying process. *Grasas y Aceites* 49, 271–274.

Pantzaris, T.P. (1999) Palm oil in frying. In: Boskou, D. and Elmadfa, I. (eds), *Frying of Food. Oxidation, Nutrient and Non-nutrient Antioxidants, Biologically Active Compounds and High Temperatures.* Technomic Publishing Co Inc, Lancaster, Pennsylvania, USA, pp. 223–252.

Papadopoulos, G. and Boskou, D. (1991) Antioxidant effect of natural phenols on olive oil. *Journal of American Oil Chemists' Society* 68, 669–706.

Parras, M. (2001) Socioeconomía del olivar y del aceite de oliva. In: Mataix, J. (ed.) *Aceite de oliva virgen: nuestro patrimonio alimentario*. Vol. 1, Universidad de Granada and Puleva Food (Instituto Omega 3), Granada, pp. 77–97.

Parras, M. and Torres, F.J. (1995) El consumo de aceite de oliva en la hostelería y la restauración. Fundación para la Promoción y el Desarrollo del Olivar y del Aceite de Oliva, Jaén.

Pérez-Camino, M.C., Márquez-Ruiz, G., Ruiz-Méndez, M.V. and Dobarganes, M.C. (1990) Deep fat frying of frozen prefried foods. *Journal of Food Science* 56, 1644–1650.

Perkins, E.G. (1996) Volatile odor and flavor components formed in deep frying. In: Perkins, E.G. and Erickson, M.D. (eds) *Deep Frying. Chemistry, Nutrition, and Practical Applications*, AOCS Press, Champaign, Illinois, pp. 43–48.

Pokorný, J. (1998) Substrate influence on the frying process. *Grasas y Aceites* 49, 2265–270.

Pokorný, J. (1999) Changes of nutrients at frying temperatures. In: Boskou, D. and Edmalfa, I. (eds) *Frying of Food. Oxidation, Nutrient and Non-nutrient Antioxidants, Biologically Active Compounds and High Temperatures*, Technomic Publishing Co. Inc., Lancaster, Pennsylvania, USA, pp. 69–103.

Porter, N.A., Cadwell, S.E. and Mills, K.A. (1995) Mechanisms of free radical oxidation of unsaturated lipids. *Lipids* 30, 277–290.

Pravasani, J.I. and Calvelo, A. (1986) Minimum cooking time for potato strip frying. *Journal of Food Science* 51, 614–617.

Quaglia, G.B. and Bucarelli, F.M. (2001) Effective process control in frying. In: Rossell, J.B. (ed.) *Frying. Improving quality.* Woodhead Publishing Limited, Cambridge, England, pp. 236–265.

Quiles, J.L., Ramírez-Tortosa, M.C., Ibañez, S., González, A., Duthie, G.G., Huertas, J.R. and Mataix, J. (1999) Vitamin E supplementation increases the stability and the in vivo antioxidant capacity of refined olive oil. *Free Radical Research* 31, 129–135.

Real Academia Española (1992) Diccionario de la Lengua Española. Vigésima primera edición.

Romero, A., Cuesta, C. and Sánchez-Muniz, F.J. (1998) Effect of oil replenishment during deep-fat frying of frozen foods in sunflower oil and high-oleic acid sunflower oil. *Journal of American Oil Chemists' Society* 75, 161–167.

Romero, A., Sánchez-Muniz, F.J. and Cuesta, C. (1999) Does frequent replenishment with fresh oil permit the frying of potatoes indefinitely? *Journal of Agricultural and Food Chemistry* 47, 1168–1173.

Romero, A., Cuesta, C. and Sánchez-Muniz, F.J. (2000) *Trans* fatty acid production in deep fat frying of frozen foods with different oils and frying modalities. *Nutrition Research* 20, 599–608.

Romero, A., Sánchez-Muniz, F.J. and Cuesta, C. (2001) Utilización de freidora doméstica entre universitarios madrileños. Aceptación de alimentos congelados fritos en aceite de oliva virgen extra, girasol y girasol alto oleico. *Grasas y Aceites* 52, 38–44.

Romero, A., Cuesta, C. and Sánchez-Muniz, F.J. (2003) Cyclic FA monomers in high-oleic acid sunflower oil and extra virgin olive oil used in repeated frying of fresh potatoes. *Journal of American Oil Chemists' Society* 80, 437–442.

Ruiz-Roso, B. and Varela, G. (2001) Health issues. In: Rossell, J.B. (ed.) *Frying. Improving Quality*. Woodhead Publishing Limited, Cambridge, England, pp. 59–84.

Sánchez-Muniz, F.J. and Bastida, S. (1997) Ácidos grasos omega-3 y protección cardiovascular. Consideraciones sobre su consumo y recomendaciones para la población española. *Revista de Nutrición Práctica* 1, 123–138.

Sánchez-Muniz, F.J. and Bastida, S. (1999) Nutrición y Lípidos. Biodisponibilidad de ácidos grasos. *Revista de Nutrición Práctica* 4, 48–64.

Sánchez-Muniz, F.J. and Bastida, S. (2003) Frying oil discarding: Polar content vs. oligomer content determinations. In: *Modern Aspects of Nutrition. Present Knowledge and*

Future Perspectives. Forum of Nutrition, Vol. 56, Karger, Basel, pp. 345–347.

Sánchez-Muniz, F.J. and Cuesta, C. (1998) Lipid metabolism in experimental animals. *Grasas y Aceites* 49, 340–346.

Sánchez-Muniz, F.J. and Sánchez-Montero, J.M. (1999) Enzymatic methods for the study of thermally oxidized oils and fats. In: Boskou, D. and Elmadfa, I. (eds) *Frying of Food. Oxidation, Nutrient and Non-nutrient Antioxidants, Biologically Active Compounds and High Temperatures*, Technomic Publishing Co. Inc., Lancaster, Pennsylvania, USA, pp. 105–141.

Sánchez-Muniz, F.J., Medina, R., Higón, E. and Viejo, J.M. (1990) Aceites de oliva y girasol y manteca de cerdo en frituras repetidas de sardinas. Valoración del rendimiento y grado de alteración, *Grasas y Aceites* 41, 256–262.

Sánchez-Muniz, F.J., Viejo, J.M. and Medina, R. (1992a) Deep-frying of sardines in different culinary fats. Changes in the fatty acid composition of sardines and frying fats. *Journal of Agricultural and Food Chemistry* 40, 2252–2256.

Sánchez-Muniz, F.J., Higón, E., Cava, F. and Viejo, J.M. (1992b) Prevention of dietary hypercholesterolemia in rats using sunflower-oil-fried sardines. Effects on cholesterol and serum enzymes. *Journal of Agricultural and Food Chemistry* 40, 2226–2231.

Sánchez-Muniz, F.J., Cuesta, C., López-Varela, S., Garrido-Polonio, M.C. and Arroyo, R. (1993a) Evaluation of the thermal oxidation rate of sunflower oil using various frying treatment. In: Applewhite, T.H. (ed.) *Proceedings of the World Conference on Oil Seed Technology and Utilization.* AOCS Press, Champaign, Illinois, pp. 448–453.

Sánchez-Muniz, F.J., Cuesta, C. and Garrido-Polonio, M.C. (1993b) Sunflower oil used for frying. Combination of column, gas and high-performance size-exclusion chromatography for its evaluation. *Journal of American Oil Chemists' Society* 70, 235–240.

Sánchez-Muniz, F.J., Cuesta, C., Garrido-Polonio, M.C. and Arroyo, R. (1994) Fritura de patatas en aceite de girasol. Estudio comparativo del grado de alteración del aceite de la freidora y del extraído de las patatas. *Grasas y Aceites* 45, 300–305.

Sánchez-Muniz, F.J., Cava, F., Viejo, J.M., Bastida, S., Higón, E. and Marcos, A. (1996) Olive oil-fried sardines in the prevention of dietary hypercholesterolemia in rats. Effects on some serum lipids and cell-damage marker enzymes. *Nutrition Research* 16, 111–121.

Sánchez-Muniz, F.J., López-Varela, S., Garrido-Polonio, M.C. and Cuesta, C. (1998) Dietary effects on growth, liver peroxides, and serum and lipoprotein lipids in rats fed a thermoxidised and polymerised sunflower oil. *Journal of the Science of Food and Agriculture* 76, 364–372.

Sánchez-Muniz, F.J., García-Linares, M.C., García-Arias, M.T., Batida, S. and Viejo, J. (2003) Fat and protein from olive oil fried sardines interact to normalize serum lipoproteins and liver lipids in hypercholesterolemic rats. *Journal of Nutrition* 133, 2302–2308.

Sebedio, J.L., Bonpunt, A., Grandgirard, A. and Prevost, J. (1990) Deep fat frying of frozen prefried French fries: Influence of the amount of linolenic acid in the frying medium. *Journal of Agricultural and Food Chemistry* 38, 1862–1867.

Skjöldebrand, C. (1984) Introduction to process group A (Frying, grilling, boiling). In: Varela, G. (ed.) *Thermal processing and quality of foods.* Elsevier Applied Science Publisher Ltd, New York, USA, pp. 313–317.

Skjöldebrand, C., Ohlsson, Th., O'Sullivan, K. and Turner, M. (1984) Reheating of food in catering. In: Varela, G. (ed.) *Thermal Processing and Quality of Foods*, Elsevier Applied Science Publisher Ltd, New York, USA, pp. 425–431.

Sumnu, G. (2001) A review on microwave baking of foods. *International Journal of Food Science and Technology* 6, 117–127.

Tsimikas, S., Philis-Tsimikas, A., Alexopoulos, S., Sigari, F., Lee, C. and Reaven, P.D. (1999) LDL isolated from Greek subjects on a typical diet or from American subjects on an oleate-supplemented diet induces less monocyte chemotaxis and adhesion when exposed to oxidative stress. *Arteriosclerosis Thrombosis and Vascular Biology* 19, 122–130.

Vaquero, M.P. (1998) Minerals. *Grasas y Aceites* 49, 352–358.

Varela, G. (1988) Current facts about the frying of food. In: Varela, G., Bender, A.E. and Morton, I.D. (eds) *Frying of Food. Principles, Changes, New Approaches*, Ellis Horwood Ltd, Chichester, England, pp. 9–25.

Varela, G. and Ruiz-Roso, B. (1998) Influence of the frying process on the real fat intake. *Grasas y Aceites* 49, 366–369.

Waltking, A.E. and Wessels, H.J. (1981) Chromatographic separation of polar and non polar components on frying fats. *Journal of Association of Official Analytical Chemists* 64, 1329–1330.

5 Antioxidant Properties of Olive Oil Phenolics

FRANCESCO VISIOLI, SIMONA GRANDE, PAOLA BOGANI AND CLAUDIO GALLI

University of Milan, Department of Pharmacological Sciences, Milan, Italy

1. Introduction

There is now substantial evidence indicating that serum cholesterol and blood pressure (the 'classic' risk factors for atherosclerosis and coronary heart disease – CHD) do not differ significantly between populations of the Mediterranean basin and those of other North-European and Western countries (Parfitt *et al.*, 1994; Mancini and Rubba, 2000). Conversely, mortality from CHD is significantly lower in Mediterranean countries. This apparent contradiction led to the formulation of an antioxidant/atherosclerosis hypothesis, which has stimulated experimental and epidemiological studies on the possible role of antioxidants including, but not limited to, vitamins in the relative protection from CHD observed in the Mediterranean area. Indeed, a growing body of data indicates that oxidative processes, mostly involving circulating lipids/lipoproteins, are implicated in the early phases of atherosclerotic plaque development (Kritharides and Stocker, 2002). Reactive species responsible for these phenomena include oxygen radicals and oxygen-containing oxidants (ROS), reactive nitrogen species (RNS) and hypochlorous acid-based species (RCS) (Carr *et al.*, 2000). It is thus of primary importance to study the interactions between such reactive species and compounds that are ingested with the diet and that might be partly responsible for the observed protection from oxidative modification of lipoproteins. Such molecules include the family of compounds termed 'polyphenols' or 'flavonoids', which comprises more than 5000 species identified thus far. Approximately 10 years ago, our laboratory became interested in the pharmacological activities of olive phenols, thanks to the availability of some molecules isolated from extra virgin olive oil (see below). Following our initial observations and publications, several other laboratories worldwide undertook investigations that confirmed and expanded our results.

This chapter reviews the evidence that indicates how olive phenols are endowed with potent biological activities that might in part explain the protective properties of olive oil when consumed as the principal source of fat.

 Olive Oil and Health
(eds J.L. Quiles, M.C. Ramírez-Tortosa and P. Yaqoob)

2. Is Oleic Acid Fully Responsible for Cardioprotection Associated with Olive Oil Consumption?

Olive oil is abundant in oleic acid, a monounsaturated fatty acid (18:1n-9), which ranges from 56 to 84% of total fatty acids. Linoleic acid (18:2n-6), the major essential fatty acid and the most abundant polyunsaturate in our diet, is present in concentrations of between 3 and 21% (usually 7–10%) (Boskou, 2000). Although predominant consumption of oleic acid has been granted to have health effects, the effects of monounsaturated fatty acids (MUFA) on circulating lipids and lipoprotein are still relatively controversial. Some studies reviewed by Mensink *et al.* (Mensink *et al.*, 2003; Mensink and Katan, 1992) attributed a direct, although modest, cholesterol-lowering effect to MUFA alone, when they equicalorically replaced carbohydrates. Yet the major effects of high monounsaturated fatty acid intakes on serum cholesterol are generally attributed to the associated replacement of saturated fatty acids (Hegsted *et al.*, 1993; Gardner and Kraemer, 1995). Furthermore, MUFA increase the levels of the protective high-density lipoprotein (HDL) more than polyunsaturates (PUFAs) when these two classes of fatty acids replace carbohydrates in the diet (Mensink and Katan, 1992). Finally, other studies suggested a neutral effect of MUFA or even a total- and LDL-cholesterol lowering activity. An often-overlooked observation is that oleic acid is one of the predominant fatty acids in highly consumed animal foods, such as poultry and pork. As a result, the percentage of oleic acid in the Mediterranean diet is only slightly higher than that of other kinds of Western diets, including the American diet (Dougherty *et al.*, 1987), and although quantitative data are scarce, it is erroneous to believe that the Mediterranean diet is strikingly higher in monounsaturated fatty acids (MUFA) as compared with other diets. Therefore, it is unlikely that oleic acid content is the primary agent responsible for the healthful properties of olive oil. It should also be stressed that, based on current knowledge, it is very difficult to significantly alter plasma concentrations of oleic acid, a non-essential fatty acid that the body synthesizes to match its needs. In fact, there is only one study (to our knowledge) that correlates supplementation with oleic acid with health effects and with variations of its plasma concentrations (Williams *et al.*, 1999).

It is also noteworthy that several kinds of seed oil, obtained through genetic selection, are rich in monounsaturates and are now available in the market; examples include sunflower, soybean and rapeseed oils. Some of these oils also contain appreciable amounts of omega-3 fatty acids, namely alpha-linolenic acid (18:3n-3), which eventually adds nutritional value (from the fatty acid point of view).

3. Olive Oil Minor Constituents

Environmental stressors such as UV radiation and relatively high temperatures (common in the Mediterranean basin) stimulate secondary metabolism in fruits and vegetables, including olives and grapes. This leads to enhanced production of phenolic compounds with protective (antioxidant) properties. As opposed to

vegetable oils, olive oil is not extracted by the use of solvents; rather, it is obtained from the whole fruit by means of physical pressure, without the use of chemicals. As a result, lipophyliccomponents originally present in the drupe are transferred to the oil, and in this way the oil retains most of the organoleptic properties of olives, e.g. bitterness.

In addition to several 'minor' constituents of virgin olive oil, such as vitamins (alpha- and gamma-tocopherols, around 200 ppm, and carotenoids), phytosterols, pigments, terpenic acids, flavonoids such as luteolin and quercetin, and squalene, its substantial content of phenolic compounds, usually termed polyphenols (Boskou, 2000), distinguish extra virgin olive oil from other vegetable oils.

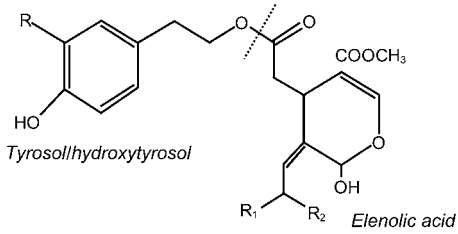
3.1. Olive oil phenolic components

The amount of phenolic compounds in olive oil depends on several factors, including cultivar, degree of maturation, possible infestation by the olive fly *Dacus Olea*, and climate (Boskou, 2000). Above all, the procedures that lead to olive oil production play the major role in determining the final phenolics concentration. For example, olives should be hand-picked at the right moment (when the skin colour changes from pale green to dark brown), stored in shallow layers in a cool environment to avoid overheating and fermentation, brought immediately to the mill and swiftly processed in a clean plant, including crushing and pressing at temperatures lower than 25–30°C. The resultant oil is very flavourful due to its high concentration of polyphenols.

It is noteworthy that olive oil production is associated with large volumes of 'waste water', resulting from the water contained in the olives and that employed to dilute the olive paste. This waste water is produced in extremely large quantities (~800,000 tonnes/year in Italy) and represents an economic burden to the mill, as it has to be properly disposed of, being a by-product of industrial origin. Due to the fact that a considerable amount of phenols, according to their partition coefficient, end up in the waste water, several projects have been undertaken to establish an economically advantageous procedure to recover the most important phenols. For example, a series of experiments performed by Visioli *et al.* (Visioli *et al.*, 1995a,b, 1999) demonstrated that waste water extracts have powerful (in the ppm range) antioxidant activity and might therefore be recovered and employed in preservative chemistry as a cheap, as yet unused, source of natural antioxidants (see below). Indeed, supplements containing hydroxytyrosol extracted from waste waters are commercially available.

3.2. Characteristics of phenolic-rich olive oils

The secoiridoid oleuropein (CAS RN Number 32619-42-4) is the bitter principle of olives and is found in olive oil, mainly in its aglycone form, as the sugar moiety makes it insoluble in oil (Fig. 5.1). Oleuropein (highly concentrated in the drupe) is hydrolysed during maturation and storage of the oil and yields several simpler



R	R_1	R_2	
OH	H	H	Oleuropein
H	H	H	Ligstroside
H	OH	OH	10-hydroxyligstroside
OH	OH	OH	10-hydroxyoleuropein

Fig. 5.1. Chemical structures of the most representative olive oil phenolics. The simple phenol hydroxytyrosol derives from the complex phenol oleuropein by hydrolysis where indicated. Note that the most potent antioxidant exhibits catecholic (ortho-diphenolic) structures.

molecules (simple phenols) that build up the complex and rich taste of olive oil. These oxidative modifications of phenolic compounds are often advantageous, as they enhance the aroma and flavour of olive oil and other foods. As many *in vitro* experiments are still performed with phenolic glycosides, it is worth mentioning that whereas olives contain complex phenols in rather polar and hydrophylic forms, olive oil contains such phenols in aglyconic forms, i.e. the more lipid-soluble residue of their molecule.

Phenol-rich oils have a bitter and pungent taste. In fact, complex interactions between the 'minor constituents' and the taste buds result in astringency and bitterness, sometimes excessive and unpleasant as in the case of some 'green' oils derived from unripe olives. Conversely, 'sweet' oils are almost devoid of phenols. This is confirmed by panel tests – adopted to evaluate the organoleptic quality of the oil and to assess its flaws – in which oils produced from greener olives usually obtain higher scores because of their 'fruity' and complex aroma, provided by their high phenol content. Flavours consequent to the presence of phenols are wide-ranging and include fruity and mineral aromas as well as flowery components. In synthesis, high phenol levels in virgin olive oils are very likely to exhibit a high stability and a strong, fruity flavour, indicating a high, but not necessarily the most preferred, organoleptic quality of the oil.

4. Olive Oil Phenolics as Antioxidants

4.1. *In vitro* studies

The recognition of LDL oxidation as a factor in the progression of atherosclerosis, and therefore CHD and the recent availability of pure compounds, namely hydroxytyrosol (2- (3,4 dihydroxyphenyl)ethanol) and oleuropein, stimulated research on the potential cardioprotective properties of these molecules.

As information on the *in vivo* dynamics of LDL oxidation is lacking, experiments can be performed *in vitro* by incubating isolated LDL with various oxidative agents, including chemicals such as transition metal ions and azo compounds, cultured cells such as macrophages and endothelial cells, or by physical means such as UV light radiation (Visioli and Galli, 1997). Several markers of oxidative stress must be taken into account, as they provide differential information on the oxidative modifications of lipids and apolipoproteins (Visioli and Galli, 1997).

Both hydroxytyrosol (HT) and oleuropein (OE) are potent (at concentrations of 10^{-6} to 10^{-4} M) inhibitors of copper sulphate-induced oxidation of LDL, in a dose-dependent manner. The protective effects of HT and OE can be evaluated by the assessment of various markers, such as a reduced formation of short-chain aldehydes (evaluated as thiobarbituric acid-reacting substances, TBARS) and of lipid peroxides, by a higher vitamin E content in the residual LDL (indicating sparing of endogenous antioxidants), and by a reduced formation of malondialdehyde-lysine and 4-hydroxynonenal-lysine adducts, indicating protection of the apoprotein layer (Visioli *et al.*, 1995a; Visioli and Galli, 1998).

Other antioxidant activities of hydroxytyrosol and oleuropein, which have been proven to be more effective than BHT or vitamin E, were demonstrated by the use of metal-independent oxidative systems (Visioli *et al.*, 1995a, 1998a) and stable free radicals, such as DPPH (Visioli and Galli, 1998), in a series of experiments that demonstrated both a strong metal-chelation and a free-radical scavenging action. In particular, both HT and OE are scavengers of superoxide anions generated by either human polymorphonuclear cells or by the xanthine/xanthine oxidase system (Visioli *et al.*, 1998a); it is noteworthy that, in these experimental set-ups, both vitamin E and BHT were found to be inactive. Relevant to both CHD and inflammation (interlinked phenomena), it is of interest that a scavenging effect of hydroxytyrosol and oleuropein was demonstrated with respect to hypochlorous acid (Visioli *et al.*, 1998), a potent oxidant (and furthermore, a chlorinating substance) produced *in vivo* at the site of inflammation (Aruoma and Halliwell, 1987) and a major component of chlorine-based bleaches that can often come into contact with food during manufacturing. As previously mentioned, the HOCl-scavenging property of hydroxytyrosol bears important consequences in terms of protection from atherosclerosis: the formation of chloramines via the myeloperoxidase-catalyzed formation of HOCl and subsequent chlorination of apoB-100 has been identified as an initiating agent in LDL lipid peroxidation (Carr *et al.*, 2000).

As far as cellular protection from oxidative stress is concerned, Manna *et al.* (1997) demonstrated an antioxidant effect of hydroxytyrosol (but not of tyrosol) in a model of oxidative stress induced in intestinal epithelial cells. In this experimental model tyrosol, which lacks the ortho-diphenolic (catecholic) structure, was found to be ineffective, as it was in the models of LDL oxidation described above (Visioli *et al.*, unpublished data). The same group described a protective effect of hydroxytyrosol toward hydrogen peroxide-induced damage to human erythrocytes.

It is well-known that the antioxidant properties of *o*-diphenols are related to hydrogen-donation, i.e. their ability to improve radical stability by forming an

intramolecular hydrogen bond between the free hydrogens of their hydroxyl group and their phenoxyl radicals. In turn, of all the phenolic components of foods and olive oils, only catechols are of biological interest, as they are the only ones endowed with antioxidant activities (Bors and Michel, 2002).

Deiana and collaborators (Deiana *et al.*, 1999) demonstrated that hydroxytyrosol protects chemically-induced DNA and aminoacid modifications at low concentrations, i.e. 50 mM. These concentrations were able to scavenge peroxynitrite and therefore to prevent ONOO—dependent DNA damage and tyrosine nitration; further, in a model of copper-induced DNA damage, the prooxidant activities of hydroxytyrosol (always a matter of concern in antioxidant chemistry and due to its copper-reducing properties) were demonstrated only at non-physiological concentrations (>500 μm and were 40-fold weaker than those of ascorbate (Deiana *et al.*, 1999). Other studies by Quiles and co-workers demonstrated inhibition of DNA oxidation in cultured prostate cells (Quiles *et al.*, 2002b), strengthening the notion that the observed lower incidence of cancer in the Mediterranean area might be in part due to olive oil consumption.

One of the most interesting aspects of the pharmacology of olive oil phenolics is that they are amphiphilic and partition between the lipid (oil) and water (waste water) phases; actually, most of them are found in the waste water. Thus, their activities on enzymes potentially sensitive to phenolic compounds were tested in a variety of cellular models, i.e. platelets, leukocytes, macrophages. Indeed, the activity of many of these enzymes depends on the intracellular 'peroxide tone', which can be modulated by dietary polyphenols (Ellis and Triggle, 2003). Also, there is literature that shows the anti-inflammatory activities of polyphenols and flavonoids (O'Leary *et al.*, 2004).

Hydroxytyrosol was proven to inhibit chemically-induced platelet aggregation, the accumulation of the pro-aggregant agent thromboxane in human serum, the production of the pro-inflammatory molecules leukotrienes by activated human leukocytes, and the inhibition of arachidonate lipoxygenase (reviewed in Visioli *et al.*, 2002). In turn, hydroxytyrosol exhibits potent (EC_{50}) in the 10^{-5} M range) anti-thrombotic and anti-inflammatory properties. Interestingly, these activities have been confirmed *in vivo*, by evaluating thromboxane B_2 formation after administration of phenol-rich olive oils or pure hydroxytyrosol (Visioli and Galli, 2003; Visioli *et al.*, 2005).

Moreover, when added to murine macrophages together with a bacterial lipopolysaccharide, oleuropein increases the functional activity of these immune-competent cells, as evaluated by a significant increase (+ 58.7 ± 4.6%) in the production of the bactericidal and cytostatic factor nitric oxide (Visioli *et al.*, 1998b). This increase was due to a direct tonic effect of oleuropein on the inducible form of the enzyme nitric oxide synthase (iNOS), as demonstrated by Western blot analysis of cell sonicates and by the coincubation of LPS-challenged cells with the iNOS inhibitor L-nitromethylarginine methylester (Visioli *et al.*, 1998b). Macrophage-derived nitric oxide during acute sepsis and inflammation represents an adaptive response of the organism that reacts to the endotoxin challenge by increasing the production of this mediator. Thus, this iNOS-enhancing activity of oleuropein can be viewed as potentially upregulating the immune system.

4.2. *In vivo* studies

In vitro studies are indispensable and build up the body of evidence that prompts *in vivo* experiments. The latter are usually more labour intensive and bear ethical limitations. Recent experiments demonstrated that olive oil phenolics are dose-dependently absorbed in animals and humans and that they are excreted in the urine as glucuronide conjugates (Visioli *et al.*, 2000a; Vissers *et al.*, 2002; Miro-Casas *et al.*, 2003a,b). Interestingly, increasing amounts of phenolics administered with olive oil stimulated the rate of conjugation with glucuronic acid (Visioli *et al.*, 2000a). Finally, the post-prandial absorption of olive oil phenolics and their incorporation into human lipoproteins has been reported by Bonanome and co-workers (Bonanome *et al.*, 2000).

Moreover, we have recently been able to demonstrate that hydroxytyrosol, administered to rats as the only bioactive component of an olive mill waste water extract, is able to increase plasma antioxidant capacity (Visioli *et al.*, 2001). Also, a low dose of waste water-derived hydroxytyrosol, i.e. only 414 mg/rat, is able to inhibit passive smoking-induced oxidative stress in rats, as demonstrated by a reduced urinary excretion of the F_2-isoprostane 8-*iso*-$PGF_{2\alpha}$ ($iPF_{2\alpha}$-III) (Visioli *et al.*, 2000c).

Finally (Table 5.1), a dose-dependent inverse correlation between the rate of 8-*iso*-$PGF_{2\alpha}$ excretion and increasing amounts of phenolics ingested with olive oil was observed in human volunteers (Visioli *et al.*, 2000b); interestingly, the urinary levels of 8-*iso*-$PGF_{2\alpha}$ inversely correlated with those of homovanillyl alcohol, i.e. a catechol-*O*-methyl-transferase (COMT)-derived metabolite of hydroxytyrosol, suggesting that the latter enters into cellular compartments where it exerts its antioxidant activity. To date, these data represent the first, albeit limited, experimental evidence of a healthful effect of olive oil minor components on human health.

Although evidence of the *in vivo* antioxidant effects of extra virgin olive oil supplementation is accumulating (Visioli *et al.*, 2000a,b, 2001, 2005; Ochoa, 2002; Quiles *et al.*, 2002a; Weinbrenner *et al.*, 2004), it is worth mentioning that other investigators did not find significant effects of virgin olive oil supplementation to humans (Vissers *et al.*, 2001a,b), possibly because of differences in

Table 5.1. Urinary concentrations of 8-iso-$PGF_{2\alpha}$ ($iPF_{2\alpha}$-III), hydroxytyrosol (HT) and homovanillyl alcohol (HValc) in subjects who were administered four olive oil samples with increasing concentrations of phenolic compounds.

Oil	A	B	C	D
8-iso-$PGF_{2\alpha}$	273 ± 67.4	228 ± 67.8	180 ± 28.2	184 ± 49.7
HT	312 ± 265	598 ± 253	873 ± 323	1024 ± 377
HValc	367 ± 177	749 ± 556	1137 ± 267	1567 ± 707

Oil samples with increasing (from A to D) phenolics concentrations were administered to human volunteers as described in Visioli *et al.* (2000b). Data are means ± SD, n = 6. 8-iso-$PGF_{2\alpha}$ concentrations are pg/mg creatinine, HT and HValc concentrations are expressed as μg/24-hour urine. The negative correlations between 8-iso-$PGF_{2\alpha}$ excretion and the administered oleuropein and hydroxytyrosol were statistically significant at the following p values: $p = 0.043$ for oleuropein, and $p = 0.046$ for hydroxytyrosol. From Visioli *et al.* (2000b).

methodologies and parameters evaluated. Thus, further experiments are necessary to elucidate the potential of olive oil phenolics to exert cardioprotection via increased antioxidant capacity. Finally, recent experimental data suggest differences in the uptake and metabolism of hydroxytyrosol between rats and humans, indicating that caution is required when extrapolating findings from animal studies to humans (Visioli *et al.*, 2003).

5. Conclusions

The contribution of excessive free radical formation to the onset of certain pathologies, such as atherosclerosis and cancer, suggests that a higher dietary intake of fruits and vegetables, i.e. food with a substantial proportion of antioxidant vitamins, flavonoids and polyphenols, might play a protective role. Indeed, the observation that in the Mediterranean area there is a lower incidence of CHD and certain types of cancers led to the hypothesis that a diet rich in grain, legumes, fresh fruits and vegetables, wine in moderate amounts and olive oil had beneficial effects on human health. As a consequence, the contribution of natural antioxidant and other components of the diet, such as fibre, to this effect is actively investigated.

Based on data presented in this chapter, consumption of a phenol-rich olive oil could lead to the intake of biologically-active compounds in quantities that have been correlated with a reduced risk of developing CHD (Hertog *et al.*, 1993, 1995). Other indirect effects of using virgin olive oil include the fact that a phenol-rich olive oil can be used in small quantities to dress foods, thus reducing the overall caloric density. Further, good-tasting olive oil could increase the consumption of fresh, raw vegetables, thus providing an additional, indirect benefit.

In conclusion, the pharmacological properties of olive oil phenolics described in this chapter provide new insights on the mechanisms by which good-quality olive oil may contribute to lower CHD mortality.

6. References

Aruoma, O.I. and Halliwell, B. (1987) Action of hypochlorous acid on the antioxidant protective enzymes superoxide dismutase, catalase and glutathione peroxidase. *Biochemical Journal* 248, 973–976.

Bonanome, A., Pagnan, A., Caruso, D., Toia, A., Xamin, A., Fedeli, E., Berra, B., Zamburlini, A., Ursini, F. and Galli, C. (2000) Evidence of postprandial absorption of olive oil phenols in humans. *Nutrition Metabolism and Cardiovascular Disease* 10, 111–120.

Bors, W. and Michel, C. (2002) Chemistry of the antioxidant effect of polyphenols. *Annals of New York Academy of Sciences* 957, 57–69.

Boskou, D. (2000) Olive oil. *World Review Nutrition and Dietetics* 87, 56–77.

Carr, A., Mccall, M.R. and Frei, B. (2000) Oxidation of LDL by myeloperoxidase and reactive nitrogen species. *Arteriosclerosis Thrombosis and Vascular Biology* 20, 1716–1723.

Deiana, M., Aruoma, O.I., Bianchi, M.D.P., Spencer, J.P.E., Kaur, H., Halliwell, B., Aeschbach, R., Banni, S., Dessi, M.A. and Corongiu, F.P. (1999) Inhibition of peroxynitrite dependent DNA base modification and tyrosine nitration by the extra virgin olive oil-derived antioxidant hydroxytyrosol. *Free Radicals Biology & Medicine* 26, 762–769.

Dougherty, R.M., Galli, C., Ferro-Luzzi, A. and Iacono, J.M. (1987) Lipid and phospholipid fatty acid composition of plasma, red blood

cells, and platelets and how they are affected by dietary lipids: a study of normal subjects from Italy, Finland, and the USA. *American Journal of Clinical Nutrition* 45, 443–455.

Ellis, A. and Triggle, C.R. (2003) Endothelium-derived reactive oxygen species: their relationship to endothelium-dependent hyperpolarization and vascular tone. *Canadian Journal of Physiology and Pharmacology* 81, 1013–1028.

Gardner, C.D. and Kraemer, H.C. (1995) Monounsaturated versus polyunsaturated dietary fat and serum lipids. A meta-analysis. *Arteriosclerosis Thrombosis and Vascular Biology* 15, 1917–1927.

Hegsted, D.M., Ausman, L.M., Johnson, J.A. and Dallal, G.E. (1993) Dietary fat and serum lipids: an evaluation of the experimental data. *American Journal of Clinical Nutrition* 57, 875–883.

Hertog, M.G., Feskens, E.J., Hollman, P.C., Katan, M.B. and Kromhout, D. (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* 342, 1007–1011.

Hertog, M.G.L., Kromhout, D., Aravanis, C., Blackburn, H., Buzina, R., Fidanza, F., Giampaoli, S., Jansen, A., Menotti, A., Nedeljkovic, S., Pekkarinen, M., Simic, B.S., Toshima, H., Feskens, E.J.M., Hollman, P.C.H. and Katan, M.B. (1995) Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Archives of Internal Medicine* 155, 381–386.

Kritharides, L. and Stocker, R. (2002) The use of antioxidant supplements in coronary heart disease *Atherosclerosis* 164, 211–219.

Mancini, M. and Rubba, P. (2000) The Mediterranean diet in Italy. In: Simopoulos, A.P. and Visioli, F. (eds) *Mediterranean Diets*. Karger, Basel, pp. 114–126.

Manna, C., Galletti, P., Cucciolla, V., Moltedo, O., Leone, A. and Zappia, V. (1997) The protective effect of the olive oil polyphenol (3,4-dihydroxyphenyl)-ethanol counteracts reactive oxygen metabolite-induced cytotoxicity in Caco-2 cells. *Journal of Nutrition* 127, 286–292.

Mensink, R.P. and Katan, M.B. (1992) Effect of dietary fatty acids on serum lipids and lipoproteins. *Arteriosclerosis Thrombosis and Vascular Biology* 12, 911–919.

Mensink, R.P., Zock, P.L., Kester, A.D. and Katan, M.B. (2003) Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials *American Journal of Clinical Nutrition* 77, 1146–1155.

Miro-Casas, E., Covas, M.I., Farre, M., Fito, M., Ortuno, J., Weinbrenner, T., Roset, P. and de la Torre, R. (2003a) Hydroxytyrosol Disposition in Humans. *Clinical Chemistry* 49, 945–952.

Miro-Casas, E., Covas, M.I., Fito, M., Farre-Albadalejo, M., Marrugat, J. and de la Torre, R. (2003b) Tyrosol and hydroxytyrosol are absorbed from moderate and sustained doses of virgin olive oil in humans. *European Journal of Clinical Nutrition* 57, 186–190.

Ochoa, J.J., Quiles, J.L., Ramirez-Tortosa, M.C., Mataix, J. and Huertas, J.R. (2002) Dietary oils high in oleic acid but with different unsaponifiable fraction contents have different effects in fatty acid composition and peroxidation in rabbit LDL. *Nutrition* 18, 60–65.

O'Leary, K.A., de Pascual-Tereasa, S., Needs, P.W., Bao, Y.P., O'Brien, N.M. and Williamson, G. (2004) Effect of flavonoids and vitamin E on cyclooxygenase-2 (COX-2) transcription. *Mutation Research* 551, 245–254.

Parfitt, V.J., Rubba, P., Bolton, C., Marotta, G., Hartog, M. and Mancini, M. (1994) A comparison of antioxidant status and free radical peroxidation of plasma lipoproteins in healthy young persons from Naples and Bristol. *European Heart Journal* 15, 871–876.

Quiles, J.L., Huertas, J.R., Battino, M., Ramirez-Tortosa, M.C., Cassinello, M., Mataix, J., Lopez-Frias, M. and Manas, M. (2002a) The intake of fried virgin olive or sunflower oils differentially induces oxidative stress in rat liver microsomes. *British Journal of Nutrition* 88, 57–65.

Quiles, J.L., Farquharson, A.J., Simpson, D.K., Grant, I. and Wahle, K.W. (2002b) Olive oil phenolics: effects on DNA oxidation and redox enzyme mRNA in prostate cells. *British Journal of Nutrition* 88, 225–234.

Visioli, F. and Galli, C. (1997) Evaluating oxidation processes in relation to cardiovascular disease: a current review of oxidant/antioxidant methodology. *Nutrition Metabolism and Cardiovascular Research* 7, 459–466.

Visioli, F. and Galli, C. (1998) The effect of minor constituents of olive oil on cardiovascular disease: new findings. *Nutrition Reviews* 56, 142–147.

Visioli, F. and Galli, C. (2003) Olives and their production waste products as sources of bioactive compounds. *Current Topics in Nutrition Research* 1, 85–88.

Visioli, F., Bellomo, G., Montedoro, G. and Galli, C. (1995a) Low density lipoprotein oxidation is inhibited in vitro by olive oil constituents. *Atherosclerosis* 117, 25–32.

Visioli, F., Vincerif, F. and Galli, C. (1995b) 'Waste waters' from olive oil production are rich in natural antioxidants. *Experientia* 51, 32–34.

Visioli, F., Bellomo, G. and Galli, C. (1998a) Free radical-scavenging properties of olive oil polyphenols. *Biochemical and Biophysical Research Communications* 247, 60–64.

Visioli, F., Bellosta, S. and Galli, C. (1998b) Oleuropein, the bitter principle of olives, enhances nitric oxide production by mouse macrophages. *Life Science* 62, 541–546.

Visioli, F., Romani, A., Mulinacci, N., Zarini, S., Conte, D., Vincieri, F.F. and Galli, C. (1999) Antioxidant and other biological activities of olive mill waste waters. *Journal of Agricultural and Food Chemistry* 47, 3397–3401.

Visioli, F., Galli, C., Bornet, F., Mattei, A., Patelli, R., Galli, G. and Caruso, D. (2000a) Olive oil phenolics are dose-dependently absorbed in humans. *FEBS Letters* 468, 159–160.

Visioli, F., Caruso, D., Galli, C., Viappiani, S., Galli, G. and Sala, A. (2000b) Olive oils rich in natural catecholic phenols decrease isoprostane excretion in humans. *Biochemical and Biophysical Research Communications* 278, 797–799.

Visioli, F., Galli, C., Plasmati, E., Viappiani, S., Hernandez, A., Colombo, C. and Sala, A. (2000c) Olive phenol hydroxytyrosol prevents passive smoking-induced oxidative stress. *Circulation* 102, 2169–2171.

Visioli, F., Caruso, D., Plasmati, E., Patelli, R., Mulinacci, N., Romani, A., Galli, G. and Galli, C. (2001) Hydroxytyrosol, as a component of olive mill waste water, is dose-dependently absorbed and increases the antioxidant capacity of rat plasma. *Free Radical Research* 34, 301–305.

Visioli, F., Poli, A. and Galli, C. (2002) Antioxidant and other biological activities of phenols from olives and olive oil. *Medicinal Research Reviews* 22, 65–75.

Visioli, F., Galli, C., Grande, S., Colonnelli, K., Patelli, C., Galli, G. and Caruso, D. (2003) Hydroxytyrosol excretion differs between rats and humans and depends on the vehicle of administration. *Journal of Nutrition* 133, 2612–2615.

Visioli, F., Caruso, D., Grande, S., Bosisio, R., Villa, M., Galli, G., Sirtori, C. and Galli, C. (2005) Virgin Olive Oil Study (VOLOS): vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients. *European Journal of Nutrition* 44, 121–7.

Vissers, M.N., Zock, P.L., Leenen, R., Roodenburg, A.J., van Putte, K.P. and Katan, M.B. (2001a) Effect of consumption of phenols from olives and extra virgin olive oil on LDL oxidizability in healthy humans. *Free Radical Research* 35, 619–629.

Vissers, M.N., Zock, P.L., Wiseman, S.A., Meyboom, S. and Katan, M.B. (2001b) Effect of phenol-rich extra virgin olive oil on markers of oxidation in healthy volunteers. *European Journal of Clinical Nutrition* 55, 334–341.

Vissers, M.N., Zock, P.L., Roodenburg, A.J., Leenen, R. and Katan, M.B. (2002) Olive oil phenols are absorbed in humans. *Journal of Nutrition* 132, 409–417.

Weinbrenner, T., Fito, M., de la Torre, R., Saez, G.T., Rijken, P., Tormos, C., Coolen, S., Albaladejo, M.F., Abanades, S., Schroder, H., Marrugat, J. and Covas, M.I. (2004) Olive oils high in phenolic compounds modulate oxidative/antioxidative status in men. *Journal of Nutrition* 134, 2314–2321.

Williams, C.M., Francis-Knapper, J.A., Webb, D., Brookes, C.A., Zampelas, A., Tredger, J.A., Wright, J., Meijer, G., Calder, P.C., Yaqoob, P., Roche, H. and Gibney, M.J. (1999) Cholesterol reduction using manufactured foods high in monounsaturated fatty acids: a randomized crossover study. *British Journal of Nutrition* 81, 439–446.

6 Olive Oil and Mitochondrial Oxidative Stress: Studies on Adriamycin Toxicity, Physical Exercise and Ageing

JOSÉ L. QUILES, JULIO J. OCHOA, JESÚS R. HUERTAS, MAGDALENA LÓPEZ-FRÍAS AND JOSÉ MATAIX

Institute of Nutrition and Food Technology, Department of Physiology, University of Granada, Granada, Spain

1. Oxidative Stress and Mitochondria

During recent years it has been ascertained that although reactive oxygen species (ROS) are produced through a large number of pathways of the aerobic metabolism, the main source of these species is the mitochondria (Lenaz, 1998; Halliwell and Gutteridge, 1999; Cadenas and Davies, 2000; Sastre *et al.*, 2000; Salvioli *et al.*, 2001; Van Remmen and Richardson, 2001). The inner mitochondrial membrane is very different from other biological membranes since its protein content is greater than 80% (most biological membranes do not exceed 50%). Because of its importance and significance in the context of oxidative stress the protein complexes integrated in the mitochondrial electron transport chain (mtETC) deserve a special mention. In aerobic organisms, the mtETC produces the energy needed to support life. Basically, metabolites from food are oxidized through the loss of electrons that are accepted by electronic carriers like the nicotinamide adenine dinucleotide (NAD^+) and flavines (flavin mononucleotide, FMN, and flavin adenine dinucleotide, FAD). The reduced nicotinamide adenine dinucleotide (NADH) and the reduced flavines ($FMNH_2$ and $FADH_2$) are oxidized again by oxygen, producing great amounts of ATP. Oxidation is carried out by small jumps in which energy is gradually liberated (Lenaz, 1998).

mtETC is mainly composed (Fig. 6.1) of five complexes (Quiles, 1995; Lenaz, 1998; Cadenas and Davies, 2000): (i) Complex I, NADH dehydrogenase complex; (ii) Complex II, succinate dehydrogenase; (iii) Complex III, bc_1 complex; (iv) Complex IV, cytochrome c oxidase (CcOx); (v) Complex V, ATPase. The mtETC fraction that metabolises oxygen is Complex IV. This enzyme uses four molecules of reduced cytochrome c to remove one electron to each one and to donate them

 Olive Oil and Health
(eds J.L. Quiles, M.C. Ramírez-Tortosa and P. Yaqoob)

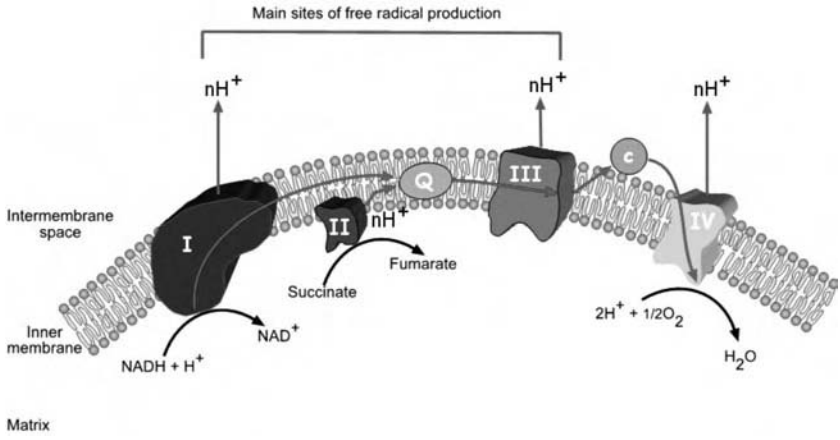


Fig. 6.1. Mitochondrial electron transport chain (mtETC) featuring proteic complexes and main sites of reactive oxygen species (ROS) production.

to an oxygen molecule. This tetra-electronic reduction of the oxygen is not performed in a single step, but must be done electron by electron. Because of this gradual reduction, the protein complex must ensure that partially oxidized oxygen, which is highly toxic, will not leak into the medium before it is transformed to water. Superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) respectively are the products of the monovalent and bivalent reduction of oxygen. Both species are usually produced during aerobic metabolism, mainly at the mitochondrial level (Cadenas and Davies, 2000). It has been estimated that 1–5% of the oxygen consumed by mitochondria is not fully reduced to water. In turn, this small percentage of oxygen is transformed to $O_2^{\bullet -}$, which spontaneously, or as the result of the action of superoxide dismutase enzymes, is transformed to H_2O_2.

Although CcOx is the enzyme involved in the oxygen reduction, it generates almost no free radicals. Instead, the two main sites of free radicals production in the mtETC are Complex I and Complex II (Cadenas *et al.*, 1977; Ksenzenko *et al.*, 1983; Shimomura *et al.*, 1985; Cross and Jones, 1991). What happens is that during the transfer from one complex to the other, some electrons escape and directly join to surrounding oxygen, resulting in the generation of $O_2^{\bullet -}$. Moreover, at the external mitochondrial membrane there is an additional source of ROS. This source originates from the deamination process of biogenic amines by monoamine oxidases, which through a bi-electronic reduction produce H_2O_2 from O_2 (Hauptman *et al.*, 1996) (Fig. 6.2). The physiological level of ROS production by the mtETC depends on the metabolic state of mitochondria. Thus, the state of mitochondrial rest (state 4), characterized by a low respiration level and no ADP availability, is associated with a high rate of ROS production, probably as a consequence of the high degree of reduction of the chain components. The active mitochondrial state (state 3), characterized by high oxygen expenditure and elevated ADP availability, shows a relatively low ROS production. In the state of anoxia (state 5), distinguished by a limitation in the oxygen delivery and absence of respiration, no ROS production is observed (Cadenas and Davies, 2000).

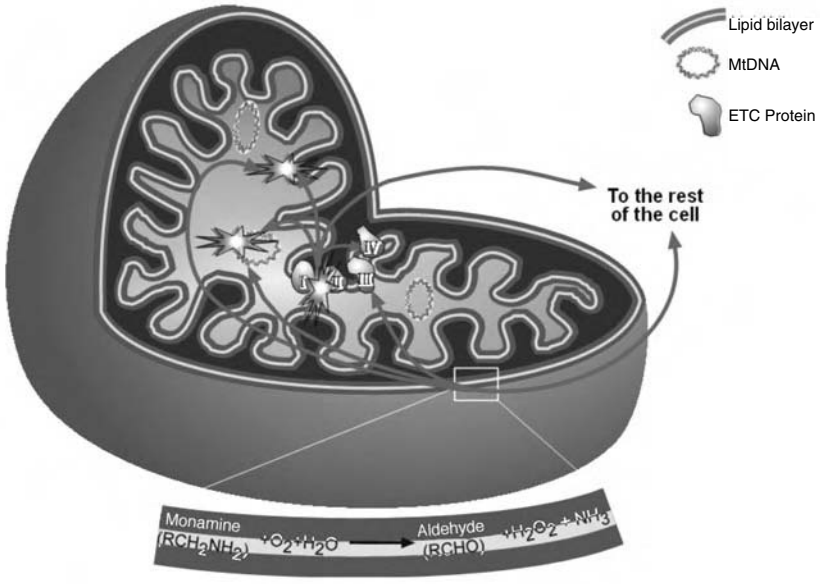


Fig. 6.2. Mitochondrial sites of ROS production and main targets.

Biological membranes are overall very sensitive to oxidative stress because of the presence of carbon-carbon double bonds in the lipid tails of its phospholipids (Montine *et al.*, 2002). Oxidative damage to membrane lipids may be directly generated through initiation by ROS as hydroxyl radicals or the superoxide anion, or indirectly by some products of the same lipid peroxidation like some highly reactive aldehydes that maximize the phenomenon (Esterbauer *et al.*, 1991). Irrespective of the mechanism, oxidative damage of membrane lipids leads to its alteration and to changes in membrane fluidity, and as a consequence of all these changes to alterations in membrane function (Halliwell and Gutteridge, 1999). Moreover, cardiolipin, which is a specific highly unsaturated mitochondrial lipid, is consequently highly prone to oxidation. Cardiolipin oxidation is extremely important to mitochondria since it is involved in the function of mtETC proteins such as CcOx and the adenine nucleotide transporter (ANT) (Paradies *et al.*, 1998). Since lipids and proteins are physically very close, oxidative damage to mitochondrial proteins, as a result of direct oxidative stress or as a consequence of lipid peroxidation, may lead to cross-linking, degradation and loss of function of such proteins. Several membrane proteins – ATPase, ANT, CcOx, etc. – are easily inactivated by oxidative stress. Moreover, protein oxidation leads to the opening of the permeability transition pore, a key step in the process of apoptosis. In summary, mtETC protein alteration has, as a direct consequence, the loss of mitochondrial functionality and, indirectly, a rise in ROS production (Lippe *et al.*, 1991; Forsmark-Andree *et al.*, 1997).

Mitochondria have their own genome (Fig. 6.3), which is different in structure and organization from the nuclear genome. It is composed of a variable number of copies of identical circular double-stranded DNA (up to ten copies). It is localized in the mitochondrial matrix, near to specific areas of the inner mitochondrial membrane (i.e. close to the main source of ROS). It has a small size

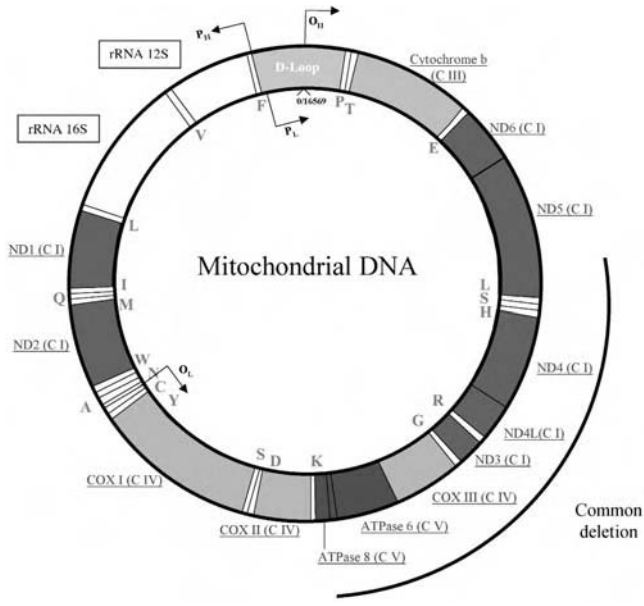


Fig. 6.3. Mitochondrial DNA (mtDNA) showing different transcription products and the site for the so-called 'common deletion'. Larger letters refer to transference RNA (tRNA). Underlined letters refer to messenger RNA (mRNA), showing within brackets the mtETC in which they are involved. Boxed letters refer to ribosomic RNA (rRNA).

(16.5 Kb) and codes for 13 mitochondrial proteins: seven subunits from Complex I, one protein from Complex III, three proteins from Complex IV, two from Complex V (ATPase), 22 trRNA and two rRNA (Lenaz, 1998; Cadenas and Davies, 2000; Van Remmen and Richardson, 2001). In contrast to nuclear DNA, mitochondrial DNA (mtDNA) is not protected by histones and it has traditionally been considered to be highly susceptible to oxidative attack (Richter *et al.*, 1988). For a long time, it was considered that mitochondria did not have a system to repair damaged DNA. Nowadays, the existence of such a system is reported, although it is not yet well known (Bohr and Anson, 1999).

There are many reports suggesting that oxidative damage to mtDNA is more important from the point of view of ageing than that to lipids and proteins. This is due to the fact that division of mtDNA amplifies the physiological consequences of the exerted damage. Furthermore, oxidative damage to mtDNA might be even more important than the damage to the nuclear DNA as the entire mitochondrial genome codes for genes that are actually expressed, while the nuclear genome contains a huge amount of non-transcribed sequences (Van Remmen and Richardson, 2001). Oxidative stress may affect mtDNA in several ways; among the most typical are oxidative alterations to bases, an increase in the number of deletions and the occurrence of punctual mutations. At the moment, the most popular approach to studying oxidative alterations to bases is by the analysis of 8-hydroxy-2-deoxyguanosine by HPLC attached to electrochemical detection. Using this procedure, several labs have reported higher levels of this biomarker at the mitochondrial level with respect to the values found in the nucleus during ageing (Chung *et al.*, 1992; Argawal and Sohal, 1994). Concerning DNA deletions, it has been described that there is an

increase in the frequency of these events with ageing in a wide variety of post-mitotic tissues from several species, including humans, monkeys, rodents and nematodes (Yoneda *et al.*, 1995). Moreover, the increase in the percentage of deletions has been directly correlated with oxidative damage. Among those studied, there is one deletion which has been termed the 'common deletion' because of its frequency and because it increases two- to threefold with ageing in some tissues, such as the brain (Cortopassi *et al.*, 1992). Nonetheless, since the percentage increase of the level of deletions does not exceed 2–3%, the true physiological significance of this phenomenon from the point of view of ageing is unclear (Van Remmen and Richardson, 2001). mtDNA mutations are the basis for a significant number of human pathologies. This has opened up a new and exciting field in mitochondrial research. mtDNA has a maternal transmission; moreover, there are many copies of the molecule in a single cell (polyplasmy) and there is a possibility that a mutation experiences different degrees of heteroplasmy. All these questions imply that a lesion is manifested only when around 80% of all the mtDNA in the cell becomes mutated (Lenaz, 1998; Michikawa *et al.*, 1999).

2. Dietary Fat Type and Mitochondrial Oxidative Stress

Dietary fat type influences several biochemical parameters at the mitochondrial membrane level (Mataix *et al.*, 1998; Quiles *et al.*, 1999a). The importance of fatty acids resides in the fact that mitochondrial membrane (as with other biological membranes) adapts its lipid composition to some extent in response to dietary fat (Huertas *et al.*, 1991a; Quiles *et al.*, 1999c; Ochoa-Herrera *et al.*, 2001). Thus, humans and animals fed on olive oil based diets have membranes richer in oleic acid than those fed on sunflower oil based diets, whose membranes are richer in linoleic acid. In addition, adaptations of the electron transport system in response to dietary fat type have been widely reported (Huertas *et al.*, 1991b; Quiles *et al.*, 2001; Battino *et al.*, 2002a). Moreover, oxidative stress is related to biological membrane composition. In that sense, a polyunsaturated fat source (e.g. sunflower oil) will lead to membranes becoming more prone to oxidation than a saturated (animal fat) or a monounsaturated (e.g. olive oil) source. This has been widely demonstrated under a wide range of physiological and pathological situations using both animal models and humans (Quiles *et al.*, 1999b, 2002b; Ramírez-Tortosa *et al.*, 1999; Battino *et al.*, 2002b; Ochoa *et al.*, 2002). Table 6.1 shows fatty acid profiles of typical oils used in most of the experiments described in the following sections.

3. Olive Oil in the Prevention of Adriamycin-mediated Oxidative Stress

3.1. Importance of adriamycin in mitochondrial oxidative stress

Adriamycin or doxorubicin is an anthracycline antibiotic obtained from *Streptomyces peucetius* that has been used for more than 30 years for the treatment of a wide variety of cancers (breast and oesophageal carcinomas, osteosarcoma,

Table 6.1. Typical fatty-acid profile of edible oils used in our experiments.

Fatty-acid composition	Virgin olive oil g/100 g	Sunflower oil g/100 g	Corn oil g/100 g
C16:0	8.9	7.2	12.6
C16:1n7	1.1	0.2	0.2
C18:0	1.9	4.5	1.9
C18:1n9	78.7	32.1	24.1
C18:2n6	8.4	54.3	60.1
C18:3n3	0.9	0.1	1.0
Total saturated	10.9	12.9	14.6
Total unsaturated	89.1	87.1	85.4
Total monounsaturated	79.8	32.3	24.3
Total polyunsaturated	9.3	54.8	61.1
MUFA:PUFA	8.6	0.6	0.4

Kaposi's sarcoma, soft-tissue sarcomas, and Hodgkin's and non-Hodgkin's lymphomas). Adriamycin is very important in the treatment of cancer patients, although its use may be complicated by the occurrence of acute and chronic side effects. The acute side effects, which may develop within minutes after intravenous administration of the drug, are nausea, vomiting, myelosuppression and arrhythmias. These effects tend to be reversible and clinically manageable (Lefrak *et al.*, 1973; Singal *et al.*, 1987). The chronic side effects, which may develop several weeks or months after repetitive doxorubicin administration, are represented by cardiovascular signs indicative of chronic cardiomyopathy and in last term congestive heart failure. These effects are irreversible and have a grave prognosis (Buja *et al.*, 1973; Singal and Iliskovic, 1998).

Several mechanisms are proposed to account for these effects of this anthracycline, both in terms of anti-cancer action and cardiac and other-organs toxicity. The capacity of adriamycin to inhibit DNA synthesis has been proposed as a mechanism of action of adriamycin (Gewirtz, 1999). This mechanism may be related to DNA intercalation or inhibition of DNA polymerase activity (Tanaka and Yoshida, 1980). Topoisomerase II is likely to be one of the primary targets for the activity of anthracycline antibiotics (Gewirtz, 1999). The induction by adriamycin of strand breaks in the DNA of L1210 leukemic cells was described more than 20 years ago (Ross *et al.*, 1978). These strand breaks were protein-associated and slowly and incompletely repaired after removal of cells from the presence of the drug. Another mode of action of adriamycin in terms of alterations in DNA is the induction of enzymatic or chemically activated DNA adducts (Cullinane *et al.*, 1994) and DNA cross-linking (Skladanowski and Konopa, 1994).

It is widely accepted that oxidative stress and the production of free radicals are involved in doxorubicin action, both in terms of anti-tumour effects and cardiotoxicity (Gewirtz, 1999; Singal *et al.*, 2000). Thus, it has been reported that adriamycin leads to direct oxidative injury to DNA (Gutteridge and Quinlan, 1985; Feinstein *et al.*, 1993) and generates lipid peroxidation (Huertas *et al.*, 1991a,b; 1992; Mataix *et al.*, 1997; Quiles *et al.*, 1999a,b). Two different path-

ways for free radical formation by adriamycin have been described. The first implicates the formation of a semiquinone free radical by the action of several NADPH-dependent reductases that produce a one-electron reduction of the doxorubicin to the corresponding doxorubicin semiquinone (Olson and Mushlin, 1990; De Beer *et al.*, 2001). In the presence of oxygen, redox cycling of adriamycin-derived quinone-semiquinone yields superoxide radicals (Singal *et al.*, 2000). In the second mechanism, adriamycin free radicals come from a non-enzymatic mechanism that involves reactions with iron. For example, Fe^{3+} reacts with adriamycin in a redox reaction after which the iron atom accepts an electron and a Fe^{2+}-doxorubicin free radical complex is produced (De Beer *et al.*, 2001). This iron–adriamycin complex can reduce oxygen to hydrogen peroxide and other active oxygen species (Gianni *et al.*, 1985; Sinha *et al.*, 1990).

In relation to adriamycin cardiotoxicity, it is worth remembering that the heart is very sensitive to free radical damage for many reasons, including the fact that it has a high rate of oxidative metabolism and a lower amount of antioxidant defences compared with other organs, such as the liver. Additionally, it has been reported that doxorubicin has a very high affinity to cardiolipin, a phospholipid species mainly present in the mitochondrial membranes of the heart, which results in the accumulation of doxorubicin inside cardiac cells (Goormaghtigh and Ruysschaert, 1984). This may be enhanced by a very unsaturated diet, which results in cardiolipin becoming enriched with highly peroxidable unsaturated fatty acids (Huertas *et al.*, 1991b).

3.2. Virgin olive oil and adriamycin

Because of the usefulness of doxorubicin in chemotherapy for the treatment of many types of cancer, researchers have expended great efforts in trying to prevent or attenuate the side effects of adriamycin administration. In this sense, several strategies have been adopted, including dosage optimization, synthesis and use of analogues or combined therapy (Singal *et al.*, 2000; Quiles *et al.*, 2002a). In relation to combined therapy, although anti-tumour action of adriamycin may be mediated by a wide number of mechanisms, free radical production is among the main causes of cardiotoxicity mediated by this drug. This fact allows the use of strategies to reduce the toxic effects of doxorubicin without interfering with its anti-tumour properties. The most immediate approach has been the combination of drug delivery together with an antioxidant in order to reduce oxidative stress (Singal *et al.*, 2000). Many antioxidants have been assayed with very different results. Among these molecules, metal ion chelators such as transferrins, metallothionein, desferrioxamine or proteins that oxidize ferrous ions, such as caeruloplasmin, have been widely investigated in relation to adriamycin. Also, numerous studies have been developed using low-molecular-mass agents that scavenge reactive oxygen species and that are synthesized *in vivo* as bilirubin, sex hormones, melatonin, uric acid or lipoic acid.

Our laboratory has been working on the effects of antioxidant-rich virgin olive oil in relation to adriamycin toxicity in rats during the last 15 years (Mataix, 2001). Several aspects of metabolism, mainly those related to

mitochondrial composition, function and free radicals generation, have been studied in relation to adriamycin and the intake of virgin olive oil or other types of edible oils. Thus, we found that the use of virgin olive oil as dietary fat (8 g/100 g of diet) attenuates the toxic effects of adriamycin (20 mg/kg intraperitoneally, daily for 4 days before sacrifice of the rats) at several points in the electron transport chain (Huertas *et al.*, 1991b) and produced lower levels of peroxides in the mitochondria and microsomes of liver (Huertas *et al.*, 1991a; Mataix *et al.*, 1997).

To study the relative importance of the antioxidants present in virgin olive oil we conducted an experiment comparing virgin olive oil (8 g/100 g of diet) with low-antioxidant 'refined' olive oil (8 g/100 g of diet). Results from these experiments (Quiles *et al.*, 1999c,d) showed that supplementation with vitamin E of refined olive oil equivalent to the levels found in virgin olive oil (this is the only supplementation to olive oil that is allowed by European Union laws) markedly improved the protective effect of this edible oil against adriamycin toxicity in rats. In fact (Table 6.2), the levels of hydroperoxides produced in liver mitochondria and microsomes by the injection of adriamycin (10 mg/kg intraperitoneally, daily for 2 days before to sacrifice of the rats) were significantly higher in rats fed on 'refined' olive oil compared with virgin olive oil and with the refined olive oil supplemented with vitamin E, which produced similar hydroperoxide levels to those in rats fed on virgin olive oil. The antioxidant profiles (α-tocopherol and coenzyme Q) were also improved by the vitamin E supplementation. From these experiments it is possible to conclude that dietary fat type modulates the toxic effects of adriamycin at the level of mitochondrial free radical production, which could be important in the chemotherapeutic treatment of patients with cancer. We are currently working on the hypothesis that the use of virgin olive oil, or some of its components, could perhaps attenuate the toxic-side effects of adriamycin treatment.

4. Oxidative Stress Related to Physical Exercise and Olive Oil

It is becoming increasingly clear that physical activity plays a critical role in growth and development. In addition, exercise is believed to be beneficial to

Table 6.2. Effect of adriamycin injection (two doses of 10 mg/kg) on hydroperoxides, coenzyme Q_9 and α-tocopherol content in liver mitochondrial membranes of rats fed on different dietary fats.

	Hydroperoxides (nmol/mg)	Coenzyme Q_9 (pmol/mg)	α-tocopherol (μmol/mg)
Virgin olive	7.6 ± 0.6[a]	506.1 ± 28.6[b]	1271.5 ± 104.4[b]
Sunflower	10.1 ± 0.7[b]	431.5 ± 27.1[a]	870.8 ± 76.8[a]
Refined olive	20.9 ± 2.9[d]	393.4 ± 45.6[a]	847.6 ± 91.6[a]
Refined olive + tocopherol	13.4 ± 2.3[c]	584.5 ± 36.4[b]	1453.8 ± 120.3[b]

Results are mean ± SE of eight animals. For each parameter, means not sharing superscript letters are statistically different ($P < 0.05$).

improve quality of life, retard age-related decline of physiological functions and prevent age-related diseases. In that sense, epidemiologic studies have shown that aerobic exercise reduces cardiovascular morbidity and mortality in the general population (Higashi and Yoshizumi, 2004). However, sport practice is also a possible mechanism by which free radicals are generated and has been widely recognized as capable of producing peroxidative damage, sometimes of severe intensity.

4.1. Oxidative stress and physical exercise

During the resting state organisms produce free radicals continuously, but normally at a rate that the body's antioxidant defence system can control. However, physical exercise elicits a 10- to 20-fold increase in whole body oxygen consumption and oxygen consumption at the level of skeletal muscle increases 100- to 200-fold (Sen, 1995; Mastaloudis *et al.*, 2004). This increase in oxygen utilization may result in an increased production of free radicals at rates that exceed the antioxidant defence system and therefore give rise to a situation of oxidative stress.

A creditable amount of data indicates that exercise at different intensities can cause increased free radical production. For example, Reid *et al.* (1992), using dichlorofluorescein (DCFH) as an intracellular probe, showed a high production of reactive oxygen species (ROS) *in vitro* by diaphragm fibre bundles after muscular contraction, suggesting that it could contribute to low-frequency fatigue. Other authors, using the same probe, demonstrated that ROS production rate was significantly increased in the homogenate of *vastus lateralis* muscle from exhaustively exercised young and old rats (Ji, 1999). In an *in vivo* model, O'Neill *et al.* (1996) reported increased hydroxyl radical production in contracting cat triceps muscle in proportion to maximal tension development. Therefore, there is evidence that strenuous *in vivo* and *in vitro* exercise can enhance free radicals production in skeletal muscle, and, potentially, in other tissues such as heart (Ji, 1999).

These free radicals produced during exercise may cause lipid, protein and/or DNA damage. Since a first report by Dillard *et al.* (1978), indicated that strenuous physical exercise induces oxidative damage to lipids in various tissues, several studies have demonstrated exercise-induced lipid peroxidation in response to various models of exercise, including endurance running, frequently using aldehydes, especially malondialdehyde (MDA), as markers to indicate lipid peroxidation in response to exercise. However, results describing the effect of exercise on lipid peroxidation are sometimes inconsistent, which is possibly due to the wide variety of methods employed (some being more specific than others) and the different conditions (e.g. intensity, type, duration, training protocols, etc.) used in different studies (Sen, 1995; Balakrishnan and Anuradha, 1998; Chiaradia *et al.*, 1998; Urso and Clarkson, 2003). Recently, there has been growing interest in exercise-induced DNA damage due to its potential involvement in various disease states. Although information regarding this damage is scarce, it has been shown that urinary 8-hydroxy-deoxyguanosine excretion is increased after

exercise and that the ratio of urinary oxidized nucleosides per creatinine after 10 hours of marathon running is increased 1.3-fold above rest (Sen, 1995; Urso and Clarkson, 2003). Using the comet assay technique, various forms of exercise have been demonstrated to produce similar relative amounts of DNA damage, with some evidence that untrained individuals experience greater damage than trained subjects (Mastaloudis *et al.*, 2004).

4.2. Free radicals sources during physical exercise

Free radicals can be produced during exercise from several potential cellular sources. Some sources may be more important than others in a certain organ, at a specific time, or with a specific exercise mode. However, these sources are not mutually exclusive and can be activated either simultaneously or by phases during and after an acute bout of strenuous exercise.

The main source for free radical generation is the mitochondria, due to electron leakage from the electron transport chain as a direct result of the increased electron flow needed to meet the higher energetic needs caused by physical exercise. This hypothesis is supported by indirect data mainly showing mitochondrial oxidative damage. Mitochondrial lipid peroxidation is enhanced after exercise, accompanied by loss of protein content and inactivation of oxidative enzymes (Mataix *et al.*, 1998; Ji, 1999). In addition, it has been shown that regular exercise increases the content of cytochromes a $+a_3$, b and $c+c_1$ and the activity of cytochrome c oxidase, in liver and skeletal muscle of rats (Quiles *et al.*, 2001), as shown in Fig. 6.4.

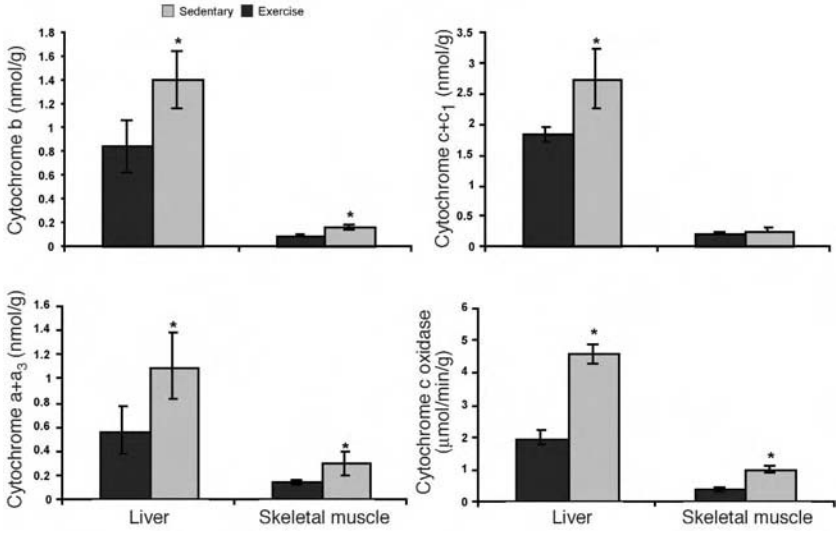


Fig. 6.4. Content of cytochromes b, $a+a_3$ and $c+c_1$ and activity of cytochrome oxidase in liver and skeletal muscle mitochondria of sedentary and exercised rats. Statistical significances ($P < 0.05$): * sedentary *vs* exercise.

Many forms of physical activity initiate ischemia-reperfusion episodes. During these episodes, xanthine oxidase-catalyzed reactions are important sources of free radicals (Fig. 6.5). Hypoxanthine has been reported to accumulate after intensive muscular concentration, and uric acid concentration was shown to increase in both contracting arm muscle and in the plasma, suggesting that xanthine oxidase was activated. It has been shown that strenuous exercise increased peroxyl radical production and xanthine oxidase activity in the plasma of horses (Ji, 1999; Urso and Clarkson, 2003).

Strenuous exercise can elicit muscle injury accompanied by an inflammatory response, characterized by increased protease and lysozomal enzyme activities in working muscle. This inflammatory response, with activation of polymorphoneutrophils, is another source of free radicals in some types of exercise. This activation can be caused by ROS or mechanical processes. However, this source of free radicals does not appear to occur during normal dynamic exercise and, given the time required for neuthrophil infiltration, it seems to be more important as a secondary source of free radical production during the recovery period following heavy exercise (Ji, 1999; Miyazaki *et al.*, 2001).

Autooxidation of catecholamines, circulating levels of which have been observed to increase during prolonged exercise, and elevated cytochrome P_{450} system activity are further potential sources of free radicals during exercise (Reid *et al.*, 1992; Ji, 1999; Urso and Clarkson, 2003).

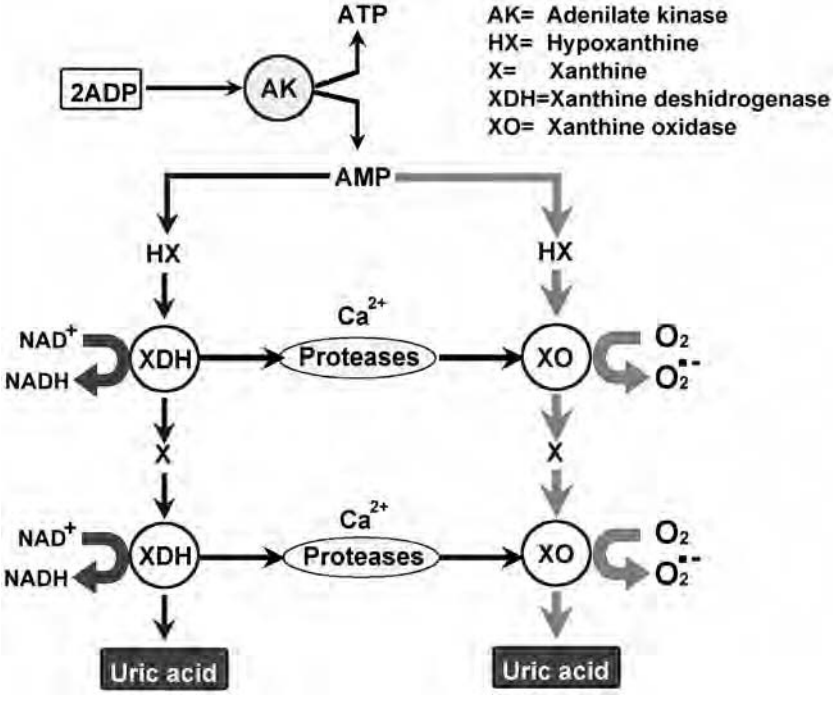


Fig. 6.5. Free radical production by xanthine oxidase during ischaemia-reperfusion.

4.3. Antioxidants and physical exercise

The extent of oxidative damage during physical exercise is determined not only by the level of free radical generation, but also by the defence capacity exerted by antioxidants. Vitamin E seems to be essential for normal cell function during exercise. The content of vitamin E seems not to be affected significantly by an acute bout of exercise, suggesting that physiological levels of tissue vitamin E allow adequate protection against exercise-induced free radical generation. However, the protective margin may be relatively small, since its concentration has been shown to decrease in rat skeletal muscle, liver and heart after chronic exercise (Mataix *et al.*, 1998; Ji, 1999). Glutathione is also important in exercise. GSH oxidation in various tissues has proven to be a consistent index of exercise-induced oxidative stress. Exhaustive exercise increases the level of GSSG and decreases the level of total glutathione in tissues such as the skeletal muscle, heart and liver. In addition, plasma levels of GSSG are remarkably higher in exhaustively exercised experimental animals (Sen, 1995; Urso and Clarkson, 2003). The activities of antioxidant enzymes, which have been considered to provide the primary defence against ROS generation during exercise, have been reported to increase in their activities in both animal and human studies (Ji, 1999; Urso and Clarkson, 2003). For example, an acute bout of exercise has been shown to increase superoxide dismutase (SOD) activity in a number of tissues including liver, skeletal muscle, heart and red blood cells, probably due to a response to the increased $O_2^{\bullet -}$ production during exercise. Similar results have been reported for glutathione peroxidase (GPx) and to a lesser degree for catalase (Ji, 1999; Urso and Clarkson, 2003).

4.4. Training and exercise-induced oxidative stress

Training seems to increase the activity of some of the aforementioned antioxidant enzymes and therefore improve the response to exercise-induced oxidative stress. For example, in volleyball players the resting muscle total and mitochondrial SOD activities were higher in trained players when compared with untrained individuals, also at rest (Urso and Clarkson, 2003). Results obtained for catalase and glutathione peroxidase (GPx) were similar although with less consistency (Ji, 1999; Miyazaki *et al.*, 2001). Other studies have not found a significant effect of training on exercise-induced oxidative stress, which seems to be due to the type of training. Thus, Criswell *et al.* (1993) studied the effect of 12-week interval training on upregulation of muscle antioxidant defences and showed that a 5 min interval of high-intensity training was better than moderate-intensity continuous exercise. Another study showed that aerobic training increased GPx activity in erythrocytes with a subsequent decrease in plasma thiobarbituric acid reactive substances (TBARS) levels. However, anaerobic training had no effect on this process (Selamoglu *et al.*, 2000). Thus, the effect of training on antioxidant systems can be very different depending on the training protocol. However, in general, it has been observed that training decreases oxidative damage during exercise, but does not completely prevent it.

4.5. Virgin olive oil and physical exercise-related oxidative stress

Most of the published studies investigating diet and exercise-induced oxidative stress have focused mostly on antioxidant vitamins, especially vitamin E. However, dietary lipid sources have been practically ignored, despite the fact that dietary lipid sources are able to modulate membrane susceptibility to oxidative stress, as described above, and despite the fact that lipid sources are known to be an important source of antioxidants.

It has been observed that dietary lipid sources affect swimming performance of Atlantic salmon. This study showed that low dietary n-3 polyunsaturated fatty acid/saturated fatty acid and n-3 polyunsaturated fatty acids:arachidonic acid ratios negatively affect swimming performance (Wagner *et al.*, 2004). In another study it was shown that high dietary levels of oleic acid increase significantly the swimming performance of Atlantic salmon (McKenzie *et al.*, 1998). However, Wagner *et al.* (2004) showed opposite results with respect to high dietary levels of oleic acid. It is important to indicate that in both studies the authors used different combinations of dietary lipid sources and in any of these studies they used olive oil as the lipid source of oleic acid. In addition, they did not study the effect of these dietary lipid sources on exercise-induced lipid peroxidation.

Our research group has been working for several years on the effect on exercise of dietary lipid sources with different degrees of unsaturation (Mataix *et al.*, 1998; Quiles *et al.*, 1994, 1998, 1999a,b, 2003). These studies were designed to investigate whether degree of unsaturation of dietary lipids could promote or depress free radical generation resulting from physical activity. Briefly, two groups of rats were fed with diets differing only in the dietary fat type: virgin olive oil or sunflower oil. Each group was subdivided into four subgroups in relation to the physical exercise assigned. Subgroup 1 consisted of sedentary rats. Subgroup 2 consisted of rats subjected to a programme of exercise based on training sessions on a treadmill without inclination during 8 weeks (in the last 6 weeks the rats were running in conditions equivalent to 65–70% of their VO_2max). The animals in this subgroup were sacrificed 24 h after the last training sessions. Subgroup 3 consisted of rats performing the same training programme as subgroup 2, although on the last day, just before being sacrificed, rats were subjected to a special running session to exhaustion, then they were immediately sacrificed. Subgroup 4 consisted of rats performing the same training programme as subgroup 3 but after exhaustion they were given a 30-min rest before being sacrificed. Thus, subgroup 2 represents a regular exercise model and subgroups 3 and 4 combine a regular model with an acute one.

Several aspects of metabolism in plasma and in different tissues (liver, skeletal muscle and heart) were studied, mainly related to mitochondrial composition, function and free radicals generation. With respect to lipid profile, results showed a good adaptation in both plasma and mitochondrial membranes to the different fatty acid profile of the dietary lipid sources used, with the heart mitochondria showing the greatest resistance to changes (Mataix *et al.*, 1998; Quiles *et al.*, 1999a, 2003). Exercise induced a decrease in plasma

triacylglycerol and cholesterol concentration, with the groups fed on a diet rich in olive oil having the lowest values. This could be very important in terms of cardiovascular disease. In addition, physical activity reduced the levels of monounsaturated fatty acids and increased the proportion of n-3 polyunsaturated fatty acids, which may contribute to the antithrombotic state and lower production of proinflammatory prostanoids attributed to physical exercise (Quiles *et al.*, 2003). In a similar way, physical exercise modified the fatty acid profile of the mitochondrial membranes, which could be due to several mechanisms such as fluidity regulation, changes in eicosanoid metabolism or differences in the availability or oxidation rate of the different fatty acids, among others (Quiles *et al.*, 1999a).

On the other hand, as we have mentioned, regular exercise gave rise to an increase in mitochondrial membrane hydroperoxide content in both liver and skeletal muscle (Mataix *et al.*, 1998). Heart mitochondria showed a smaller change in hydroperoxide content after exercise, which, as noted before, has been observed in other studies (Ji, 1999). These results are enhanced after the exhaustive effort (subgroups with regular training model plus acute model). Thus, in addition to lipid peroxidation caused by the normal training programme, there is an additional effect due to a short exhausting session. It is important to consider that the exhaustion programme is not performed separately but rather on animals that had been previously trained for 8 weeks, which suggests that although training may decrease the oxidative damage it cannot prevent it during an episode of acute exercise and therefore cannot entirely prevent possible peroxidative damage. These results are also highly dependent on the dietary lipid source. Hydroperoxide contents (Fig. 6.6) of rats fed with olive oil were lower than those obtained in rats fed on a polyunsaturated fatty acid-rich diet, being approximately 2-fold lower in liver both after regular exercise and after regular exercise plus acute exercise. These results are very important because they clearly show that, depending on the dietary lipid sources, any form of exercise will almost double peroxidative damage. Plasma hydroperoxides showed a similar pattern (Quiles *et al.*, 1998).

Another aspect studied is the interactions between physical exercise and the type of dietary fat on the concentrations of different antioxidants, such as coenzyme Q and retinol in the plasma and mitochondrial membranes of liver and skeletal muscle in rats (Quiles *et al.*, 1999b). Coenzyme Q levels were strongly affected by physical exercise and, to a lesser degree, by dietary lipid source. Sedentary rats fed on a sunflower oil diet showed the highest levels of this molecule in plasma. However, after regular exercise these animals showed a sharp decrease in its content, which was not seen in the olive oil group. Exhaustive exercise resulted in a similar decrease in plasma coenzyme Q level in both groups. However, mitochondrial membrane coenzyme Q content, which increases after exercise, especially in skeletal muscle after exhaustive exercise, was higher in animals fed on sunflower oil as the dietary lipid source. The lower levels of coenzyme Q in plasma, especially after exhaustive exercise, appear to show the increased metabolic demand of this molecule of tissues more directly involved in physical exercise. This demand seems to respond, on the one hand, to the extra need for protection against oxidative stress, which is

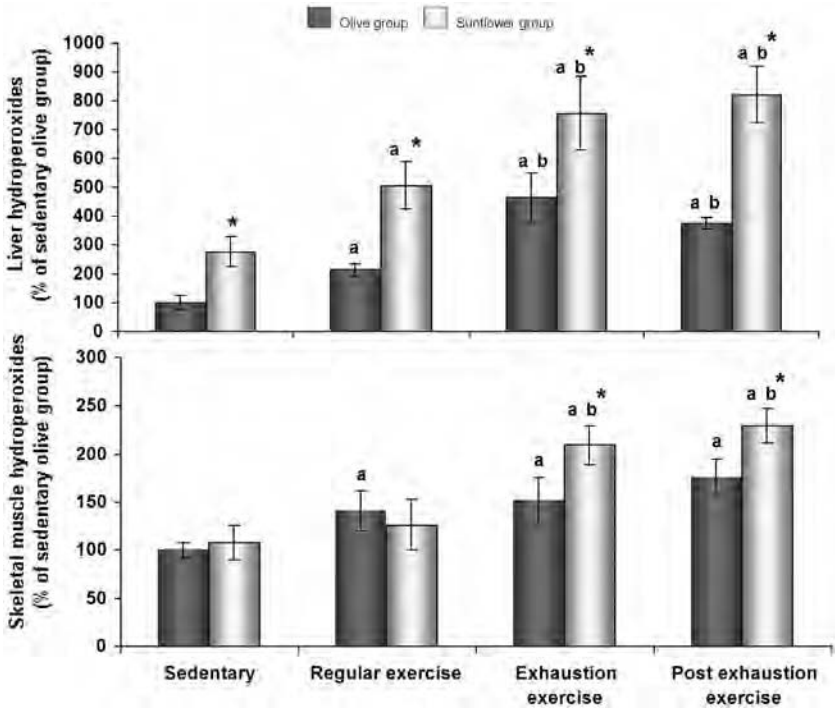


Fig. 6.6. Hydroperoxide level in liver and skeletal muscle membrane mitochondria of sedentary, regular exercise, exhaustion exercise and post exhaustion exercise rats fed on virgin olive oil and sunflower oil. Results are expressed as per cent with respect to the values obtained in sedentary virgin olive oil group. Statistical significances ($P < 0.05$): * sedentary *vs* exercise. a: regular exercise, exhaustion exercise or post exhaustion exercise *vs* sedentary rats in each dietary group; b: exhaustion exercise or post exhaustion exercise *vs* exercise rats in each dietary group; c: post exhaustion exercise *vs* exhaustion exercise rats in each dietary group.

in accord with the highest levels of hydroperoxides shown by rats fed with sunflower oil. On the other hand, this demand of coenzyme Q responds to a general increase in mitochondria and mitochondrial components induced by endurance exercise to answer the increased metabolic demands. This last aspect is supported by the fact that there is an increase in the content of cytochromes $a+a_3$, b and $c+c_1$ and in the activity of cytochrome oxidase observed in exercised animals (Quiles *et al.*, 2001). This increase in the content and/or activity of several components of the mitochondrial electron transport chain has been shown to be higher in animals fed with sunflower oil, which could be correlated with the higher content of hydroperoxides in these animals (Quiles *et al.*, 2001).

Retinol follows a similar pattern, since physical training plus acute exercise led to a general enhancement of mitochondrial retinol levels in liver and skeletal muscle, which was in parallel with the decrease in plasma levels. The greatest differences between dietary groups were shown in skeletal muscle after exhaustive exercise, as shown in Fig. 6.7. Vitamin E levels were 2-fold higher in sedentary animals fed polyunsaturated fatty acids, which is probably due to the fact

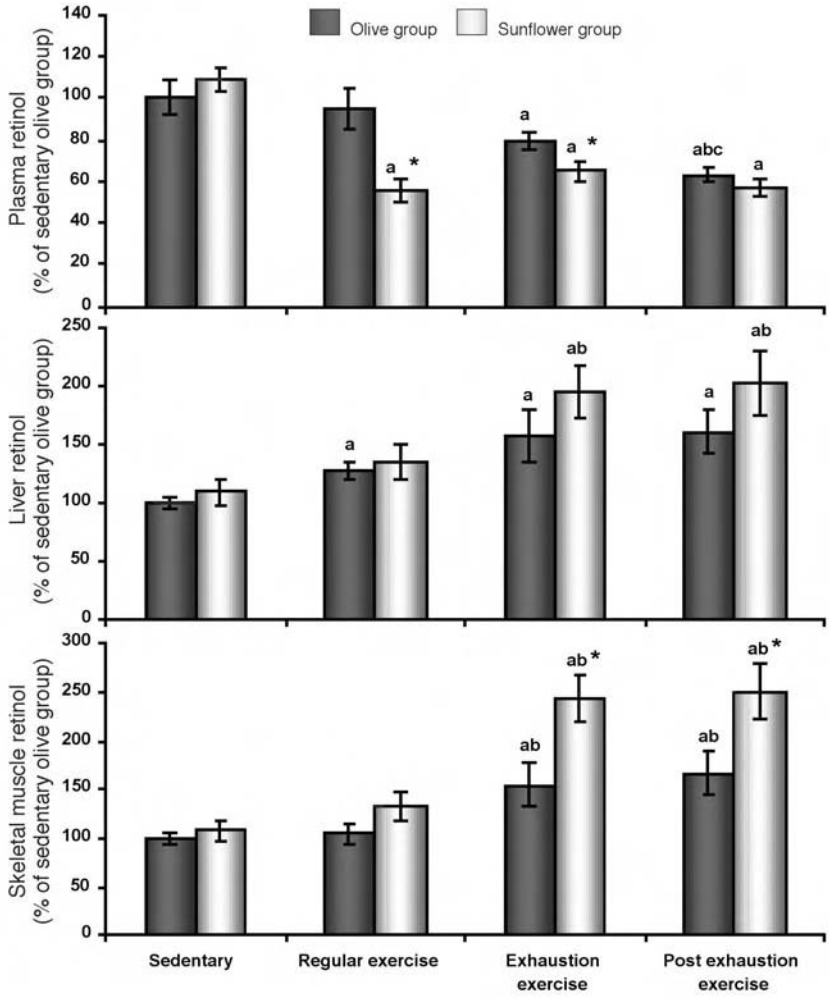


Fig. 6.7. Retinol levels in plasma and liver and skeletal muscle membrane mitochondria of sedentary, regular exercise, exhaustion exercise and post exhaustion exercise rats fed on virgin olive oil and sunflower oil. Results are expressed as per cent with respect to the values obtained in sedentary virgin olive oil group. Statistical significances ($P < 0.05$): * sedentary *vs* exercise. a: regular exercise, exhaustion exercise or post exhaustion exercise *vs* sedentary rats in each dietary group; b: exhaustion exercise or post exhaustion exercise *vs* exercise rats in each dietary group; c: post exhaustion exercise *vs* exhaustion exercise rats in each dietary group.

the polyunsaturated fatty acids-based diets contain a higher quantity of this antioxidant. However, as for coenzyme Q, physical training decreased vitamin E levels to a greater degree in animals fed on sunflower oil. This vitamin tended to increase in liver and muscle mitochondrial membranes in rats subjected to acute exercise, especially in the skeletal muscle of rats fed on sunflower oil, as shown in Fig. 6.8. Again, there is a correlation between a high demand for antioxidants and higher levels of hydroperoxides in animals fed on the polyunsaturated dietary lipid source.

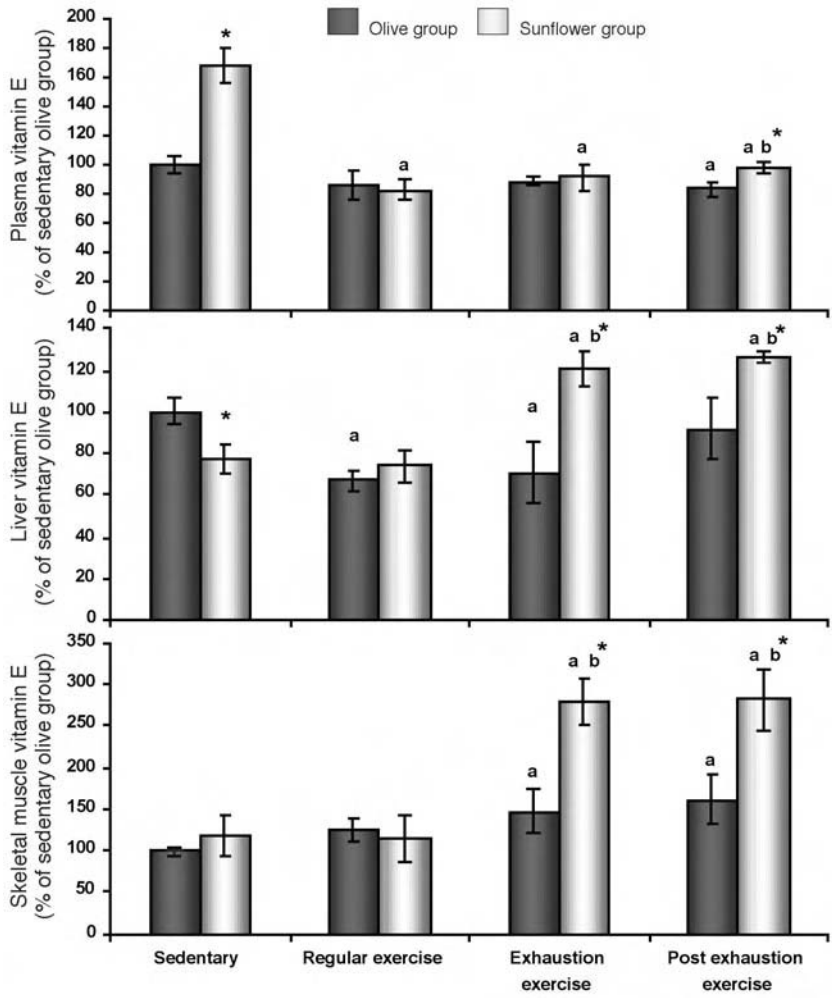


Fig. 6.8. Vitamin E levels in plasma and mitochondrial membranes of liver and skeletal muscle of sedentary, regular exercise, exhaustion exercise and post exhaustion exercise rats fed on virgin olive oil and sunflower oil. Results are expressed as per cent with respect to the values obtained in sedentary virgin olive oil group. Statistical significances ($P < 0.05$): * sedentary *vs* exercise. a: regular exercise, exhaustion exercise or post exhaustion exercise *vs* sedentary rats in each dietary group; b: exhaustion exercise or post exhaustion exercise *vs* exercise rats in each dietary group; c: post exhaustion exercise *vs* exhaustion exercise rats in each dietary group .

According to the above results, the intake of a diet rich in monounsaturated fatty acids (virgin olive oil) provides clear benefits in terms of protecting liver, skeletal muscle and heart mitochondrial membranes against exercise-induced peroxidative damage and, in addition, may improve some aspects related to the beneficial effect of exercise on several pathologies. These results show that the dietary lipid source should be considered in studies on exercise, since oxidative stress is directly modulated by this factor.

5. Olive Oil, Mitochondrial Oxidative Stress and Ageing

5.1. Concepts and theories

Ageing is a phenomenon common to all multicellular organisms that has been described as an endogenous and progressive decay in the efficacy of the physiology of the organism after the reproductive phase (Halliwell and Gutteridge, 1999; Camougrand and Rigoulet, 2001; Barja, 2002). The importance of ageing resides in the high percentage of people over 65 years (circa 20%), with a rise in the number of individuals aged over 80 years. Moreover, there is an increasing incidence of ageing-related pathologies such as Alzheimer's disease, Parkinson's disease, diabetes, certain types of cardiovascular disease, cancer and the ageing-related degenerative-maculae (the main cause of blindness in developed countries). The above-mentioned decay has been attributed to a genetic programme present in all individuals of a species or to the stochastic accumulation of errors in the somatic cells that could lead to a progressive loss of cell function (Camougrand and Rigoulet, 2001).

Since 1840, life expectancy has increased in developed countries at a rate of 3 months per year. Health has improved, but health-related costs have also been increased, with a number of new diseases that almost did not exist a century ago (Halliwell and Gutteridge, 1999; Partridge and Gems, 2002). However, if ageing is deleterious for individuals and it exists all over the world, why does it happen? The answer to that question might be that ageing is the side-effect of something else. Thus, genes that delay ageing could do so by repression of the cause that generates the damage associated with ageing. A source of such damage is reproduction. Fertility is frequently reduced both evolutionarily when ageing decreases as well as by the presence of punctual mutations that extend the life. Food seems to be another source of damage since many of the genes involved in the reduction of ageing are also related to adaptation of the organism to changes in the levels of nutrients (Masoro, 2000; Partridge and Gems, 2002).

Among the huge number of theories that have been proposed, only a few are able to explain the gradual loss of homeostasis at the end of life. Variability in the extent of the life between groups or the life extension through mutations or experimental approaches must be considered (Sohal *et al.*, 2002). Among these theories, the most accepted is the free radical theory of ageing proposed by Harman (1956). This theory enunciates that normal ageing is the result of the stochastic damage to tissues mediated by free radicals. Later, Harman focused his theory to the mitochondria as the main source and target of free radicals (Harman, 1972). In 1980 Miquel (Miquel *et al.*, 1980) proposed the mitochondrial theory of ageing (progressive damage to mitochondrial DNA by reactive oxygen species (ROS), etc.). Since it is now well known that many ROS are not free radicals, the above-mentioned theory is termed the oxidative stress theory of ageing.

5.2. The role of mitochondria in ageing

Progressive loss of mitochondrial functionality is one of the common events associated with ageing. This observation supports the consideration of these

organelles as the biological clock of ageing (Salvioli *et al.*, 2001). In fact, mitochondria have been proposed as the link between the age-dependent accumulation of oxidative damage produced by ROS and the physiological alterations associated with ageing (Van Remmen and Richardson, 2001). In that sense, several experimental observations suggest that mitochondrion is one of the main targets of the ageing process. Among those observations are (Salvioli *et al.*, 2001): (i) accumulation of deletions and punctual mutations in the mtDNA and decrease in the mtDNA copy number in some tissues; (ii) age-dependent decline in the activity of some enzymes in the mtETC; (iii) increase in the production of free radicals, probably as a consequence of the previously described alterations; (iv) alterations in the morphology of mitochondria and collapse of the mitochondrial membrane potential (ψ_{mt}).

Variations in the functionality of mitochondria may be important in determining adequate or inadequate ageing. Some insight can be gained from the study of specific population groups around the world. For example, an inherited variation of a mtDNA germ line (halogroup J) has been associated with a more adequate ageing process and an extended life in the Italian population (De Benedictis *et al.*, 2000). In another study, it has been reported that in Japan three mutations associated with a mtDNA germ line are present at a high frequency in centenarians from this part of the world (Tanaka *et al.*, 1998).

Another interesting question is the role of mitochondria as a very important element from the point of view of cell signal transduction. Thus, mitochondria may also be considered as an element of control for nuclear gene expression. In this sense, a number of adaptational or regulatory proteins have been found at the mitochondrial level or are translocated to the mitochondria to perform this role. Examples include Nur77/TR3, p53, PKCδ, JNK/SAPK, some caspases and several members of the bcl2 family, such as Bid, Bax or Bim (Finkel and Holbrook, 2000).

In relation to the role of mitochondria in the ageing process, the control of apoptosis is very important. This control is frequently lost in aged cells, which in addition are more prone to suffer from oxidative stress. ROS decrease ψ_{mt}, allowing the opening of the transition pore and the subsequent escape to the outside of calcium and other substrates. This sequence of reactions leads to apoptosis in lymphocytes, liver and the brain of aged mice (Watson *et al.*, 2000).

5.3. Oxidative stress and antioxidant defences in ageing

Several studies have reported that the levels of different markers of oxidative stress increase with ageing, although other studies did not find such an increase (Halliwell and Gutteridge, 1999). Such a disparity in the results may be due to the nature of the biological sample or in the studied biomarker. Concerning the nature of the biological sample, many studies have been carried out using tissue homogenates or whole cells. In relation to this, it should be noted that most of the oxidative stress in the cell occurs in mitochondria. Therefore, a poor choice of biological sample could give rise to misleading results, since mtDNA represents approximately 5% of nuclear DNA. Furthermore, the net oxidative stress seems

to be dependent on the sex of the animal, the species, the studied tissue, the lipid profile of mitochondrial membranes, etc. Enhanced levels of exhaled pentane and ethane related to age in old rats have been reported. In a similar way, higher levels of carbonyl radicals and 8-OhdG have been found in the brain and other tissues from rats, mice and humans (Sagai and Ichinose, 1980; Sohal and Dubey, 1994; Lee *et al.*, 1997).

According to Halliwell and Gutteridge (1999), the steady state level of oxidative stress is the result of the balance between the levels of damage and the degree of repair or replacement of damaged molecules. According to that, in terms of balance, a net increase in oxidative stress during ageing may be found in terms of higher levels of damage or as the result of defects in the repair system. In that sense, a positive correlation between the efficiency of DNA repair systems and species longevity has been observed (Barnett and King, 1995). On other hand, it has been reported that the capacity of several cell lines to degrade abnormal proteins and to repair DNA seems to diminish with ageing. Thus, conceptually, the term 'oxidative stress', as well as considering the level of ROS production and the fall in the antioxidant capacity, should be extended to the complex, and not yet well understood, framework of the damage repairing systems (Bohr and Anson, 1999).

In relation to the response of antioxidant defences, it seems that overall protection does not decline with ageing (Kellog and Fridovich, 1976; Quiles *et al.*, 2004), although several exceptions have been described.

5.4. Nutrition and ageing

Nutrition has been related to ageing for some time. That link has been focused mainly at the level of caloric restriction and supplementation with antioxidants. The role of caloric restriction, i.e. a limitation in the food intake, was first described in 1935 by McCay *et al.* (1989). Since then, caloric restriction has been demonstrated to enhance mean life span in a wide range of species and decrease the development of age-related disease in rodents (Finkel and Holbrook, 2000; Masoro, 2000). These effects are suggested to be mediated by a reduction in the level of oxidative stress. This is supported by the fact that calorically restricted mice experience less oxidative stress than their counterparts fed *ad libitum* (Finkel and Holbrook, 2000; Masoro, 2000). In addition, caloric restriction prevents many of the changes found at the level of gene expression during ageing (e.g., increase in the expression of heat shock proteins). Caloric restriction might be a powerful therapeutic tool to fight against ageing since, a priori, it fulfils with the required needs of effectiveness against oxidative stress and ageing (Roth *et al.*, 1999). Nevertheless, the possible use of caloric restriction as an anti-ageing therapy in humans would involve practical and ethical difficulties that make it almost impossible to consider feasible or desirable (Finkel and Holbrook, 2000).

As described above, oxidative stress plays a very important role in the global process of ageing. Thus, nutritional supplementation with molecules or substances endowed with antioxidant capacity should be useful as a possible anti-ageing therapy. Miquel and Economos (1979) performed some of the first studies

in this field. These authors studied the capacity of thiazolidine carboxylate to enhance vitality and mean life span in mice. Later, Furukawa *et al.* (1987) showed the role of glutathione in protection against the decline in immune function associated with ageing. Many other antioxidants (including vitamin E, vitamin C, coenzyme Q, herbal extracts rich in polyphenols and flavonoids, and others) have been tested in relation to ageing, showing more or less positive results (Halliwell and Gutteridge, 1999; Huertas *et al.*, 1999; Watson *et al.*, 2000). Although results found with these antioxidants have been successful in relation to the attenuation of the age-related oxidative stress, they had low or no success in extending life span. The reasons are not clear, but perhaps require a deeper understanding of the pharmacological properties of the studied molecules, particularly in relation to absorption, tissue distribution and metabolism. Furthermore, it should be remembered that ROS play a role in cell signalling and therefore the antioxidant dosage must be carefully adjusted in order to avoid changes in the redox state that could alter the cell function. These problems are being addressed through the use of a new generation of synthetic antioxidant substances, mimetics of the superoxide dismutase and catalase enzymes. These substances are being assayed with some success, for example in relation to the extension of longevity in mice and *Caenorabditis elegans* (Melov *et al.*, 1998; Rong *et al.*, 1999).

5.5. Olive oil, dietary fatty acids and oxidative stress: a new approach to the study of ageing

According to the above-mentioned premises, dietary fat type affects mitochondrial structure and function as well as their susceptibility to experiencing oxidative stress. Thus, if we build 'customized' biological membranes according to a particular dietary fat type, it would be possible to modify in a positive way the manner in which different organs aged. This working hypothesis represents a new approach to the study of ageing from the point of view of nutrition and it could have important implications for the study of the ageing phenomenon.

We conducted experiments using virgin olive oil or sunflower oil as the source of dietary fat in relation to mitochondrial ageing in a rat model using three different groups. The first group we used to investigate parameters related to general oxidative stress status. The second group generated findings concerning the effects of dietary fat type and ageing on liver mitochondrial DNA deletions and ultrastructural abnormalities. The third group showed differences found between mitotic and non-mitotic tissues concerning aspects of mitochondrial function and oxidative stress status during ageing and the possible effect of the studied dietary fats.

5.5.1. Effects on general oxidative stress status

Some studies suggest that fatty acids become more saturated with ageing, this being associated with a decrease in the degree of polyunsaturation (Ulmann *et al.*, 1991; Imre *et al.*, 2000). Most authors, however (e.g. Engler *et al.*, 1998), report lower levels of saturated fatty acids (SFA) with ageing as well as increased

levels of selected polyunsaturated fatty acids (PUFA). In our study PUFA were higher in animals fed on sunflower oil. Ageing increased these parameters for both dietary groups (Fig. 6.9A). These results suggest that net changes in fatty acids during ageing depend to some extent on the specific dietary fat, although a general increase in polyunsaturation is evident. Consequences of the intake of n-6 PUFA or MUFA on health are well established, apart from those related to the effects on blood lipids (Quiles *et al.*, 2004). Plasma and subcellular-membrane lipid profiles of animals fed on diets with PUFAn-6 fatty acids are associated with higher levels of oxidative stress than when animals are fed on virgin olive oil.

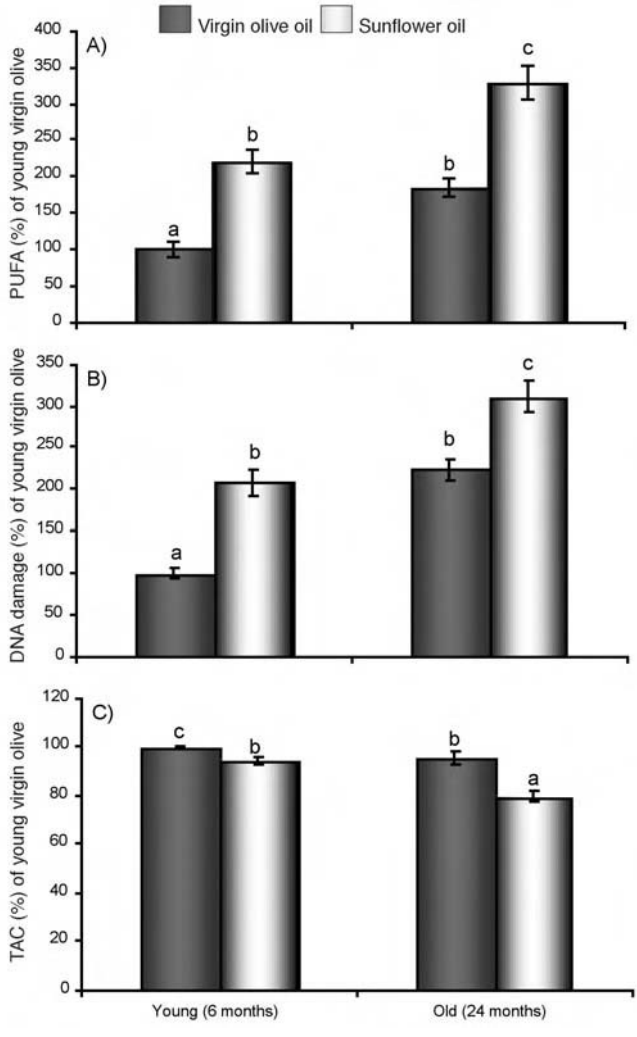


Fig. 6.9. Plasma levels of polyunsaturated fatty acids (PUFA), and total antioxidant capacity (TAC), and double strand breaks in DNA of peripheral blood lymphocytes of young (6 months old) and aged (24 months old) rats fed on virgin olive or sunflower oils based diets. For each parameter, means with different letters are statistically ($P < 0.05$) different.

Opposing results concerning free radical damage and ageing have been reported in the past years (Halliwell and Gutteridge, 1999). However, overall, it appears that free radical damage (and particularly the levels of DNA damage) increases during ageing (Halliwell and Gutteridge, 1999; Chevanne *et al.*, 2003). In our study, when studying DNA damage in peripheral blood lymphocytes (in terms of DNA double-strand breaks), as shown in Fig. 6.9B, we found the lowest levels in young animals fed on virgin olive oil, which were approximately 50% lower than the damage found in the sunflower oil group. Ageing increased levels of DNA oxidative damage in both dietary fat groups, with the highest values found in the sunflower oil group. This finding agrees with the above-mentioned assumption of higher DNA damage with ageing, and is additionally in accord with the free radical theory of ageing of Harman (2003).

The level of DNA damage can be correlated with the antioxidants present in the organism and with their lipid profile. Results support the hypothesis that greater damage occurs when the lipid profile is more polyunsaturated, as for animals fed on a sunflower oil based diet, and for old animals from both dietary groups, in which polyunsaturation increased with age. In relation to antioxidants, total antioxidant capacity may be considered as a marker of all the antioxidants present in the organism. The highest total antioxidant capacity (Fig. 6.9C) for both young and old animals was observed in animals fed on virgin olive oil. In addition we found a very good inverse correlation ($r = -0.725$; $P < 0.01$, for sunflower oil group and $r = -0.535$; $P < 0.01$, for virgin olive oil group) between DNA damage and total antioxidant capacity.

In summary, dietary fat type should be considered in studies on ageing, since the intake of oils with different polyunsaturation levels directly modulates total antioxidant capacity of plasma and DNA damage to peripheral blood lymphocytes and leads to important changes at the lipid metabolism level. In the present study there were benefits of olive oil intake, which suggest the possible use of that edible oil to provide a healthier ageing.

5.5.2. Effects on liver mitochondrial DNA deletions and ultrastructural abnormalities

The liver is the central metabolic organ of the body, therefore dietary changes can have a major impact on ageing liver and on general health (Anantharaju *et al.*, 2002). Moreover, the liver is critical in protection from oxidative damage and plays a major role in the breakdown of potentially harmful lipophilic toxins (Thomas *et al.*, 2002). Although the ageing liver appears to preserve its function relatively well (Anantharaju *et al.*, 2002), several changes have been associated with this organ during the process of ageing. We therefore investigated possible effects on the frequency of liver mitochondrial DNA deletions, oxidative stress and mitochondrial abnormalities in liver mitochondria during ageing by following the previously described model of feeding rats lifelong with two different dietary fat sources (virgin olive or sunflower oils).

There is substantial evidence from human and animal studies linking mtDNA deletions and ageing. Deletion frequency is affected by age, tissue of origin, species, the presence of some age-related diseases (such as Alzheimer's)

and also appears highly variable depending on the laboratory (Kang *et al.*, 1998). We have found an increased frequency of the so-called common deletion at the mtDNA levels in aged animals (Table 6.3), with those fed on sunflower oil being twice as frequent as those fed on virgin olive oil. This finding demonstrates that the age-related rise in mtDNA deletions can be modulated by dietary fat type. A similar effect was previously demonstrated for caloric restriction (Kang *et al.*, 1998). mtDNA deletions corresponded with the increased levels of oxidative stress with ageing in both dietary groups, although this increase was greater in animals fed on sunflower oil. We investigated whether changes in the frequency of mtDNA deletions and oxidative stress status could affect mitochondrial ultrastructure under our experimental conditions. We found that sunflower oil led to a deterioration in mitochondrial structure (Table 6.3, Fig. 6.10), as suggested by the lower percentage of cristae per μm of mitochondrial contour found in old animals fed on sunflower oil compared with the young animals fed on the same oil. Additionally, animals fed on virgin olive oil had a higher number of mitochondrial cristae at both age periods. Mitochondrial circularity (whose increase represents control loss) was higher in old animals fed on sunflower oil compared with those fed on virgin olive oil. These results demonstrate that the age-related increase in liver mtDNA deletion frequency is differentially modulated by the intake of different dietary fats, with virgin olive oil leading to a lower frequency of deletions than the n-6 polyunsaturated sunflower oil. On the other hand, mtDNA deletion frequency could be correlated with mitochondrial oxidative stress status and ultrastructural alterations.

5.5.3. Differences between mitotic and non-mitotic tissues

Ageing, understood as an endogenous and progressive phenomenon (Barja, 2002), leads throughout the life span to different disturbances in mitochondria and their components, such as mtDNA (Sohal and Dubey, 1994; Lee *et al.*, 1997; Michikawa *et al.*, 1999). These disturbances (which have a high oxidative component) have a negative impact on mitochondrial structure and function. Depending on the capacity of the affected tissue to repair the damage or to replace the altered cell, tissue function will be affected to a greater or lesser

Table 6.3. Effect of ageing and of feeding lifelong rats on virgin olive or sunflower oil based diets on liver mitochondrial DNA deletions and ultrastructural abnormalities.

	Young animals (6 months)		Old animals (24 months)	
	Virgin olive oil	Sunflower oil	Virgin olive oil	Sunflower oil
mtDNA common deletion*	100 ± 0^{a}	100 ± 0^{a}	451 ± 48^{b}	823 ± 184^{c}
Mitochondrial circularity*	100 ± 1.5^{a}	104.7 ± 1.7^{a}	102.9 ± 1.9^{a}	113.3 ± 1.1^{b}
Mitochondrial cristae number*	100 ± 3.8^{c}	78.5 ± 2.5^{b}	94.9 ± 3.2^{c}	60.7 ± 1.8^{a}

Results show means ± SEM (n = 8). For each variable; values in a row not sharing superscript letters are significantly different ($P < 0.05$). *Results are presented as percentage of change relative to young animals fed on virgin olive oil.

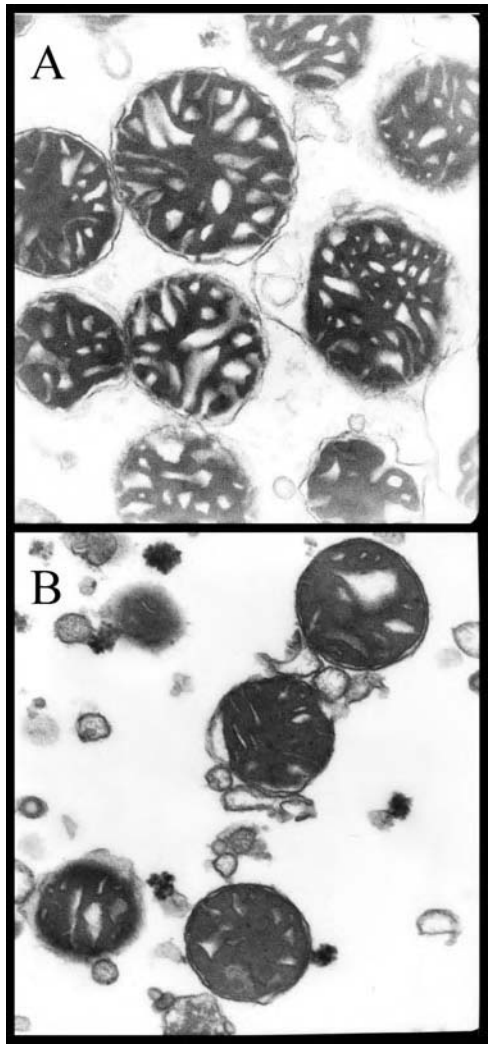


Fig. 6.10. Transmission electron microscopy (TEM) images of liver mitochondrial membranes of young (A, 6 months old) and aged (B, 24 months old) rats fed on sunflower oil based diet. Magnification: 40000 ×.

extent (Quiles *et al.*, 2002b). In this way, tissues with the ability to regenerate their cells, such as liver, appear to be able to buffer the damage, at least in part, as suggested by the lack of alterations of mitochondrial function in terms of cytochrome c oxydase activity (Fig. 6.11; Quiles *et al.*, 2002b). However, a loss in function is found in postmitotic tissues like skeletal muscle, heart or brain. These tissues lack the opportunity to replace damaged cells and are likely to have a less effective repairing system (differences between liver and heart concerning repair mechanisms for mtDNA damage have been already reported (Souza-Pinto *et al.*, 1999)). This loss in function is reflected in the substantial decrease in cytochrome c oxidase activity, which leads to the uncoupling of

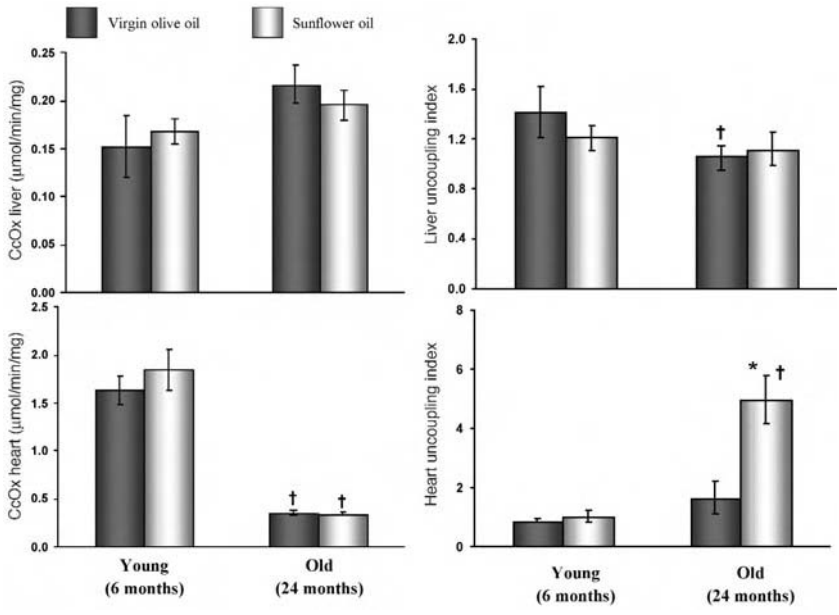


Fig. 6.11. Cytochrome c Oxidase (CcOx) and uncoupling index in liver and heart mitochondria from young (6 months old) and aged (24 months old) rats fed on virgin olive or sunflower oils based diets. Statistical significances ($P < 0.05$): * For each age group: virgin olive *vs* sunflower; † For each dietary group: young *vs* old.

the mtETC, with the further bioenergetic inefficacy and an increase in ROS production (Figs 6.11 and 6.12; Quiles *et al.*, 2002b; Ochoa *et al.*, 2003). Mitochondria from postmitotic tissues try to buffer the unfavourable situation by an increase in some elements of the mtETC, such as cytochrome b or polyunsaturated fatty acids. The increase in polyunsaturation could enhance membrane fluidity and cytochrome c oxidase activity by the presence of a more polyunsaturated cardiolipin, as has been previously suggested (Huertas *et al.*, 1991b; Quiles *et al.*, 2001). However, both actions lead to a rise in the ROS production (Fig. 6.12). The role of dietary fat in this mechanism could reside in the building of an environment more or less prone to the generation and propagation of ROS, especially when, as the result of events like ageing, failures in the mtETC start to appear. Moreover, dietary fat could modulate the phenomenon through variations in the antioxidant system and overall upregulate or attenuate the process. Thus, as postmitotic tissues are the most affected by ageing, the influence of diet should be particularly important in these tissues. Figure 6.13 shows a summary of the proposed mechanisms described in this section.

In summary, results described in the last three sections open a new and exciting way to investigate the mechanisms involved in the benefits of virgin olive oil in relation to ageing. In that sense, new studies are being developed to investigate issues such as the possible modification of the mtDNA repair systems or the changes in the nuclear and mitochondrial gene expression profile after the intake of virgin olive oil and their consequences on the ageing process.

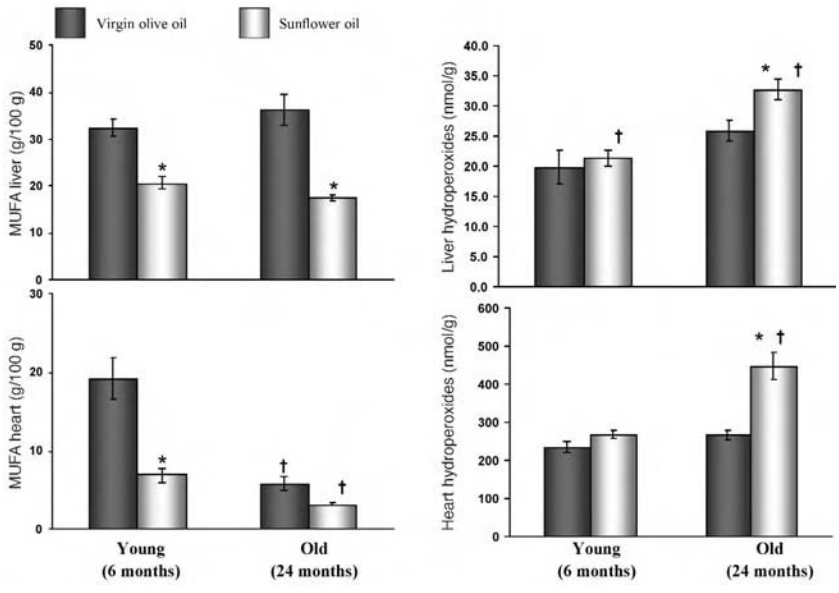


Fig. 6.12. Monounsaturated fatty acids (MUFA) and hydroperoxides in liver and heart mitochondria from young (6 months old) and aged (24 months old) rats fed on virgin olive or sunflower oils based diets. Statistical significances ($P < 0.05$): * For each age group: virgin olive *vs* sunflower oils; † For each dietary group: young *vs* old.

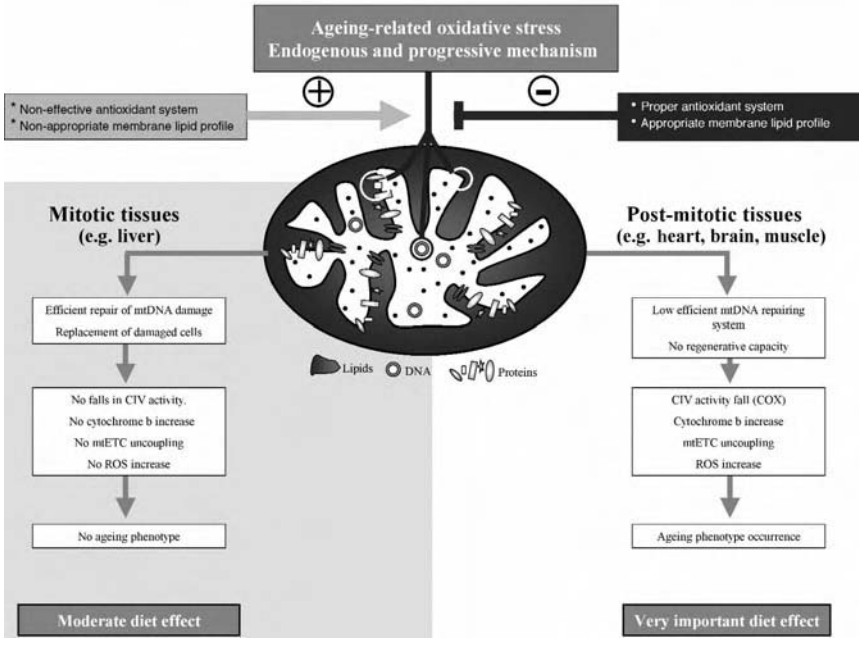


Fig. 6.13. Proposed mechanism to explain why dietary lipids can modulate the way in which an organism ages, depending on the tissue type. Such a mechanism would involve the mitochondrial-membrane lipid profile and bioenergetics, together with the particular regenerative capacity of the specific tissue.

Acknowledgements

Dr José L. Quiles and Dr Julio J. Ochoa are supported by a 'Ramón y Cajal' contract from the Spanish Ministry of Education and Science and the University of Granada. We are grateful for financial support to the Spanish Ministry of Education and Science (Grants: ALI91-1113-C03-01; ALI95-1036-C05-04; 1FD97-0457-C02-01; and AGL2003-05693) and to the International Olive Oil Council.

6. References

Agarwal, S. and Sohal, R.S. (1994) DNA oxidative damage and life expectancy in houseflies. *Proceedings of the National Academy of Sciences of the United States of America* 91, 12332–12335.

Anantharaju, A., Feller, A. and Chedid, A. (2002) Aging liver. *Gerontology* 48, 343–353.

Balakrishnan, S.D. and Anuradha, C.V. (1998) Exercise, depletion of antioxidants and antioxidant manipulation. *Cell Biochemistry and Function* 16, 269–275.

Barja, G. (2002) Rate of generation of oxidative stress-related damage and animal longevity. *Free Radical Biology and Medicine* 33, 1167–1172.

Barnett, Y.A. and King, C.M. (1995) An investigation of antioxidant status, DNA repair capacity and mutation as a function of age in humans. *Mutation Research* 338, 115–128.

Battino, M., Ferreiro, M.S., Littarru, G., Quiles, J.L., Ramírez-Tortosa, M.C., Huertas, J.R., Mataix, J., Villa, R.F. and Gorini, A. (2002a) Structural damages induced by peroxidation could account for functional impairment of heavy synaptic mitochondria. *Free Radical Research* 36, 479–484.

Battino, M., Quiles, J.L., Huertas, J.R., Ramírez-Tortosa, M.C., Cassinello, M., Mañas, M., López-Frías, M. and Mataix, J. (2002b) Feeding fried oil changes antioxidant and fatty acid pattern of rat and affects rat liver mitochondrial respiratory chain components. *Journal of Bioenergetics and Biomembranes* 34, 127–134.

Bohr, V.A. and Anson, R.M. (1999) Mitochondrial DNA repair pathways. *Journal of Bioenergetics and Biomembranes* 31, 391–398.

Buja, L.M., Ferrans, V.J., Mayers, R.J., Robert, W.C. and Henderson, E.S. (1973) Cardiac ultrastructural changes induced by daunorubicin therapy. *Cancer* 32, 771–778.

Cadenas, E. and Davies, K.J.A. (2000) Mitochondrial free radical generation, oxidative stress and aging. *Free Radical Biology and Medicine* 29, 222–230.

Cadenas, E., Boveris, A., Ragan, C.I. and Stoppani, A.O.M. (1977) Production of superoxide radicals and hydrogen peroxide by NADH-ubiquinone reductase and ubiquinol-cytochrome c reductase from beef-heart mitochondria. *Archives of Biochemistry and Biophysics* 180, 248–257.

Camougrand, N. and Rigoulet, M. (2001) Aging and oxidative stress: studies of some genes involved both in aging and in response to oxidative stress. *Respiration Physiology* 128, 393–401.

Chevanne, M., Caldini, R., Tombaccini, D., Mocali, A., Gori, G. and Paoletti, F. (2003) Comparative levels of DNA breaks and sensitivity to oxidative stress in aged and senescent human fibroblasts: a distinctive pattern for centenarians. *Biogerontology* 4, 97–104.

Chiaradia, E., Avellini, L., Rueca, F., Spaterna, A., Porciello, F., Antonioni, M.T. and Gaiti, A. (1998) Physical exercise, oxidative stress and muscle damage in racehorses. *Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology* 119, 833–836.

Chung, M.H., Kasai, H., Nishimura, S. and Yu, B.P. (1992) Protection of DNA damage by dietary restriction. *Free Radical Biology and Medicine* 12, 523–525.

Cortopassi, G.A., Shibata, D., Soong, N.W. and Arnheim, N. (1992) A pattern of accumula-

tion of a somatic deletion of mitochondrial DNA in aging human tissues. *Proceedings of the National Academy of Sciences of the United States of America* 89, 7370–7374.

Criswell, D., Powers, S., Dodd, S., Lawler, J., Edwards, W., Renshler, K. and Grinton, S. (1993) High intensity training-induced changes in skeletal muscle antioxidant enzyme activity. *Medicine and Science in Sports and Exercise* 25, 1135–1140.

Cross, A.R. and Jones, O.T. (1991) Enzymic mechanisms of superoxide production. *Biochimica et Biophysica Acta* 1057, 281–298.

Cullinane, C., Cutts, S.M., van Rosmalen, A. and Phillips, D.R. (1994) Formation of adriamycin-DNA adducts *in vitro. Nucleic Acids Research* 22, 2296–2303.

De Beer, E.L., Bottone, A.E. and Voest, E.E. (2001) Doxorubicin and mechanical performance of cardiac trabeculae after acute and chronic treatment: a review. *European Journal of Pharmacology* 415, 1–11.

De Benedictis, G., Carrieri, G., Varcasia, O., Bonafè, M. and Franceschi, C. (2000) Inherited variability of the mitochondrial genome and successful aging in humans. *Annals of the New York Academy of Sciences* 908, 208–218.

Dillard, C.J., Litov, R.E., Savin, W.M., Dumelin, E.E. and Tappel, A.L. (1978) Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation. *Journal of Applied Physiology* 45, 927–932.

Engler, M.M., Engler, M.B. and Nguyen, H. (1998) Age-related changes in plasma and tissue fatty acid composition in fischer 344 rats. *Biochemistry and Molecular Biology International* 46, 1117–1126.

Esterbauer, H., Schaur, R.J. and Zollner, H. (1991) Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. *Free Radical Biology and Medicine* 11, 81–128.

Feinstein, E., Canaani, E. and Weiner, L.M. (1993) Dependence of nucleic acid degradation on *in situ* free-radical production by adriamycin. *Biochemistry* 32, 13156–13161.

Finkel, T. and Holbrook, N.J. (2000) Oxidants, oxidative stress and the biology of ageing. *Nature*, 408, 239–247.

Forsmark-Andree, P., Lee, C.P., Dallner, G. and Ernster, L. (1997) Lipid peroxidation and changes in the ubiquinone content and the respiratory chain enzymes of submitochondrial particles. *Free Radical Biology and Medicine* 22, 391–400.

Furukawa, T., Meydani, S.N. and Blumberg, J.B. (1987) Reversal of age-associated decline in immune responsiveness by dietary glutathione supplementation in mice. *Mechanisms of Ageing and Development* 38, 107–117.

Gewirtz, D.A. (1999) A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. *Biochemical Pharmacology* 57, 727–741.

Gianni, L., Zweier, J.L., Levy, A. and Myers, C.E. (1985) Characterization of the cycle of iron-mediated electron transfer from adriamycin to molecular oxygen. *The Journal of Biological Chemistry* 260, 6820–6826.

Goormaghtigh, E. and Ruysschaert, J.M. (1984) Anthracycline glycoside–membrane interactions. *Biochimica et Biophysica Acta* 779, 271–288.

Gutteridge, J.M.C. and Quinlan, G.J. (1985) Free radical damage to deoxyribose by anthracycline, aureolic acid and aminoqui-none antitumour antibiotics. An essential requirement for iron, semiquinones and hydrogen peroxide. *Biochemical Pharmacology* 34, 4099–4103.

Halliwell, B. and Gutteridge, J.M.C. (1999) *Free radicals in biology and medicine*, 3rd edn, Oxford University Press, Oxford, 723 pp.

Harman, D. (1956) Aging: a theory based on free radical and radiation chemistry. *Journal of Gerontology* 11, 298–300.

Harman, D. (1972) The biologic clock: the mitochondria? *Journal of the American Geriatrics Society* 20, 145–147.

Harman, D. (2003) The free radical theory of aging. *Antioxidant Redox Signal* 5, 557–561.

Hauptmann, N., Grimsby, J., Shih, J.C. and Cadenas, E. (1996) The metabolism of tyramine by monoamine oxidase A/B causes oxidative damage to mitochondrial DNA. *Archives of Biochemistry and Biophysics* 335, 295–304.

Higashi, Y. and Yoshizyumi, M. (2004) Exercise

and endothelial function: Role of endothelium-derived nitric oxide and oxidative stress in healthy subjects and hypertensive patients. *Pharmacology and Therapeutics* 102, 87–96.

Huertas, J.R., Battino, M., Lenaz, G. and Mataix, F.J. (1991a) Changes in mitochondrial and microsomal rat liver coenzyme Q_9 and Q_{10} content induced by dietary fat and endogenous lipid peroxidation. *FEBS Letters* 287, 89–92.

Huertas, J.R., Battino, M., Mataix, F.J. and Lenaz, G. (1991b) Cytochrome oxidase induction after oxidative stress induced by adriamycin in liver of rats fed with dietary olive oil. *Biochemical and Biophysical Research Communications* 181, 375–382.

Huertas, J.R., Battino, M., Barzanti, V., Maranesi, M., Parenti-Castelli, G., Littarru, G.P., Turchetto, E., Mataix, F.J. and Lenaz, G. (1992) Mitochondrial and microsomal cholesterol mobilization after oxidative stress induced by adriamycin in rats fed with dietary olive and corn oil. *Life Sciences* 50, 2111–2188.

Huertas, J.R., Martínez-Velasco, E., Ibáñez, S., López-Frías, M., Ochoa, J.J., Quiles, J.L., Parenti-Castelli, G., Mataix, J. and Lenaz, G. (1999) Virgin olive oil protect heart mitochondria from peroxidative damage during aging. *BioFactors* 9, 337–343.

Imre, S., Firbas, J.H. and Noble, R.C. (2000) Reduced lipid peroxidation capacity and desaturation as biochemical markers of aging. *Archives of Gerontology and Geriatry* 31, 5–12.

Ji, L.L. (1999) Antioxidant and oxidative stress in exercise. *Proceedings of the Society for Experimental Biology and Medicine* 222, 283–292.

Kang, C.M., Kristal, B.S. and Yu, B.P. (1998) Age-related mitochondrial DNA deletions: effect of dietary restriction. *Free Radicals Biology & Medicine* 24, 148–154.

Kellog, E.W. and Fridovich, I. (1976) Superoxide dismutase in the rat and mouse as a function of age and longevity. *Journal of Gerontology* 31, 405–408.

Ksenzenko, M., Konstantinov, A.A., Khomutov, G.B., Tikhnov, A.N. and Ruuge, E.K. (1983) Effect of electron transfer inhibitors on superoxide generation in the cytochrome bc1 site of the mitochondrial respiratory chain. *FEBS Letters* 155, 19–24.

Lee, C.M., Weindruch, R. and Aiken, J.M. (1997) Age-associated alterations of the mitochondrial genome. *Free Radical Biology and Medicine* 22, 1259–1269.

Lefrak, E.A., Pitha, J., Rosenheim, S. and Gottleib, J.A. (1973) A clinicopathologic analysis of adriamycin cardiotoxicity. *Cancer* 32, 302–314.

Lenaz, G. (1998) Role of mitochondria in oxidative stress and ageing. *Biochimica et Biophysica Acta* 1366, 53–67.

Lippe, G., Comelli, M., Mazzilis, D., Sala, F.D. and Mavelli, L. (1991) The inactivation of mitochondrial F1 ATPase by H_2O_2 is mediated by iron ions not tightly bound in the protein. *Biochemical and Biophysical Research Communications* 181, 764–770.

Masoro, E.J. (2000) Caloric restriction and aging: an update. *Experimental Gerontology* 35, 299–305.

Mastaloudis, A., Yu, D., O'Donnell, R.P., Frei, B., Dashwood, R.H. and Traber, M.G. (2004) Endurance exercise results in DNA damage as detected by the comet assay. *Free Radical Biology and Medicine* 15, 966–975.

Mataix, J. (2001) Aceite de oliva virgen: nuestro patrimonio alimentario. Universidad de Granada y PULEVA Food, Granada, Spain.

Mataix, J., Mañas, M., Quiles, J.L., Battino, M., Cassinello, M., López-Frías, M. and Huertas, J.R. (1997) Coenzyme Q content depends upon oxidative stress and dietary fat unsaturation. *Molecular Aspects of Medicine* 18, 129–135.

Mataix, J., Quiles, J.L., Huertas, J.R., Battino, M. and Mañas, M. (1998) Tissue specific interactions of exercise, dietary fatty acids, and vitamin E in lipid peroxidation. *Free Radical Biology and Medicine* 24, 511–521.

McCay, C.M., Crowell, M.F. and Maynard, L.A. (1989) The effect of retarded growth upon the length of life span and upon the ultimate body size. 1935. *Nutrition* 5, 155–171.

McKenzie, D.J., Higgs, D.A., Dosanjh, B., Deacon, G. and Randall, D.J. (1998) Dietary lipid composition influences swimming performance in Atlantic salmon (Salmo salar) in seawater. *Fish Physiology and Biochemistry* 19, 111–112.

Melov, S., Schneider, J.A., Day, B.J., Hinerfeld, D., Coskun, P., Mirra, S.S., Crapo, J.D. and Wallace, D.C. (1998) A novel neurological phenotype in mice lacking mitochondrial manganese superoxide dismutase. *Nature Genetics* 18, 159–163.

Michikawa, Y., Mazzucchelli, F., Bresolin, N., Scarlato, G. and Attardi, G. (1999) Aging-dependent large accumulation of point mutations in the human mtDNA control region for replication. *Science* 286, 774–779.

Miquel, J. and Ecónomos, A.C. (1979) Favorable effects of the antioxidants sodium and magnesium thiazolidine carboxylate on the vitality and the life span of Drosophila and mice. *Experimental Gerontology* 14, 279–285.

Miquel, J., Economos, A.C., Fleming, J.E. and Johnson, J.E. (1980) Mitochondrial role in cell aging. *Experimental Gerontology* 15, 579–591.

Miyazaki, H., Oh-Isi, S., Ookawara, T., Kizaki, T., Ha, S., Haga, S., Ji, L.L. and Ohno, H. (2001) Strenuous endurance training in humans reduces oxidative stress following exhausting exercise. *European Journal of Applied Physiology* 84, 1–6.

Montine, T.J., Neely, M.D., Quinn, J.F., Flint Beal, M., Markesbery, W.R., Roberts, L.J. and Morrow, J.D. (2002) Lipid peroxidation in aging brain and alzheimer's disease. *Free Radical Biology and Medicine* 33, 620–626.

O'Neill, C.A., Stebbins, C.L., Bonigut, S., Halliwell, B. and Longhurst, J.C. (1996) Production of hydroxyl radicals in contracting skeletal muscle of cats. *Journal of Applied Physiology* 81, 1197–1206.

Ochoa, J.J., Quiles, J.L., Ramirez-Tortosa, M.C., Mataix, J. and Huertas, J.R. (2002) Dietary oils high in oleic acid but with different unsaponifiable fraction contents have different effects in lipid profile and peroxidation in rabbit-LDL. *Nutrition* 18, 60–65.

Ochoa, J.J., Quiles, J.L., Ibáñez, S., Martínez, E., López-Frías, M., Huertas, J.R. and Mataix, J. (2003) Aging-related oxidative stress depends on dietary lipid source in rat post-mitotic tissues. *Journal of Bioenergetics and Biomembranes* 35, 267–275.

Ochoa-Herrera, J.J., Huertas, J.R., Quiles, J.L. and Mataix, J. (2001) Dietary oils high in oleic acid, but with different non-glyceride contents, have different effects on lipid profiles and peroxidation in rabbit hepatic mitochondria. *The Journal of Nutritional and Biochemistry* 12, 357–364.

Olson, R.D. and Mushlin, P.S. (1990) Doxorubicin cardiotoxicity: analysis of prevailing hypotheses. *The FASEB Journal* 4, 3076–3086.

Paradies, G., Ruggiero, F.M., Petrosillo, G. and Quagliariello, E. (1998) Peroxidative damage to cardiac mitochondria: cytochrome oxidase and cardiolipin alterations. *FEBS Letters* 424, 155–158.

Partridge, L. and Gems, D. (2002) A lethal side-effect. *Nature* 418, 921.

Quiles, J.L. (1995) Comparative study of olive and sunflower oils on lipid peroxidation in rats that underwent physical exercise. PhD thesis, University of Granada, Granada, Spain.

Quiles, J.L., Huertas, J.R., Mañas, M., Battino, M., Cassinello, M., Litarru, G.P., Lenaz, G. and Mataix, F.J. (1994) Peroxidative extent and coenzyme Q levels in the rat: influence of physical training and dietary fats. *Molecular Aspects of Medicine* 15, s89–s95.

Quiles, J.L., Huertas, J.R., Mañas, M., Battino, M., Ochoa, J.J. and Mataix, J. (1998) Plasma antioxidants are strongly affected by iron-induced lipid peroxidation in rats subjected to physical exercise and different dietary fats. *Biofactors* 8, 119–127.

Quiles, J.L., Huertas, J.R., Mañas, M., Battino, M. and Mataix, J. (1999a) Physical exercise affects the lipid profile of mitochondrial membranes in rats fed with virgin olive oil or sunflower oil. *The British Journal of Nutrition* 81, 21–24.

Quiles, J.L., Huertas, J.R., Mañas, M., Ochoa, J.J., Battino, M. and Mataix, J. (1999b) Oxidative stress induced by exercise and dietary fat modulates the coenzyme Q and vitamin A balance between plasma and mitochondria. *International Journal for Vitamin and Nutrition Research* 69, 243–249.

Quiles, J.L., Ramírez-Tortosa, M.C., Ibáñez, S., González, A., Duthie, G.G., Huertas, J.R. and Mataix, J. (1999c) Vitamin E supplementation increases the stability and the *in vivo* antioxidant capacity of refined olive oil. *Free Radical Research* 31, 129–135.

Quiles, J.L., Ramírez-Tortosa, M.C., Huertas,

J.R., Ibáñez, S., Gómez, J.A., Battino, M. and Mataix, J. (1999d). Olive oil supplemented with vitamin E affects mitochondrial coenzyme Q levels in liver of rats after an oxidative stress induced by adriamycin. *Biofactors* 9, 331–336.

Quiles, J.L., Huertas, J.R., Mañas, M., Ochoa, J.J., Battino, M. and Mataix, J. (2001) Dietary fat type and regular exercise affect mitochondrial composition and function depending on specific tissue in rat. *Journal of Bioenergetics and Biomembrane* 33, 127–143.

Quiles, J.L., Huertas, J.R., Battino, M., Mataix, J. and Ramirez-Tortosa, M.C. (2002a) Antioxidant nutrients and adriamycin toxicity. *Toxicology* 180, 79–95.

Quiles, J.L., Martínez, E., Ibáñez, S., Ochoa, J.J., Martín, Y., López-Frías, M., Huertas, J.R. and Mataix, J. (2002b) Ageing-related tissue-specific alterations in mitochondrial composition and function are modulated by dietary fat type in the rat. *Journal of Bioenergetics and Biomembrane* 34, 517–524.

Quiles, J.L., Huertas, J.R., Ochoa, J.J., Battino, M., Mataix, J. and Mañas, M. (2003) Dietary fat (virgin olive oil or sunflower oil) and physical training interactions on blood lipids in the rat. *Nutrition* 19, 363–368.

Quiles, J.L., Ochoa, J.J., Ramírez-Tortosa, M.C., Battino, M., Huertas, J.R., Martín, Y. and Mataix, J. (2004) Dietary fat type (virgin olive *vs.* sunflower oils) affects age-related changes in DNA double-strand-breaks, antioxidant capacity and blood lipids in rats. *Experimental Gerontology* 39, 1189–1198.

Ramírez-Tortosa, M.C., López-Pedrosa, J.M., Suarez, A., Ros, E., Mataix, J. and Gil, A. (1999) Olive oil and fish oil enriched diets modify plasma lipids and susceptibility of low density lipoprotein to oxidative modification in free-living male patients with peripheral vascular disease: the Spanish Nutrition Study. *The British Journal of Nutrition* 82, 31–39.

Reid, M.B., Haack, K.E., Franchek, K.M., Valberg, P.A., Kobzik, L. and West, S. (1992) Reactive oxygen in skeletal muscle I. Intracellular oxidant kinetics and fatigue in vitro. *Journal of Applied Physiology* 73, 1797–1804.

Richter, C., Park, J.W. and Ames, B.N. (1988) Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proceedings of the National academy of Sciences of the United States of America* 85, 6465–6467.

Rong, Y., Doctrow, S.R., Tocco, G. and Baudry, M. (1999) EUK-134, a synthetic superoxide dismutse and catalase mimetic, prevents oxidative stress and attenuates kainate-induced neuropathology. *Proceedings of the National Academy of Sciences of the United States of America* 96, 9897–9902.

Ross, W.R., Glaubiger, D.L., Kohn, K.W. (1978) Protein-associated DNA breaks in cells treated with adriamycin or ellipticine. *Biochimica et Biophysica Acta* 519, 23–30.

Roth, G.S., Ingram, D.K. and Lane, M.A. (1999) Calorie restriction in primates: will it work and how will we know? *Journal of the American Geriatrics Society* 47, 896–903.

Sagai, M. and Ichinose, T. (1980) Age-related changes in lipid peroxidation as measured by ethane, ethylene, butane and pentane in respired gases of rats. *Life Sciences* 27, 731–738.

Salvioli, S., Bonafè, M., Capri, M., Monti, D. and Franceschi, C. (2001) Mitochondria, aging and longevity – a new perspective. *FEBS Letters* 492, 9–13.

Sastre, J., Pallardó, F.V., García de la Asunción, J. and Viña, J. (2000) Mitochondria, oxidative stress and aging. *Free Radical Research* 32, 189–198.

Selamoglu, S., Turgay, F., Kayatekin, B.M., Gonenc, S. and Yslegen, C. (2000) Aerobic and anerobic training effects on the antioxidant enzymes of the blood. *Acta Physiologica Hungarica* 87, 267–273.

Sen, C.K. (1995) Oxidants and antioxidants in exercise. *Journal of Applied Physiology* 79, 675–686.

Shimomura, Y., Nishikimi, M. and Ozawa, T. (1985) Novel purification of cytochrome c1 from mitochondrial Complex III. Reconstitution of antimycin-insensitive electron transfer with the iron-sulfur protein and cytochrome c1. *The Journal of Biological Chemistry* 260, 15075–15080.

Singal, P.K. and Iliskovic, N. (1998) Adriamycin cardiomyopathy. *The New England Journal of Medicine* 339, 900–905.

Singal, P.K., Deally, C.M.R. and Weinberg, L.E.

(1987) Subcellular effects of adriamycin in the heart: a concise review. *Journal of Molecular and Cellular Cardiology* 19, 817–828.

Singal, P.K., Li, T., Kumar, D., Danelisen, I. and Iliskovic, N. (2000) Adriamycin-induced heart-failure: mechanism and modulation. *Molecular and Cellular Biochemistry* 207, 77.85.

Sinha, B.K. and Polliti, P.M. (1990) Anthracyclines. *Cancer Chemotherapy and Biological Response Modifiers* 11, 45–57.

Skladanowski, A. and Konopa, J. (1994) Interstrand DNA crosslinking induced by anthracyclines in tumour cells. *Biochemical Pharmacology* 47, 2269–2278.

Sohal, R.S. and Dubey, A. (1994) Mitochondrial oxidative damage, hydrogen peroxide release, and aging. *Free Radical Biology and Medicine* 16, 621–626.

Sohal, R.S., Mockett, R.J., and Orr, W.C. (2002) Mechanisms of aging: an appraisal of the oxidative stress hypothesis. *Free Radical Biology and Medicine* 33, 575–586.

Souza-Pinto, N., Croteau, D.L., Hudson, E.K., Heansford, E.G. and Bohr, V.A. (1999) Age-associated increase in 8-oxo-deoxyguanosine glycosylase/AP lyase activity in rat mitochondria. *Nucleic Acids Research* 27, 1935–1942.

Tanaka, M. and Yoshida, S. (1980) Mechanism of the inhibition of calf thymus DNA polymerases a and b by daunomycin and adriamycin. *Journal of Biochemistry* 87, 911–918.

Tanaka, M., Gong, J., Zhang, J., Yamada, Y., Borgeld, H. and Yagi, K. (1998) Mitochondrial genotype associated with longevity. *Lancet* 351, 185–186.

Thomas, R.P., Guigneaux, M., Wood, T. and Mark Evers, B. (2002) Age-associated changes in gene expression patterns in the liver. *Journal of Gastrointestinal Surgery* 6, 445–454.

Ulmann, L., Blond, J.P., Maniongui, C., Poisson, J.P., Duran, G., Bezard, J. and Pascal, G. (1991) Effects of age and dietary fatty acids on desaturase activities and on fatty acid composition of liver microsomal phospholipids of adult rats. *Lipids* 26, 127–133.

Urso, M.L. and Clarkson, P.C. (2003) Oxidative stress, exercise, and antioxidant supplementation. *Toxicology* 189, 41–54.

Van Remmen, H. and Richardson, A. (2001) Oxidative damage to mitochondria and aging. *Experimental Gerontology* 36, 957–968.

Wagner, G.N., Balfry, S.K., Higgs, D.A., Lall, S.P. and Farrel, A.P. (2004) Dietary fatty acid composition affects the repeat swimming performance of Atlantic salmon in seawater. *Comparative Biochemistry and Physiology Part A* 137, 567–576.

Watson, W.H., Cai, J. and Jones, D.P. (2000) Diet and apoptosis. *Annual Review of Nutrition* 20, 485–505.

Yoneda, M., Katsumata, K., Hayakawa, M., Tanaka, M. and Ozawa, T. (1995) Oxygen stress induces an apoptotic cell death associated with fragmentation of mitochondrial genome. *Biochemical and Biophysical Research Communications* 209, 723–729.

7 Epidemiology of Olive Oil and Cardiovascular Disease

MARIKA MASSARO, EGERIA SCODITTI, MARIA ANNUNZIATA CARLUCCIO AND RAFFAELE DE CATERINA

Laboratory for Thrombosis and Vascular Research, CNR Institute of Clinical Physiology, Pisa, and Institute of Cardiology, 'G d'Annunzio' University, Chieti, Italy

1. Introduction

The importance of dietary patterns in the aetiology of cardiovascular disease is now an established acquisition (Keys, 1975). Over the past 30 years, many epidemiological studies have suggested the existence of a causal link between high dietary intake of saturated fat (and, in part, dietary cholesterol) and serum cholesterol levels (Kato *et al.*, 1973; Keys, 1980; McGee *et al.*, 1984; Kushi *et al.*, 1985; Posner *et al.*, 1991), this last largely considered the major risk factor for coronary heart disease (CHD) (Brown and Goldstein, 1996). It was therefore hypothesized that reduction of plasma cholesterol by dietary means might reduce the risk of coronary heart disease ('The diet-heart' hypothesis). However, only trials that have reproduced Mediterranean or Asian-vegetarian dietary patterns, despite being not particularly low in total and saturated fats, have clearly shown significant reduction in CHD mortality and morbidity, and this has occurred mostly independently of serum cholesterol lowering (Burr *et al.*, 1989; Singh *et al.*, 1992; de Lorgeril *et al.*, 1994).

These clinical results encourage the adoption of Mediterranean dietary patterns. However, such promotion also needs to be solidly based on epidemiological findings. Moreover, speaking about 'the' Mediterranean diet, or trying to isolate the 'key factor' responsible for its health benefits is likely to be a difficult if not impossible task, given that there is not such a thing as a single 'Mediterranean diet', and dietary components are in any case quite diverse. This is the reason why it would appear more logical to speak of 'Mediterranean lifestyle behaviours' (Simopoulos and Pavlou, 2001; Ness, 2002).

At least until the early 1960s, the traditional diet of countries surrounding the Mediterranean Sea featured a high intake of plant foods, whole-grain cereals,

 Olive Oil and Health (eds J.L. Quiles, M.C. Ramírez-Tortosa and P. Yaqoob)

legumes and nuts compared with most Western diets, and abundantly used olive oil, with a very large proportion – about 29% – of daily caloric intake derived from monounsaturated fatty acids (Table 7.1) (Nestle, 1995). It was therefore not an accident that for centuries olive oil, largely produced in Greece and other Mediterranean countries, has also been treasured in those regions as a functional food for its healing properties, in addition to being a source of calories, finally prompting its extension beyond Mediterranean regions. Many authors started to recognize olive oil as one of the key elements in the cardioprotection and longevity of Mediterranean regions. Among these was Ancel Keys, the first world-known supporter of Mediterranean diets, who wrote (Keys, 1987):

> ... I am reminded of Elie Metchnikoff, the successor to Louis Pasteur as director of the Institute Pasteur. He became fascinated with longevity and visited Greece on that account. He concluded that centenarians were ten times more common in Greece than in France. We may discount his theory that the credit should go to yogurt, a foodstuff then unknown in other parts of Europe. In any case, from many survey on the island of Crete, starting in 1957, I have the impression that centenarians are common among farmers, whose breakfast is often only a wineglass of olive oil ...

Despite the complexity of teasing out the healthy role of olive oil from those of other Mediterranean nutrients, such an exercise is not futile for several reasons. One is that a widespread adoption of Mediterranean dietary patterns in the Western world would have deep implications for agriculture and the environment. Until now, the European Union has invested more than 35 million euros in promoting olive oil consumption in its member states. Another is that a solid scientific basis of the knowledge of active principles in Mediterranean diets would help in devising rational choices, in individual nations, to promote specific food items in Mediterranean countries and beyond. We will therefore here review the epidemiological background linking Mediterranean dietary styles in general, and olive oil in particular, to cardioprotection, in order to provide the scientific basis for political actions in such directions.

Table 7.1. Per cent of total energy contributed by major food groups in the diet of Crete compared with their availability in the food supplies of Greece and USA in 1948–1949 (modified from Nestle, 1995).

Food group	Crete	Greece	USA
Foods (%)			
Cereals	34	61	25
Pulses, nuts and potatoes	11	8	6
Vegetables and fruits	11	5	6
Meat, fish and eggs	4	3	19
Dairy products	3	4	14
Oils and fats	29	15	15
Sugar and honey	2	4	15
Energy (kcal/day)	2547	2477	3129

2. Ecological Studies: the Seven Countries Study and the 'Albanian Paradox'

The basic observation underlying the current interest in the adoption of Mediterranean-type dietary habits is that, at least until the early 1960s, adults living in the regions around the Mediterranean Sea had rates of chronic diseases that were among the lowest, as well as life expectancies that were among the highest, in the world. Such favourable statistics could not be explained by the educational level, the financial status or health care conditions, because all socioeconomic indicators in those regions were often lower than in more industrialized countries, where, conversely, the incidence of CHD was higher (Nestle, 1995).

The observed low rates of heart disease in the Mediterranean area spurred the starting of a series of ecological investigations on dietary and other risk factors for CHD (Keys, 1970). Ancel Keys, the promoter of such studies, was particularly interested in the question of whether differences in the occurrence of CHD could be explained by differences in the dietary fat content, on the basis of his original assumption that serum cholesterol concentrations could be the intermediate link with CHD (Keys, 1970). His earlier investigations had in fact indicated that dietary, but not racial (genetic), differences were primarily responsible for variations in serum cholesterol concentrations (Keys *et al.*, 1958).

Against this background, the international research programme of the 'Seven Countries Study' was launched (Keys, 1970). In this cooperative study, 16 cohorts were selected in seven culturally different countries: the USA, Finland, Greece, Italy, Japan, Yugoslavia and the Netherlands. A total of 12,770 men, aged 40–59 years, were enrolled. At entry, age-standardized CHD prevalence rates were inferred from electrocardiographic evidence of previous myocardial infarction and resulted many times higher in the USA and in Finland than in Greece, Italy, Yugoslavia and Japan. Among 12,529 men judged to be free from CHD at entry, in the first 5-year follow-up, age-standardized CHD incidence rates differed largely among study cohorts, the uppermost and lowermost extremes being in Finland, and in Japan and Greece respectively (Fig. 7.1). Examination of risk factors already at that time believed to be of great importance showed that cigarette smoking, sedentary lifestyle habits and body weight could not explain the inter-cohort differences in CHD incidence, which could be directly and strongly related solely to the distribution of serum cholesterol values and to the amount of dietary calories provided by saturated fats (Keys, 1970). However, in looking at the behaviour of the individual cohorts, the 5- and 10-year follow-up data suggested a strong associations between serum cholesterol and CHD mortality especially in the USA and Northern Europe, while in Southern Europe and Japan a much weaker association was present (Keys, 1970, 1980). In agreement with these original data, the 25-year mortality follow-up of the same cohorts more convincingly showed that, although some association between total serum cholesterol and CHD mortality was present in the various populations, the absolute CHD mortality was strikingly different: at serum cholesterol level of 200 mg/dl, CHD mortality rates was 4–5% in Japan and Mediterranean Southern Europe, 12% in the USA and 15% in Northern Europe. In addition to variations in the absolute levels

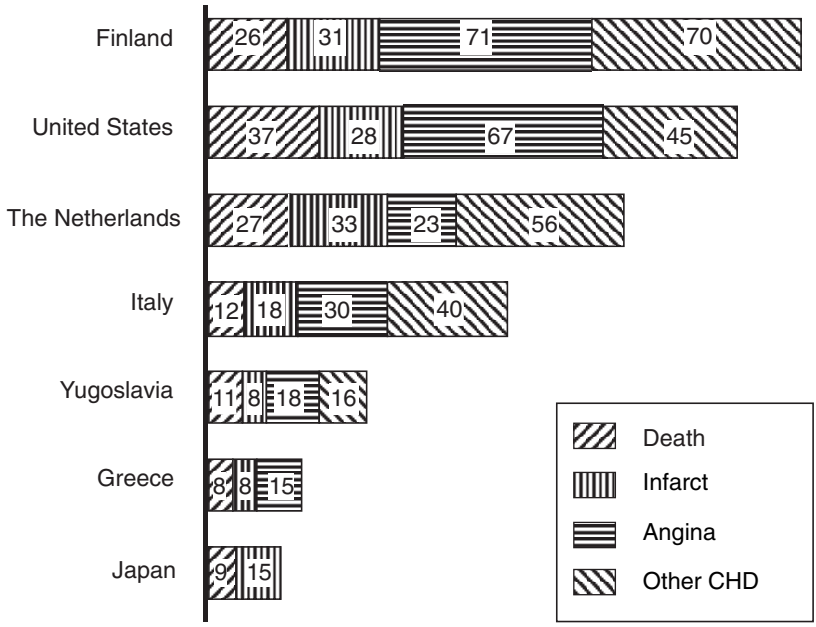


Fig. 7.1. Age-standardized average yearly coronary heart disease (CHD) incidence rates in seven countries (modified from Keys, 1970). Age-standardized average yearly CHD incidence rates per 10,000 of 12,529 men aged 40–59 judged to be free of CHD at the beginning, followed-up for 5 years. Age-standardized CHD incidence rates differed largely among study cohorts, the uppermost and lowermost extremes being Finland, Japan and Greece, respectively.

conferred by the same amount of total cholesterol, the slope of the relationship between serum cholesterol and mortality varied substantially, with little or no increase in mortality by large cholesterol changes in Japan and Mediterranean Europe (Menotti *et al.*, 1993; Verschuren *et al.*, 1995) (Fig. 7.2). These data reinforced the concept that the typical diet in Mediterranean southern Europe was responsible for the low incidence of CHD, and that this could occur independently of serum cholesterol levels.

But what was the 'Mediterranean dietary protective factor'? Although in the first 5-year follow-up of the Seven Countries Study the existence of a negative correlation between CHD incidence in 13 cohorts and the average percentage of calories derived from olive oil monounsaturated fatty acids was already clearly evident ($r = -0.42$) (Fig. 7.3) (Keys, 1970), the cardioprotective effect by monounsaturated fatty acids was rather underestimated. This evidence was confirmed in the 15-year follow up (Keys *et al.*, 1986), when, for the first time, an active role for olive oil in cardiovascular protection was strongly suggested.

This hypothesis is also supported by the simple observation that in Crete (Greece, one of the Mediterranean cohorts in the Seven Countries Study), the abandonment of the traditional dietary habits for more 'Westernized' dietary styles coincided with a rapid increase in the incidence of chronic diseases. Such

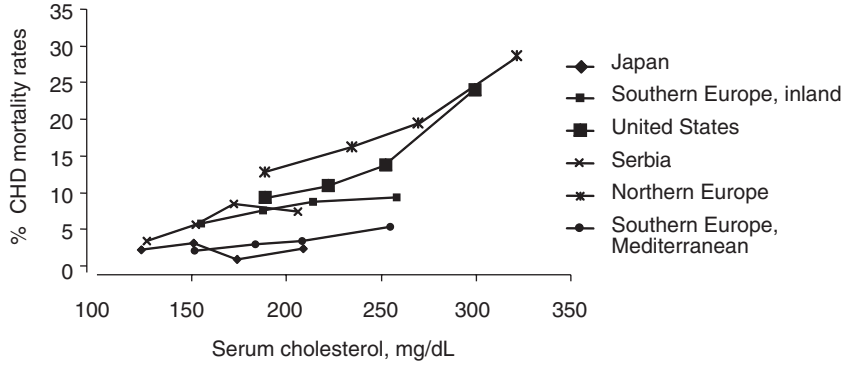


Fig. 7.2. Twenty-five-year coronary heart disease (CHD) mortality rates per baseline cholesterol quartiles (redrawn from Verschuren *et al.*, 1995). Rates were adjusted for age, smoking and systolic blood pressure. The figure indicates that serum cholesterol can explain only partially the variability in mortality rates among different countries. For an absolute cholesterol value of 200 mg/dL, CHD mortality rates varied from 4% to 5% in Japan and Mediterranean Southern Europe to 15% in Northern Europe. Also notice that the slope of the relationship between serum cholesterol and CHD mortality rates is much steeper for Northern Europe and the United States than for Southern Mediterranean countries and Japan.

Fig. 7.3. The relationship between coronary heart disease (CHD) incidence rates and the average percentage of calories from monounsaturated fatty acids in 13 cohorts in the Seven Countries Study (redrawn from Keys, 1970). Age-standardized 5-year incidence rate of CHD among men CHD-free at entry is plotted against the average percentage of total dietary calories provided by monounsaturared fatty acids. A negative correlation between CHD incidence rates and the percentage of calories from monounsaturated fatty acids was found (correlation coefficient, $r = -0.42$).

an increase has been related to a reduced intake of monounsaturated fatty acids, particularly oleic acid, and to the increased consumption of saturated fatty acids, which globally also translated into an increase of serum cholesterol from 181 mg/dl in 1962 to 247 mg/dl in 1988 (Kafatos *et al.*, 1991).

Another association between olive oil consumption and cardiovascular protection is nowadays known as the 'Albanian paradox' (Gjonca and Bobak, 1997). At present in Albania, despite miserable economic conditions with the highest infant mortality rates in Europe, the mortality rate from cardiovascular disease is similar to those of the much more developed Mediterranean countries. The paradox seems to be interpretable on the basis of the typical Albanian dietary habits, resembling the old Mediterranean model. In particular, there is an uneven geographical distribution of mortality, with a striking north-south gradient, the mortality rate in the north-eastern districts being almost twice as high as that of the south-western regions. This mortality pattern has been stable over time, being already present in the 1950s, when the earliest data were available, and remaining identical today. In the hilly north-eastern Albania most foods and fats are of animal origin. By contrast, the diet in south-western Albania is typically Mediterranean, with high intakes of olive oil, fresh fruit and vegetables. Moreover, the geographical pattern of olive production is inversely related to the distribution of mortality rates. Since the consumption of olive oil is highest in areas where it is produced, the low mortality rates recorded in south-western Albania further support a cardioprotective role for olive oil (Gjonca and Bobak, 1997).

These seminal observations, although fundamental to recognize the health-promoting properties of Mediterranean diets, do not, however, clarify the cardioprotective role of olive oil or its constituents, in an indisputable fashion. To this aim, cohort and case-control studies, with adequate statistical power, need to be considered, to determine more conclusively the relationship between a risk or a protective factor (in this case olive oil intake) and the incidence of CHD.

3. Cohort Studies (see Table 7.2)

Since there are not many cohort studies specifically investigating the relationship of olive oil with cardiovascular disease, we will here review also surveys examining the role of monounsaturated fatty acids, independently of their alimentary origin.

After Keys' studies, the first two prospective surveys relating monounsaturated fatty acids to CHD were published in 1991 (Posner *et al.*, 1991) and in 1995 (Esrey *et al.*, 1996).

Posner and co-workers carried out a sub-analysis of the well-known Framingham study (Stokes *et al.*, 1987), specifically investigating the association between CHD incidence and the intake of saturated, monounsaturated, polyunsaturated fatty acids and cholesterol (Posner *et al.*, 1991). The Framingham study was initiated in 1948 with a cohort of 5209 men and women aged 30–62 years selected in the small city of Framingham (Massachusetts). These people were followed up every two years to assess the incidence of cardiovascular disease and cancer (Stokes *et al.*, 1987). Such a monitoring is still in progress. From 1966 to 1969, male subjects aged 45–65 years (n = 859) were also invited to participate in a dietary assessment, by a 24-hour recall technique. Men judged to be free from any cardiovascular disease (n = 813) were divided into two age groups: 45–55 years (n = 420) and 56–65 years (n = 393). They were followed-up for 16 years,

Table 7.2. Summary of the key characteristics of cohort studies on the intake of olive oil or monounsaturated fats and the risk of coronary heart disease (CHD).

First author, year	Population (n)	Follow-up (y)	CHD events	Intake of MUFA	Intake of olive oil	Dietary assessment method	Does olive oil or MUFA protect against CHD risk?	Score points
Posner, 1991	859 M	16	213	R	NR	24-h recall	No	4
Esrey, 1996	4,546 M F	12	92	R	NR	24-h recall	No	2
Pietinen, 1997	21,930 M	6	1399	R	NR	SFFQ	Yes	4
Hu, 1997	80,082 F	14	939	R	NR	SFFQ	Yes	5
Trichopoulou, 2003	22,043 M F	3.07	54	R	R	SFFQ	Yes	3

Abbreviations: M = males; F = females; SFFQ = semiquantitative food frequency questionnaires; MUFA = monounsaturated fatty acids; R = recorded; NR = not recorded; score points: studies scoring 5–6 points are considered of high quality, those scoring 3–4 points of intermediate, and those scoring 2 points or less of insufficient quality. Points were scored according to dietary assessment (3 points for cross-check dietary history, 2 for semiquantitative FFQ, 1 for non-quantitative FFQ, 4-day food records and repeated 24-h recalls and 0 for unpresented details), CHD death ascertainment (1 point if based on individual medical records, 0 if based on death certificates), number of CHD death (1 point if ≥ 100, 0 if ≤ 100), and statistical presentation (1 point if multivariate analyses were included, 0 if lacking) as indicated in Marckmann and Gronbaek (1999).

during which time 99 incident cases of CHD in the younger cohort and 114 in the older one were recorded. The results were rather unexpected. In the younger age group, a significant positive association between CHD incidence and the proportion of energy from monounsaturated fats were found using both univariate and multivariate analysis, the latter taking into account total energy intake, serum cholesterol, physical activity, systolic blood pressure, left ventricular hypertrophy, smoking, glucose intolerance and weight. A very high intake of energy from total fat (almost 40% in both groups), and a very strong correlation between the intake of saturated and monounsaturated fats in both groups ($r = 0.81$ and 0.86 in the younger and older age groups respectively) was also found.

Esrey and co-workers found similar results (Esrey *et al.*, 1996). Here 4546 North American men and women aged over 30 years and initially free from CHD were followed up for 12 years. Dietary intake was measured at entry using the 24-hour recall technique. Proportional hazards analyses, controlling for total energy intake, indicated that increasing percentages of energy intake as monounsaturated fats, as well as total fats and saturated fats were all significant risk factors for CHD mortality among men and women aged 30–59 years (Relative Risks – RR – = 1.08, 95% CI: 1.01–1.16; RR = 1.04, 95% CI: 1.01–1.08; RR = 1.11, 95% CI: 1.04–1.18, respectively). The strength of these associations was not decreased after adjustment for specific classes of serum lipids, thus suggesting an independent relationship with CHD mortality.

The results of these two early prospective studies therefore appear to be deeply in conflict with the international comparisons of the Seven Countries Study (Keys, 1970), but some limitations of both studies have to be taken into account. Neither study clearly reported the origin of fat intake, nor did they

adjust for the intake of other types of fats. This adjustment, as shown below, is of primary importance in a country, such as the USA, where both studies were conducted, in which monounsaturated fats derive from animal food also rich in saturated and *trans*-unsaturated fats.

Also rather interesting were the results obtained from a large cohort study conducted in Finland (Pietinen *et al.*, 1997). Here 21,930 smoking men, aged 50–69 years and free from previous myocardial infarction, stroke, diabetes and history of angina, were followed-up for 6 years. Dietary intake pertaining to the year preceding the enrolment was assessed by a food-frequency questionnaire including 276 food items. Food intake data were also updated at the end of the follow-up. The types of dietary fat used were carefully taken into consideration and the fatty acid composition of several foods determined by chemical analyses at the University of Helsinki. The endpoints of the study were the occurrence of a first non-fatal myocardial infarction and death due to CHD. After adjustments for age, treatment with vitamins A, C and E, smoking, Body Mass Index (BMI), blood pressure, intake of energy, alcohol, fibre, education and physical activity, an inverse association between CHD risk and the intake of monounsaturated fatty acids was found (RR = 0.73 for the uppermost quintile of intake, 95% CI: 0.56–0.95, p for trend = 0.06) (Pietinen *et al.*, 1997).

Almost at the same time, another report, from Hu and co-workers, best emphasized the protective role for monounsaturated fatty acids (Hu *et al.*, 1997). In this study, 80,082 women (nurses), aged 34–59 years, free from CHD, stroke, cancer, hypercholesterolemia and diabetes at baseline, were prospectively followed up for 14 years. Dietary information was obtained by a food-frequency questionnaire (including 116 food items) at baseline, and updated during the follow-up every 2 years. The primary endpoints of the study were non-fatal and fatal CHD after the completion of the first questionnaire (1980). For the statistical analysis, women were divided into five groups according to quintiles of per cent of energy obtained from each type of fat, and the model for multivariate analysis included age, BMI, smoking, menopausal status, a family (parental) history of acute myocardial infarction before the age of 65, the use of multivitamin or vitamin E supplements, the use of alcohol, history of hypertension, the use of aspirin, physical exercise, the percentage of energy obtained from proteins, and the total energy intake. When data were analysed using univariate analysis adjusting for age, the intake of monounsaturated fatty acids, as well as the intake of animal-derived fat and saturated fatty acids, were found to be associated with an increased risk for CHD events (for the highest quintile of monounsaturated fatty acid intake, RR = 1.30; 95% CI: 1.07–1.59, p for trend = 0.004), and similar results were obtained at multivariate analysis (for the highest quintile of monounsaturated fatty acids intake, RR = 1.18; 95% CI: 0.95–1.46; p for trend = 0.14). This study was conducted in the USA, where the major food sources of monounsaturated fatty acids, unlike the Mediterranean area, are beef, dairy products and partially hydrogenated vegetable oils. It was therefore postulated that the protective effect of monounsaturated fats was not apparent because of the presence of other covariates. For this reason the authors, hypothesising that such food sources for monounsaturated fatty acids in the study could also contain saturated, *trans*-unsaturated and polyunsaturated fat, re-analysed the data including all such four types of fat in the

multivariate analysis. In agreement with their hypothesis, some (but weak and non-significant) inverse association with the risk of CHD was shown for monounsaturated fatty acids from the second to the fifth quintile of dietary intake in this adjusted analysis (for the highest intake of monounsaturated fatty acids, RR = 0.95, 95% CI: 0.64–1.39, p for trend = 0.57). An inverse association became clearer when the percentages of energy obtained from monounsaturated fats were treated as a continuous variable, with adjustments for the intake of other types of fat, and when the effect was compared with those of an equivalent amount of energy from carbohydrates. In this condition, a clear positive association with the intake of saturated and *trans*-unsaturated fats was found, together – this time – with a significant inverse association with monounsaturated fats (RR = 0.81, 95% CI: 0.65–1.00, p for trend = 0.05) (Hu *et al.*, 1997).

Although several cohort studies have been conducted relating longevity to adherence to Mediterranean dietary habits and reporting encouraging results for the intake of olive oil (Kouris-Blazos *et al.*, 1999; Fortes *et al.*, 2000; Lasheras *et al.*, 2000), only one has been conducted relating the intake of olive oil to CHD mortality (Trichopoulou *et al.*, 2003). This was a population-based cohort study involving males and females aged 20–86 years, recruited from all regions of Greece. At the beginning, 25,917 subjects with complete information on dietary, life-style and antropometric variables were included, and actively followed-up for 3.7 years until July 2002. Out of the initial cohort, 3874 participants were excluded because of a diagnosis of CHD, diabetes mellitus or cancer at enrolment, and the remaining 22,043 participants were finally included in the analysis. Dietary intake during the year preceding the enrolment was assessed by a food-frequency questionnaire including 150 foods and beverages common in Greece, and classified into 18 dietary variables: vegetables, legumes, fruits and nuts, dairy products, cereals, meat, fish, eggs, potatoes, olive oil, sweets, non-alcoholic beverages, monounsaturated lipids, saturated lipids, polyunsaturated lipids, percentage of energy from saturated lipids, ratio of monounsaturated to saturated lipids and energy intake. A scale indicating the degree of adherence to traditional Mediterranean diet ('Mediterranean dietary score') was constructed, assigning a value of 1 to foods considered beneficial and 0 to those considered detrimental. Dates and causes of death were obtained from death certificates and mortalities from all causes, CHD and cancer were recorded. Hazard ratios (HR) were calculated for deaths from any cause and deaths from CHD, in relation to daily intakes of selected dietary variables and to Mediterranean diet scores. The only individual measures that were predictive of total mortality were the ratio of monounsaturated to saturated lipids and the intake of fruits and nuts, also after adjustments for age, sex, years of education, smoking, BMI and total energy intake. For the ratio of monounsaturated to saturated lipids, HR = 0.86, 95% CI: 0.76–0.98 for any increase of 0.5 in the daily intake. Data were not significant for a relationship of olive oil intake with overall mortality (HR = 0.96, 95% CI: 0.83–1.10, for an increase of 20 g/day), but it is difficult to imagine alternative mechanisms for the significant protective effect of increasing ratios of monounsaturated to saturated lipids, olive oil being the major source of monounsaturated fat in Mediterranean countries. Similarly, a two-point increment in the Mediterranean dietary score was demonstrated to be inversely associated to CHD mortality (HR = 0.67, 95% CI: 0.4–0.94).

4. Case-control Studies (see Table 7.3)

Besides the outstanding international comparisons and ecological correlations by Keys and co-worker (Keys, 1970, 1980; Keys *et al.*, 1986; Menotti *et al.*, 1993) and the not completely unequivocal data from cohort studies, there is little direct evidence from more analytical epidemiological studies relating olive oil to CHD. Therefore great emphasis has been given to the recent results obtained from a case-control study conducted in Pamplona (Spain), which, for the first time, has reported an unquestionable protective effect by olive oil (Fernandez-Jarne *et al.*, 2002). This was a hospital-based case-control study recruiting 171 patients (138 male, 33 female) aged <80 years, who were survivors of a first acute myocardial infarction, and 171 age-, gender- and hospital-matched controls admitted for minor surgery, trauma or genito-urinary disease. To minimize bias, patients with a previous history of angina, or a diagnosis of CHD or other major cardiovascular disease were excluded from the case group. Cases and controls were interviewed in the same fashion, as soon as possible after the admitting event, to assess their dietary intake during the previous year with a food-frequency questionnaire investigating 136 food items. Quintiles of olive oil intake were defined and compared, for patients and controls, for several potential confounding variables. Crude olive oil intake unadjusted or adjusted for total energy was used as an independent variable. When quintiles of crude olive oil intake adjusted for smoking, BMI, hypertension, hypercholesterolemia, diabetes, physical activity, marital status, occupation and study level were used, the uppermost quintile of olive oil intake (median intake = 54.3 g/day) was associated with a 64% relative risk reduction compared with the first quintile (median intake: 7.2 g/day) (Odds Ratio – OR – = 0.36, 95% CI: 0.12–1.08, *p* for trend 0.05). Further adjustment for other nutrients (intake of saturated and *trans*-fatty acids and total fibre, treated as continuous variables) led to an even clearer association with cardiovascular protection, with OR = 0.26 (95% CI: 0.08–0.85, *p* for trend 0.02). When models were fitted using energy-adjusted olive oil intake, the risk-reduction was even more evident, with OR <1 in all four upper quintiles (Table 7.4). The reduction in the risk of a first myocardial infarction was greater than 75% for the uppermost quintile, with OR = 0.22, 95% CI: 0.07–0.67, *p* for trend 0.03 after adjusting for non-dietary confounders, and OR = 0.18, 95% CI: 0.05–0.63, *p* for trend 0.03 after adjustment for both dietary and non-dietary confounders (Fernandez-Jarne *et al.*, 2002).

The results of this study are in contrast, however, with previous reports by Gramenzi (Gramenzi *et al.*, 1990), Tzonou (Tzonou *et al.*, 1996) and Bertuzzi (Bertuzzi *et al.*, 2002) (see Table 7.4).

The study published by Gramenzi *et al.* (1990) was a hospital based, case-control study conducted on 287 women, aged 22–69 years, who had had an acute myocardial infarction and admitted to one of 30 coronary care units in Northern Italy, and 649 controls, aged 21–69 years, with acute disorders unrelated to CHD. Cases and controls were interviewed with a structured questionnaire to obtain information on the frequency of consumption of ten food items and beverages (coffee and alcohol). No information was given about the amount and the type of oil consumed. At univariate analysis, no relationship between

Table 7.3. Key characteristics of case-control studies of the intake of olive oil or monounsaturated fats and the risk of coronary heart disease (CHD).

	Fernandez-Jarne, 2002	Bertuzzi, 2002	Tzonou, 1993	Gramenzi, 1990
Country:	Spain	Italy	Greece	Italy
Age of cases (years, median)	64	61	58	49
Number of cases	171	507	329	287
Number of controls	171	478	570	649
Gender of cases	139 M, 32 F	378 M, 129 F	283 M, 46 F	287 F
Index exposure	Olive oil (quintiles)	Olive oil (quintiles)	MUFA (quintiles)	All oils (tertiles)
Case definition	First myocardial infarction	First myocardial infarction	Myocardial infarction and positive arteriogram	Myocardial infarction
Number of centres	3	Not reported	1	30
Matched design	Yes	No	No	No
Criteria of exclusion[1]	Yes	No	No	No
Dietary assessment method	SFFQ	SFFQ	SFFQ	SFFQ
Food items	136	78	110	10
Nutrient database	Spanish tables	Italian tables	University of Massachusetts	Not used
Adjustment for total energy	Residuals method	Calorie as an independent term	Quintiles of caloric intake as an independent term	No

Abbreviations: M = males; F = females; MUFA = monounsaturated fatty acid; CHD = coronary heart disease; SFFQ = semi-quantitative food frequency questionnaire.
[1]Criteria of exclusion: previous angina pectoris, previous CHD diagnosis or other prior diagnosis of major cardiovascular disease.

Table 7.4. Distribution of 171 cases and 171 controls and corresponding Odds Ratios (OR) with 95% CI for a first myocardial infarction according to energy-adjusted olive oil intake in the report by Fernandez-Jarne *et al.* (2002).

Quintile of intake	Cases (n)	Controls (n)	Median intake (g/day)	Multivariate adjusted OR (95% CI)
1	40	28	6.01	1 (ref.)
2	31	38	13.06	0.45 (0.16–1.25)
3	30	38	21.00	0.44 (0.18–1.07)
4	40	29	30.09.00	0.70 (0.24–2.02)
5	30	38	52.02.00	0.18 (0.05–0.63)
Trend test *p*-value				0.03

Conditional logistic regression (age-, hospital- and gender-matched pairs) adjusted for smoking, body mass index, high blood pressure, high blood cholesterol, diabetes, marital status, occupation, study level, leisure-time physical activity, % of energy derived from saturated *trans*-fat, total fibre consumption, folic acid intake, vitamin C intake, glycaemic load and ethanol intake (adding a quadratic term for non-linear relationship).

the risk of myocardial infarction and the frequency of oil consumption was found. This study clearly had limitations in the inaccuracy of the dietary assessment technique in terms of quality (only ten food items) and estimates of total amount, the lack of adjustment for total energy and the lack of a matched design. Moreover it was conducted in Northern Italy, with mostly continental dietary habits (i.e. richer in animal fat and poorer in vegetable fats like olive oil (Gruppo di Ricerca ATS-RF2, 1980). The study also did not report whether cases were primary or secondary coronary events.

Bertuzzi and co-workers analysed 507 patients (378 men and 129 women aged 25–79 years) surviving a first acute myocardial infarction, and 478 controls (297 men and 181 women, aged 25–79 years), admitted to the same hospitals for a wide spectrum of acute conditions unrelated to known or potential CHD (Bertuzzi *et al.*, 2002). Cases and controls were interviewed to assess the dietary intake during the previous 2 years, with the use of a food-frequency questionnaire including 78 food items plus additional questions to assess the type of fat used for seasoning and cooking. The mean amount of olive oil intake in the entire population was 35.6 g/day. Once more, quintiles of olive oil intake were defined. Compared with the lowest quintile of olive oil intake, the OR for an acute myocardial infarction adjusted for age, sex, education, BMI, cholesterol, smoking, intake of coffee, alcohol, calories, butter, margarine, seed oil, physical activity, diabetes, hyperlipidemia, hypertension and family history of acute myocardial infarction in first-degree relatives were 0.90, 1.01, 0.86 and 1.48, with no clear trend of any relationship with olive oil consumption. Data were not, however, adjusted for the total energy intake, which made the inverse association with olive oil more clearly apparent in the study by Fernandez-Jarne (2002), and there was no matched design (Table 7.3).

Similarly to the above reported studies, Tzonou and co-workers conducted a hospital-based case-control study in Greece, on 329 cases of CHD diagnosed either by coronary angiography or on the basis of an electrocardiographically-confirmed first myocardial infarction, including 284 males and 45 females, age-ranging 49 to 70, and 570 controls (324 males, 246 females) admitted to the same hospital for disease unrelated to nutrition or CHD. Cases and controls were interviewed to assess the dietary intake during the previous year with the use of a food-frequency questionnaire including 110 food items and beverages. No relation between monounsaturated fat intake (expressed in quintiles of assumption) and CHD risk emerged from this study, even after adjustments for gender, age, education, physical exercise, duration of 'siesta', smoking habits, alcohol and total energy intake, as well as the intake of saturated and polyunsaturated fat (Tzonou *et al.*, 1996). The variance of these results from the others reported above might be due, as pointed out by the same authors, to the lack of adjustment for *trans*-isomers of fatty acids, whose pro-atherogenic effects have been clearly shown (Hu *et al.*, 1997). Furthermore, the authors here used a table for determination of food composition (University of Massachusetts nutrient data base) deriving from another country because that was the only computerized data base available at the time of the analysis. Table 7.3 reports a comparison of the most important features in the most relevant case-control studies relating olive oil or its derivates to CHD risk.

5. Clinical Studies

To the best of our knowledge, the first clinical trial testing the use of olive oil in the treatment of CHD was published by Rose in 1965 (Rose *et al.*, 1965). This was a randomized double-blind, controlled trial in which 80 patients aged <70 years surviving after a myocardial infarction or suffering from angina pectoris were enrolled. Beside receiving conventional pharmacological treatment, patients were assigned to one or two experimental groups – olive oil or corn oil, 80 g/day – or a control group. Patients in both experimental groups were instructed to avoid saturated fats, namely fried foods, fatty meat, sausages, pastry, ice-cream, cheese and cakes, and to limit the use of milk, eggs and butter. No advice on dietary fat was given to control patients. The prescribed daily dose of oil, irrespective of the type, was found too heavy by many patients, so that, after 1 year, the estimated average intake fell to around 60% of the initially prescribed dose. Although the trial was planned to be of 3 years duration, by the end of 2 years only about 50% of the enrolled patients remained in the trial, the remainder having died or been excluded because of the occurrence of a reinfarction or lost to follow-up. Therefore the results from the first 2 years of follow-up were reported, at which time the proportions of patients remaining alive and free of reinfarction were 75%, 57% and 52 % for the control, olive and corn oil groups, respectively. The results therefore suggested no beneficial effect of oil treatments. However, the small sample size and the lack of information on the composition of the entire data have to be kept in mind.

After the Seven Countries Study, the report that has best emphasized the cardioprotective role of the 'Mediterranean diet' has been the 'Lyon Diet Heart Study' (de Lorgeril *et al.*, 1994). This was a prospective, randomized, single-blinded, multicentre, secondary prevention trial, testing the cardioprotection of a Mediterranean-type diet. Here 605 patients of both sexes, surviving a first acute myocardial infarction and aged <70 years were enrolled within 6 months from the index event. Patients were assigned to either a control (n =303) or an experimental (n = 302) group. In the control group patients were advised to follow the prudent *Step I American Heart Association* diet, while in the experimental group they were advised to adopt a Mediterranean-type diet. Since the patients would not have accepted olive oil as the only fat source, this was integrated with a canola oil-based margarine, the composition of which was comparable to olive oil, but slightly enriched in linoleic and highly enriched in α-linolenic acid, to better resemble the Cretan diet (one of the healthiest cohorts in the Seven Countries Study). Diet adherence was evaluated by 24-hour recalls and food frequency questionnaire, and the plasma total fatty acid composition was assessed annually. After only 27 months, a reduction in coronary events and cardiac deaths of the order of 70% was observed in the experimental group (Fig. 7.4) (de Lorgeril *et al.*, 1994), and this benefit persisted in the longer-term (46-month) follow-up (de Lorgeril *et al.*, 1999). To confirm compliance with the experimental dietary regimen, a significant increase in the relative proportions of oleic acid and omega-3 fatty acids was observed in plasma lipids. Although olive oil was not specifically recommended, the increased incorporation of oleic acid in plasma lipids obviously suggests the use of olive oil in the experimental group

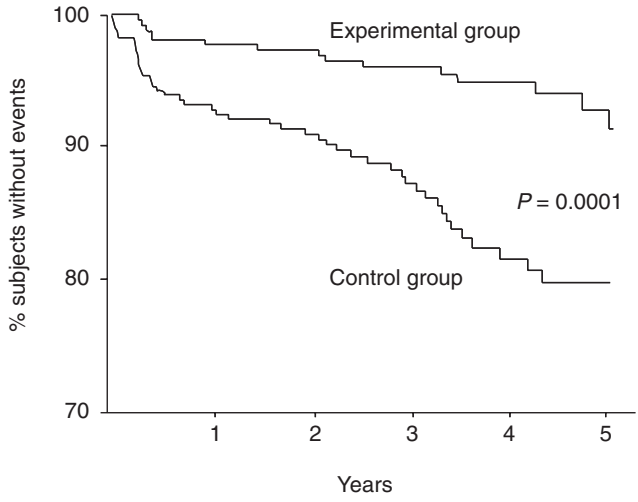


Fig. 7.4. Survival curves in the Lyon Heart Study (from de Lorgeril *et al.*, 1994). Cumulative survival without cardiac death and non-fatal acute myocardial infarction comparing an experimental (Mediterranean diet) group and control subject. The very early appearance of the protective effect of the Mediterranean diet is evident.

and, by inference, a role of olive oil in the cardioprotection observed (de Lorgeril *et al.*, 1999). In this study, the per cent reduction in cardiovascular morbidity and mortality by far exceeded that reported in any trial testing the effects of cholesterol-lowering agents in secondary prevention of myocardial infarction (e.g. the 35% reduction in cardiovascular events achieved in the POSCH Study (Buchwald *et al.*, 1990) and the 34% reduction observed in the Scandinavian Simvastatin Survival Study (4S) (Scandinavian Simvastatin Survival Study Group, 1994)).

Finally, a sub-analysis from the GISSI-Prevenzione data base (GISSI-prevenzione Investigators, 1999) investigated the protective effect associated with the use of some Mediterranean foods (Barzi *et al.*, 2003). The GISSI-Prevenzione study was a clinical trial designed to test the beneficial effect of omega-3 fatty acids and vitamin E supplementation. To this aim 11,323 patients surviving a first myocardial infarction were recruited in 172 centres across Italy, and randomly assigned to receive a supplement of omega-3 fatty acids (1 g/day), vitamin E (300 mg/day), both, or none (control), for 3.5 years. In addition, each patient received dietary advice to increase the consumption of Mediterranean foods, considered cardioprotective, namely olive oil, fruit, raw and cooked vegetables, and fish, the intake of which was assessed. Dietary information was obtained at entry and after 6, 18 and 42 months by a food frequency questionnaire. Data were analysed using methods appropriate for a cohort study, taking the treatment allocation during the trial as a confounding variable, and estimating the OR for death, over the entire period of the follow-up, for each of the five foods advised, after adjustment for other non-dietary confounding factors (sex, smoking, hypertension, diabetes, intermittent claudication, electrical instability, left ventricular dysfunction, ischemia, use of aspirin, ACE inhibitors and

β-blockers), as well as for all other food sources. Increasing frequency of olive oil consumption was here associated with a decreased risk of death after overall adjustments, with OR = 0.77, 95% CI: 0.62–0.95 and OR = 0.76, 95% CI: 0.64–0.91 for categories of 'often' and 'regular' consumption, respectively, compared with the 'sometimes- or never-' consumed category, thus suggesting a clear protective effect by olive oil (Barzi *et al.*, 2003). It should, however, be pointed out that this was a sub-analysis of a study prospectively designed to test the hypothesis of a cardioprotective effect of omega-3 fatty acids or vitamin E, and therefore did not test the olive oil hypothesis prospectively.

6. Studies *In Vitro*, in Experimental Animals and on Intermediate Endpoints in Humans

In contrast with the data from epidemiological and clinical trials, not often easy to interpret, a huge mass of biochemical and metabolic data clearly support the hypothesis of a cardiovascular protective role for olive oil. In comparison with saturated fat, olive oil has been shown to reduce low-density lipoprotein (LDL) cholesterol (Mattson and Grundy, 1985; Mensink and Katan, 1987, 1992), and increase the levels of high-density lipoprotein (HDL) cholesterol (Mattson and Grundy, 1985). In addition, olive oil is relatively resistant to oxidation for its high content in monounsaturated fatty acid and polyphenolic constituents, these latter potent scavengers of superoxide radicals (Visioli *et al.*, 1998). Such constituents, beside being potential inhibitors of LDL oxidation (Fito *et al.*, 2000), have been shown to directly affect the early phases of atherosclerosis (Massaro *et al.*, 1999). Using an *in vitro* model of early atherogenesis, consisting of human endothelial cells in culture challenged by proinflammatory stimuli (cytokines, bacterial lipopolysaccharide or oxidized LDL), we have shown that the administration of nutritionally relevant concentrations of oleic acid (Massaro *et al.*, 2002) or polyphenolic compounds such as oleuropein (Carluccio *et al.*, 2003) to endothelial cells reduces the stimulated expression of pro-atherogenic endothelial leukocyte adhesion molecules. In particular, oleic acid, when accumulated in membrane phospholipids, likely acts through a quenching of reactive oxygen species (ROS), the intracellular production of which is elicited by proinflammatory stimuli (Massaro *et al.*, 2002). These effects could well explain the reduction in atherosclerosis progression elicited by olive oil in animal models (Mangiapane *et al.*, 1999). Furthermore, olive oil favourably affects the haemostatic system (Junker *et al.*, 2001) and, in diabetic patients, improves the lipid profile (Madigan *et al.*, 2000) and the glycaemic control (Garg, 1998). Finally, some antihypertensive role has been demonstrated with the use of olive oil (Ferrara *et al.*, 2000).

7. Conclusions

Olive oil is a typical and quantitatively important food in Mediterranean countries, where the incidence of CHD, at least until the early 1960s, was very low. Traditional Mediterranean diets were based mainly on plant foods, contained

small amounts of animal foods – including fish, alcohol (mainly in the form of red wine) in moderation, and olive oil as the main fat, in the form of monounsaturated fatty acids. In addition, such diets were well balanced with respect to the intake:expenditure energy ratio.

After the results of Keys' surveys (Keys, 1970, 1980; Keys *et al.*, 1986), which suggested olive oil and monounsaturated fatty acid consumption as key factors in Mediterranean cardioprotection, only a few cohort prospective studies have been conducted to further test such hypotheses. Of these, only one has been conducted in the Mediterranean area (Trichopoulou *et al.*, 2003). The other studies have been conducted in countries where the use of olive oil is very limited and sources of monounsaturated fatty acids are mostly different from olive oil, and richer in saturated and *trans*-fatty acids. Conversely, in the Mediterranean area, olive oil, typically obtained by cold-pressure techniques that ensure the preservation of the content in vitamins and phenolic compounds, is the main source of monounsaturated fatty acids. Most prospective case-control studies which do not find a clear cardiovascular protective role for olive oil (Gramenzi *et al.*, 1990; Tzonou *et al.*, 1996) are, in our opinion, flawed in their design.

The results from secondary prevention clinical trials, such as the Lyon Diet Heart Study, with an extraordinary 70% reduction in the risk of a coronary events, are highly encouraging. On the other hand, a large number of biochemical and metabolic studies continue to supply an ample mechanistic basis for the hypothesis of cardiovascular protection by olive oil, pointing both to monounsaturated fatty acids (oleic acid) in the presence of very low amounts of saturated and *trans*-fatty acids, and to anti-oxidant polyphenols as the biologically active components. Therefore, although the results of epidemiological studies have not been always consistent, the combination of epidemiological data, controlled intervention trials and biochemical and metabolic studies yield a rather convincing argument for the health benefits of olive oil consumption, especially with the adherence to a Mediterranean dietary lifestyle. Additive or synergistic effects among different nutrients are likely to occur. The health benefits of a diet rich in monounsaturated fatty acids from olive oil have been highlighted in a recent American Heart Association (AHA) Science advisory report (Kris-Etherton, 1999). This report contains recommendations for a diet moderately rich in monounsaturated fatty acids (15% of calories) and polyunsaturated fatty acids (7%), and relatively low in saturated fatty acids (8%), in large agreement with the AHA dietary guidelines for healthy American adults – the so-called 'step-1 diet' (Krauss *et al.*, 1996). However, diets higher in monounsaturated fatty acids, also exceeding 30% of calories from fat, were here considered an alternative option for managing risk factors in the prevention and treatment of CHD, provided that they do not exceed the recommendation for the saturated fatty acids intake and compromise weight control (Kris-Etherton, 1999).

It would be important – at this stage – to have results of larger, well-designed cohort studies in Mediterranean countries, using extra virgin olive oil as a source of vitamins and phenolic antioxidants, and with a balanced ratio of monounsaturated to polyunsaturated fatty acids. At least one such study, in Spain, is now in progress (Research team of the SUN (University of Navarre follow-up Study) project, 2002), and its results eagerly awaited.

8. References

Barzi, F., Woodward, M., Marfisi, R.M., Tavazzi, L., Valagussa, F. and Marchioli, R. (2003) Mediterranean diet and all-causes mortality after myocardial infarction: results from the GISSI-Prevenzione trial. *European Journal of Clinical Nutrition* 57, 604–611.

Bertuzzi, M., Tavani, A., Negri, E. and La Vecchia, C. (2002) Olive oil consumption and risk of non-fatal myocardial infarction in Italy. *International Journal of Epidemiology* 31, 1274–1277; author reply 1276–1277.

Brown, M.S. and Goldstein, J.L. (1996) Heart attacks: gone with the century? *Science* 272, 629–635.

Buchwald, H., Varco, R., Matts, J., Long, J., Fitch, L., Campbell, G., Pearce, M., Yellin, A., Edmiston, W. and Smink, R.J. (1990) Effect of partial ileal bypass surgery on mortality and morbidity from coronary heart disease in patients with hypercholesterolemia: report of the Program on the Surgical Control of the Hyperlipidemias (POSCH). *New England Journal of Medicine* 323, 946–955.

Burr, M.L., Gilbert, J.F., Hollyday, R.M., Elwood, P.C., Fehily, A.M., Rogers, S., Sweetnam, P.M. and Deadman, N.M. (1989) Effect of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial. (DART). *Lancet* II, 757–761.

Carluccio, M.A., Siculella, L., Ancora, M.A., Massaro, M., Scoditti, E., Storelli, C., Visioli, F., Distante, A. and De Caterina, R. (2003) Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of Mediterranean diet phytochemicals. *Arteriosclerosis, Thrombosis, and Vascular Biology* 23, 622–629.

de Lorgeril, M., Renaud, S., Mamelle, N., Salen, P., Martin, J.L., Monjaud, I., Guidollet, J., Touboul, P. and Dalaye, J. (1994) Mediterranean alpha-linoleic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* 343, 1454–1459.

de Lorgeril, M., Salen, P., Martin, J.L., Monjaud, I., Dalaye, J. and Mamelle, N. (1999) Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction – Final report of the Lyon Diet Heart Study. *Circulation* 99, 779–785.

Esrey, K.L., Joseph, L. and Grover, S.A. (1996) Relationship between dietary intake and coronary heart disease mortality: lipid research clinics prevalence follow-up study. *Journal of Clinical Epidemiology* 49, 211–216.

Fernandez-Jarne, E., Martinez-Losa, E., Prado-Santamaria, M., Brugarolas-Brufau, C., Serrano-Martinez, M. and Martinez-Gonzalez, M.A. (2002) Risk of first non-fatal myocardial infarction negatively associated with olive oil consumption: a case-control study in Spain. *International Journal of Epidemiology* 31, 474–480.

Ferrara, L.A., Raimondi, A.S., d'Episcopo, L., Guida, L., Dello Russo, A. and Marotta, T. (2000) Olive oil and reduced need for antihypertensive medications. *Archives of Internal Medicine* 160, 837–842.

Fito, M., Covas, M.I., Lamuela-Raventos, R.M., Vila, J., Torrents, L., de la Torre, C. and Marrugat, J. (2000) Protective effect of olive oil and its phenolic compounds against low density lipoprotein oxidation. *Lipids* 35, 633–638.

Fortes, C., Forastiere, F., Farchi, S., Rapiti, E., Pastori, G. and Perucci, C.A. (2000) Diet and overall survival in a cohort of very elderly people. *Epidemiology* 11, 440–445.

Garg, A. (1998) High-monounsaturated-fat diets for patients with diabetes mellitus: a meta-analysis. *American Journal of Clinical Nutrition* 67, 577S–582S.

GISSI-prevenzione Investigators (1999) Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* 354, 447–455.

Gjonca, A. and Bobak, M. (1997) Albanian paradox, another example of protective effect of Mediterranean lifestyle? *Lancet* 350, 1815–1817.

Gramenzi, A., Gentile, A., Fasoli, M., Negri, E., Parazzini, F. and La Vecchia, C. (1990) Association between certain foods and risk of acute myocardial infarction in women. *British Medical Journal* 300, 771–773.

Gruppo di Ricerca ATS-RF2 (1980) I fattori di rischio dell'arteriosclerosi in Italia: la fase A del progetto finalizzato del CNR, medicina preventiva arteriosclerosi RF2. *Giornale Italiano di Cardiologia* 10, 1–184.

Hu, F.B., Stampfer, M.J., Manson, J.E., Rimm, E., Colditz, G.A., Rosner, B.A., Hennekens, C.H. and Willett, W.C. (1997) Dietary fat intake and the risk of coronary heart disease in women. *New England Journal of Medicine* 337, 1491–1499.

Junker, R., Kratz, M., Neufeld, M., Erren, M., Nofer, J.R., Schulte, H., Nowak-Gottl, U., Assmann, G. and Wahrburg, U. (2001) Effects of diets containing olive oil, sunflower oil, or rapeseed oil on the hemostatic system. *Thrombosis and Haemostasis* 85, 280–286.

Kafatos, A., Kouromalis, I., Vlachonikolis, I., Theodorou, C. and Labadorios, D. (1991) Coronary-heart-disease risk factor status of the Cretan urban population in the 1980s. *American Journal of Clinical Nutrition* 54, 591–598.

Kato, H., Tillotson, J., Nichaman, M.Z., Rhoads, G.G. and Hamilton, H.B. (1973) Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California. *American Journal of Epidemiology* 97, 372–385.

Keys, A. (1970) Coronary heart disease in seven countries. *Circulation* 41, 1–211.

Keys, A. (1975) Coronary heart disease - The global picture. *Atherosclerosis* 22, 149–192.

Keys, A. (1980) *Seven Countries: A Multivariate Analysis of Death and Coronary Heart Disease.* Harvard University Press, Cambridge.

Keys, A. (1987) Olive oil and coronary heart disease. *Lancet*, 983–984.

Keys, A., Kimura, N., Kusukawa, A., Bronte-Stewart, B., Larsen, N. and Keys, M.H. (1958) Lessons from serum cholesterol studies in Japan, Hawaii and Los Angeles. *Annals of Internal Medicine* 48, 83–94.

Keys, A., Menotti, A., Karvonen, M.J., Aravanis, C., Blackburn, H., Buzina, R., Djordjevic, B.S., Dontas, A.S., Fidanza, F. and Keys, M.H. (1986) The diet and 15-year death rate in the seven countries study. *American Journal of Epidemiology* 124, 903–915.

Kouris-Blazos, A., Gnardellis, C., Wahlqvist, M.L., Trichopoulos, D., Lukito, W. and Trichopoulou, A. (1999) Are the advantages of the Mediterranean diet transferable to other populations? A cohort study in Melbourne, Australia. *British Journal of Nutrition* 82, 57–61.

Krauss, R.M., Deckelbaum, R.J., Ernst, N., Fisher, E., Howard, B.V., Knopp, R.H., Kotchen, T., Lichtenstein, A.H., McGill, H.C., Pearson, T.A., Prewitt, T.E., Stone, N.J., Horn, L.V. and Weinberg, R. (1996) Dietary guidelines for healthy American adults. A statement for health professionals from the Nutrition Committee, American Heart Association. *Circulation* 94, 1795–1800.

Kris-Etherton, P.M. (1999) AHA Science Advisory. Monounsaturated fatty acids and risk of cardiovascular disease. American Heart Association. Nutrition Committee. *Circulation* 100, 1253–1258.

Kushi, L.H., Lew, R.A., Stare, F.J., Ellison, C.R., el Lozy, M., Bourke, G., Daly, L., Graham, I., Hickey, N. and Mulcahy, R. (1985) Diet and 20-year mortality from coronary heart disease. The Ireland–Boston Diet-Heart Study. *New England Journal of Medicine* 312, 811–818.

Lasheras, C., Fernandez, S. and Patterson, A.M. (2000) Mediterranean diet and age with respect to overall survival in institutionalized, nonsmoking elderly people. *American Journal of Clinical Nutrition* 71, 987–992.

Madigan, C., Ryan, M., Owens, D., Collins, P. and Tomkin, G.H. (2000) Dietary unsaturated fatty acids in type 2 diabetes: higher levels of postprandial lipoprotein on a linoleic acid-rich sunflower oil diet compared with an oleic acid-rich olive oil diet. *Diabetes Care* 23, 1472–1477.

Mangiapane, E.H., McAteer, M.A., Benson, G.M., White, D.A. and Salter, A.M. (1999) Modulation of the regression of atherosclerosis in the hamster by dietary lipids: comparison of coconut oil and olive oil. *British Journal of Nutrition* 82, 401–409.

Marckmann, P. and Gronbaek, M. (1999) Fish consumption and coronary heart disease mortality. A systematic review of prospective cohort studies. *European Journal of Epidemiology* 53, 585–590.

Massaro, M., Carluccio, M.A. and De Caterina, R. (1999) Direct vascular antiatherogenic

effects of oleic acid: a clue to the cardioprotective effects of the Mediterranean diet. *Cardiologia* 44, 507–513.

Massaro, M., Basta, G., Lazzerini, G., Carluccio, M., Bosetti, F., Solaini, G., Visioli, F., Paolicchi, A. and De Caterina, R. (2002) Quenching of intracellular ROS generation as a mechanism for oleate-induced reduction of endothelial activation and early atherogenesis. *Thrombosis and Haemostasis* 88, 176–375.

Mattson, F.H. and Grundy, S.M. (1985) Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in men. *Journal of Lipid Research* 26, 194–202.

McGee, D.L., Reed, D.M., Yano, K., Kagan, A. and Tillotson, J. (1984) Ten-year incidence of coronary heart disease in the Honolulu Heart Program. Relationship to nutrient intake. *American Journal of Epidemiology* 119, 667–676.

Menotti, A., Keys, A., Kromhout, D., Blackburn, H., Aravanis, C., Bloemberg, B., Buzina, R., Dontas, A., Fidanza, F. and Giampaoli, S. (1993) Inter-cohort differences in coronary heart disease mortality in the 25-year follow-up of the seven countries study. *European Journal of Epidemiology* 9, 527–536.

Mensink, R.P. and Katan, M.B. (1987) Effect of dietary fatty acids versus complex carbohydrates on high-density lipoprotein in healthy men and women. *Lancet* I, 122–125.

Mensink, R.P. and Katan, M.B. (1992) Effect of dietary fatty acids on serum lipids and lipoproteins: a meta-analysis on 27 trials. *Arteriosclerosis and Thrombosis* 12, 911–919.

Ness, A.R. (2002) Is olive oil a key ingredient in the Mediterranean recipe for health? *International Journal of Epidemiology* 31, 481–482.

Nestle, M. (1995) Mediterranean diet: historical and research overview. *American Journal of Clinical Nutrition* 61, 1313s–1320s.

Pietinen, P., Ascherio, A., Korhonen, P., Hartman, A.M., Willett, W.C., Albanes, D. and Virtamo, J. (1997) Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *American Journal of Epidemiology* 145, 876–887.

Posner, B.M., Cobb, J.L., Belanger, A.J., Cupples, L.A., D'Agostino, R.B. and Stokes, J., 3rd (1991) Dietary lipid predictors of coronary heart disease in men. The Framingham Study. *Archives of Internal Medicine* 151, 1181–1187.

Research team of the SUN (University of Navarre follow-up Study) project (2002) The Mediterranean diet and cardiovascular disease: results from pilot study from the SUN project (University of Navarre follow-up Study). *Revista De Medicina Universidad de Navarra* 46, 9–16.

Rose, G.A., Thomson, W.B. and Williams, R.T. (1965) Corn oil in treatment of ischaemic heart disease. *British Medical Journal* 544, 1531–1533.

Scandinavian Simvastatin Survival Study Group (1994) Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 344, 1383–1389.

Simopoulos, A. and Pavlou, K. (2001) *Nutrition and Fitness: Diet, Genes, Physical Activity and Health.* Karger, Basel.

Singh, R.B., Rastogi, S.S., Verma, R., Laxmi, B., Singh, R., Gosh, S. and Niaz, M.A. (1992) Randomised controlled trial of cardioprotective diet in patients with recent myocardial infarction: results of one year follow-up. *British Medical Journal* 304, 1015–1018.

Stokes, J., 3rd, Kannel, W.B., Wolf, P.A., Cupples, L.A. and D'Agostino, R.B. (1987) The relative importance of selected risk factors for various manifestations of cardiovascular disease among men and women from 35 to 64 years old: 30 years of follow-up in the Framingham Study. *Circulation* 75, V65–73.

Trichopoulou, A., Costacou, T., Bamia, C. and Trichopoulos, D. (2003) Adherence to a Mediterranean diet and survival in a Greek population. *New England Journal of Medicine* 348, 2599–2608.

Tzonou, A., Lipworth, L., Kalandidi, A., Trichopoulou, A., Gamatsi, I., Hsieh, C.C., Notara, V. and Trichopoulos, D. (1996) Dietary factors and the risk of endometrial cancer: a case–control study in Greece. *British Journal of Cancer* 73, 1284–1290.

Verschuren, W.M., Jacobs, D.R., Bloemberg, B.P., Kromhout, D., Menotti, A., Aravanis, C., Blackburn, H., Buzina, R., Dontas, A.S. and Fidanza, F. (1995) Serum total cholesterol and long-term coronary heart disease mortality in different cultures. Twenty-five-year follow-up of the seven countries study. *The Journal of the American Medical Association* 274, 131–136.

Visioli, F., Bellomo, G. and Galli, C. (1998) Free radical-scavenging properties of olive oil polyphenols. *Biochemical and Biophysical Research Communications* 247, 60–64.

8 Olive Oil, Blood Lipids and Postprandial Lipaemia

JAVIER S. PERONA AND VALENTINA RUIZ-GUTIÉRREZ

Instituto de la Grasa (CSIC), Sevilla, Spain

1. Introduction

The relation between triacylglycerols (TG) and coronary heart disease (CHD) is complicated by the metabolic relation between triacylglycerol-rich lipoproteins (TRL) and high density lipoproteins (HDLs), the heterogeneity of TRL, and the imprecision with which serum TG are determined (Gotto, 1998; Rapp, 2002). The NIH Consensus Development Panel on Triglyceride, High-Density Lipoprotein, and CHD concluded in 1993 that 'For triglyceride, the data are mixed, and although strong associations are found in some studies, the evidence of a causal relationship is still incomplete' (NIH Consensus Conference, 1993).

However, serum TG concentration is often more strongly correlated with the future incidence of CHD than is serum cholesterol (Durrington, 1998). In recent years, improvements in analytical and biostatistical methods have raised the question of whether the measurement of serum TG concentration would be as useful as cholesterol measurement for the diagnosis and prediction of CHD. Elevated plasma TG concentration is therefore now being recognized as a risk factor for CHD (Roche and Gibney, 1999). It has been postulated that the atherogenic process might be enhanced by the accumulation of TRL in plasma, such as VLDL (Björkegren *et al.*, 1997; Roche and Gibney, 2000). Considering this evidence and that the TG metabolism also determines the fate of some other lipoproteins, such as LDL or HDL (Roche and Gibney, 2000), TG seem to have a central role in the pathogenesis of atherosclerosis.

Several clinical studies have shown that delayed postprandial lipaemia is an important factor in the pathogenesis and progression of CHD (Groot *et al.*, 1991) and postprandial, but not fasting, triglyceridemia is a good predictor of the presence and progression of this pathology (Patsch *et al.*, 1992). Zilversmit (1979) was the first to propose that chylomicron (CM) remnants were as atherogenic as LDL. Since then, it has been reported that elevated postprandial response is associated with the formation and increased concentrations of small, dense chylomicron remnants.

 Olive Oil and Health
(eds J.L. Quiles, M.C. Ramírez-Tortosa and P. Yaqoob)

2. Induction of Atherosclerosis by Chylomicron Remnants

2.1. The development of atherosclerosis

The development of atherosclerotic lesions begins with dysfunction of the vascular endothelium. The oxidative alteration of circulating lipoproteins that penetrate the vascular wall induces the transcription of adhesion factors by the endothelial cells, such as VCAM-1, which facilitate the adhesion of leukocytes to the endothelium. This is followed by a migration of monocytes to the subendothelial space, where they are transformed into macrophages, expressing scavenger receptors to recognize oxidized lipoproteins and phagocytose them. Eventually, macrophages become so engorged that they take on a foamy appearance. The accumulation of foam cells and the proliferation of vascular smooth muscle cells (VSMCs) leads to the formation of fatty streaks, which are the first visible lesions in the artery wall (Ross, 1993; Kadar and Glasz, 2001).

Foam cells secrete proinflammatory cytokines and reactive oxygen species (ROS) that amplify the immune response, as well as growing and coagulation factors. At the same time, these growing factors induce proliferation and migration of smooth muscle cells from tunica media to the intima, thickening it and generating a fibrous cap. As foam cells die, some by apoptosis, a necrotic core is formed in the plaque. These processes lead to the eventual occlusion of the vessel. If the fibrous cap breaks, the content of the plaque accesses blood and platelet aggregation takes place and so the formation of thrombus (Ross, 1993).

2.2. Involvement of chylomicron remnants in atherogenesis

Dietary fat is composed mainly of TG, which after digestion and absorption stimulates the production of CM. These are TRL synthesized in the intestinal enterocyte, which transport exogenous dietary TG within the circulation, causing an increase in plasma TG concentrations. CM contain apolipoprotein B-48 (apo B-48) as structural protein, and are very large, heterogeneous, lipid-rich lipoproteins, ranging in diameter from 75 to 450 nm. This size heterogeneity depends on the rate of fat absorption, type and amount of fat absorbed. The fatty acid composition of the TG present in CM reflects the composition of dietary fat (Hussain, 2000).

Recent years have seen accumulating evidence showing that exogenous TRL, CM and their remnants, are strongly atherogenic (Mamo, 1995; Tomkin and Owens, 2001; Yu and Cooper, 2001; Wilheim and Cooper, 2003). CM remnants have been shown to penetrate the artery wall, and to interact with vascular cells (Mamo, 1995; Proctor *et al.*, 2002). Delay in their clearance from the circulation has been found to correlate with the development of atherosclerotic lesions (Patsch *et al.*, 1992; Benlian *et al.*, 1996). ApoE knock out mice accumulate remnants in the plasma and develop severe atherosclerosis (Boren *et al.*, 2000). Finally, apo B48-containing lipoproteins of intestinal origin have been isolated from atherosclerotic plaque (Ghung *et al.*, 1994; Pal *et al.*, 2003).

It has been suggested that the entry of lipoproteins into the arterial wall is inversely related to the size of the particles, and for that reason, it was thought for many years that CM and CM remnants were not atherogenic since they are too large to penetrate the tissue (Stender and Zilversmit, 1981; Nordestgaard and Zilversmit, 1988; Ooi and Ooi, 1998). However, these lipoproteins are heterogenous populations which can differ substantially in size (Mamo, 1995). In fact, half of the particles recovered from atherosclerotic lesions of humans by immunoabsorption on an anti-apolipoprotein B column were found to have the size of IDL (intermediate-density lipoprotein) or, specially, VLDL remnants (Rapp *et al.*, 1989, 1994). Small TRL, including postprandial CM remnants, are believed to be the most atherogenic of all TRL particles (Sharrett *et al.*, 1995). Proctor *et al.* (2002) have also found that, although in terms of numbers of particles many fewer of the larger CM remnants than LDL are retained in the intima, the mass of cholesterol retained from the remnants is more than 2-fold greater.

Although cholesterol carried in CM remnants represents only 5% of the plasma total even after a meal, because of the rapid turnover of these particles it has been estimated that they could contribute at least three times more cholesterol to the tissues than LDL in a 24-h period (Mamo, 1995). Since the studies of Mamo and colleagues established that the remnant particles enter the artery wall and are retained in the sub-endothelial space, it is clear that the lipids they carry have the opportunity to influence atherosclerosis development by interacting directly with cells in the tissue (Proctor *et al.*, 2002).

2.3. Influence of dietary lipid composition on chylomicron formation

The process of CM synthesis in enterocytes involves the synthesis of apo B-48, and other apolipoproteins, transport of lipids to the site of CM assembly, association of lipids with the formed apolipoproteins and incorporation of the lipid mass into the lipoprotein (Hussain, 2000).

Synthesis of apo B-48 as an integral component of the rough endoplasmic reticulum (RER) membrane is believed to be the first step in CM formation but they move rapidly to the smooth endoplasmic reticulum (SER) (Cartwright and Higgins, 2001). Addition of preformed phospholipids and a small amount of TG leads to the formation of the so-called primordial particle. The next step is the lipidation of the primordial particle by means of TG transfer by MTP (microsomal TG transfer protein), followed by the fusion of the particle with stable lipid droplets within the enterocyte. Early (30–90 min) secreted CM have been reported to be small dense particles that would represent mainly primordial particles. However, as absorption progresses, the amount of less dense particles increases (Silva *et al.*, 2001).

This is the model proposed for the synthesis of large CM, but seems not to be appropriate for VLDL or small CM formation. VLDL are continuously synthesized by the liver, also in the postprandial period, and contain apo B-100 as structural protein. In fact, apo B-48 is the result of truncation of the apo B-100 gene posttranscriptionally. In contrast to CM assembly, the formation of VLDL is not sensitive to the surfactant Pluronic L81, which disperses lipid droplets inhibiting the

formation of large lipoproteins (Hussain *et al.*, 2001). Therefore, VLDL formation must be only mediated by simple addition of TG molecules by MTP, which is saturable and limits the capacity of the liver to secrete lipoproteins.

VLDL secretion is under complex regulation via insulin control (Karpe, 1999). This hormone can regulate the availability of TG for VLDL production, the release of free fatty acids from adipose tissue by the action of hormone sensitive lipase (HSL) and the expression of lipoprotein lipase (LPL), the key enzyme for TRL metabolism. In addition, the liver can synthesize TG *de novo* for VLDL formation and can incorporate lipids from uptaken VLDL and CM remnants.

In contrast, CM synthesis and secretion are much more dependent on the dietary lipid composition. Assuming the model proposed, the size of the nascent CM particles that are secreted to the lymph would be partially determined by the size of the TG droplets they fuse with. Likewise, the size and composition of the TG droplets depends on the TG composition of the diets. It has been suggested that MUFA and PUFA could form more stable lipid droplets that would enhance the recruitment of primordial particles into the active secretory pathway (Williams *et al.*, 2004). Therefore, the fatty acid composition of the diet would determine the size and composition of mature CM.

In addition, dietary fatty acids may regulate key proteins involved in CM assembly. Van Greevenbroek *et al.* (1998) have suggested that oleic acid-rich TG may activate MTP compared with linoleic or palmitic acids, producing a larger number of CM. Although very little has been investigated to date in this regard, proteins involved in fatty acid transport, TG and cholesteryl ester synthesis, lipid droplet formation and even apo B-48 would be candidates for regulation by dietary fatty acids (Williams *et al.*, 2004).

Fatty acids stimulate the secretion of TG and apo B-48 from enterocytes as observed in Caco-2 cells. Most unsaturated fatty acids are more potent than saturated fatty acids in the stimulation of lipoprotein secretion, with oleic acid (18:1, n-9) being the most potent (Field *et al.*, 1988). When unsaturated fatty acids are applied to polarized Caco-2 cells, all secreted lipoproteins are of proper VLDL/CM size (d <1.006 g/ml Sf >400). However, when the cells are incubated with saturated fatty acids the size of most of the secreted lipoproteins is in the LDL/HDL range (1.009–1.068 g/ml). van Greevenbroek *et al.* (2000) have proposed that TG species that are synthesized during incubation with unsaturated fatty acids may be more efficiently incorporated into CM. In addition, these authors had previously shown that palmitic acid suppresses *de novo* synthesis of TG in Caco-2 cells (van Greevenbroek *et al.*, 1996). Cartwright and Higgins (1999) administered high fat diets containing fish oil, sunflower oil or a blend of different oils mimicking a Western diet to primary rabbit enterocytes for 2 weeks. They found that the rates of TG and apo B-48 synthesis were higher after the sunflower oil diet, followed by the Western diet. Fish oil produced the lowest effect on lipoprotein secretion by these cells.

Comparisons of the effects of n-6 PUFA dietary oils with olive oil ingested by humans have shown comparable (Lichtenstein *et al.*, 1993; Tholstrup *et al.*, 2001; Mekki *et al.*, 2002) magnitudes of postprandial lipaemia. In regard to SFA-rich fats or oils controversy remains: when butter was compared with olive oil the postprandial TG response was exacerbated (Thomsen *et al.*, 1999) or considerably

lower (Mekki *et al.*, 2002). Such discrepancies have been attributed to the amount of fat, composition of test meals and subjects involved. Olive oil has been shown to promote apo B-48 secretion by enterocytes postprandially (de Bruin *et al.*, 1993; Higashi *et al.*, 1997; Jackson *et al.*, 2002a), which supports the suggestion that olive oil causes a higher production of CM also in humans, perhaps through enhanced expression of apo B messenger RNA editing enzyme.

Jackson *et al.* (2002a) examined the effect of the type of fat ingested on the density distribution of CM in the Svedberg flotation rate (Sf). Ingestion of olive oil resulted in a greater postprandial response in the Sf >400 fraction compared with palm or safflower oils, suggesting that olive oil ingestion causes the formation of a greater number of large apo B 48-containing particles. In addition, such effect is retained after the ingestion of a second meal 5 h later (Jackson *et al.*, 2002b). However, these authors also pointed out that the majority of particles secreted are in the Sf 60–400 fraction, rather than the Sf >400 fraction, as measured by quantification of the apo B-48 content. Therefore, according to Jackson *et al.* (2002b) the enterocyte might be releasing CM particles of varying size, including those in the range of large VLDL or chylomicron remnants (Sf 60–400).

Another explanation for the higher TG secretion by enterocytes after the intake of oleic acid-rich oils is an enhanced absorption. Yang *et al.* (1990) studied the activity of pancreatic lipase towards TG of different compositions. The most efficiently hydrolyzed fatty acid was oleic acid and the least was docosahexaenoic acid (22:6, n-3). Christensen *et al.* (1995) confirmed that the presence of PUFA in the outer positions of the glycerol backbone retards the hydrolysis of the TG. Finally, El Boustani *et al.* (1987) and Lawson and Hughes (1988) showed that once fish oil TG are hydrolysed they are completely absorbed. A number of studies have demonstrated that oleic acid is better absorbed than saturated fatty acids (Thomson, 1980; Vallot *et al.*, 1985; Roche *et al.*, 1998). Bergstedt *et al.* (1990) observed a greater efficiency in absorption and transference to lymph of triolein compared with tristearin in rats fed emulsions of these TG. These findings indicate that substrate specificity of pancreatic lipase and TG structure and enterocyte uptake are limiting in the process of TG absorption.

We have compared the fatty acid and TG incorporation into CM after the intake of two high-oleic dietary oils, such as high-oleic sunflower oil (HOSO) and olive oil. In spite of an equal TG contribution from the diet the TG concentration in CM was higher after the intake of HOSO (Fig. 8.1). This cannot be a consequence of enhanced TG absorption due to oleic acid, since the concentration of this fatty acid in both oils was similar, but to the higher triolein content of HOSO (Abia *et al.*, 2001).

In summary, diets enriched in unsaturated fatty acids, and more importantly oleic acid, promote the synthesis of large CM for enterocytes in either model investigated. The mechanism underlying this effect would probably be related to both a greater TG synthesis and accumulation in the form of big TG droplets in the cell but also a more efficient assembly of the lipoproteins by regulation of the function of the proteins involved. Van Greevenbroek *et al.* (2000) have suggested that after the intake of a meal enriched in MUFA, larger CM are secreted from the enterocyte to the circulation, which are rapidly cleared. In contrast, when saturated fatty acids are absorbed, after the large CM containing the

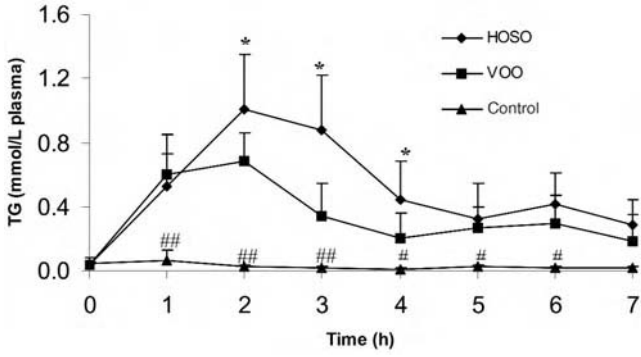


Fig. 8.1. Mean (± SD) triacylglycerol (TG) concentration during the postprandial period in the chylomicron (CM) fraction after virgin olive oil (VOO), high-oleic sunflower oil (HOSO) intake and control experiments. $n = 8$. TG from control experiment was significantly different for both VOO and HOSO-TG. Significant differences are represented by $p < 0.01$, $P < 0.05$, ANOVA.TG in the CM fraction after VOO intake as significantly different from CM-HOSO ($p < 0.05$). Data from Abia *et al.* (2001).

unsaturated fatty acids, smaller ones, containing mainly saturated fatty acids, are secreted. These smaller particles are cleared more slowly, leading to a more atherogenic lipoprotein profile.

2.4. Influence of dietary lipid composition on chylomicron clearance

The highest postprandial TG concentration in plasma occurs about 180 min after the intake of fat (Fielding *et al.*, 1996). However, it has been suggested that this TG peak would contain TG from the previous meal, which might be stored in the intestine or the lymphatic duct (Peel *et al.*, 1993; Fielding *et al.*, 1996). Once CM are in the bloodstream there is a transfer of apolipoproteins from HDL, including apo C-I, C-II, C-III and apo E (Havel *et al.*, 1973). Apo C-II acts as co-factor for lipoprotein lipase (LPL), enhancing its binding with the CM on the endothelial surface of blood vessels and unloading of its massive TG content (Pastch, 1987). CM particle half-life in plasma is certainly longer than the half-life of its constituent TG. The remaining particles are called CM-remnants and may have a half-life similar to VLDL of the same size (Grundy and Mok, 1976; Lichtenstein *et al.*, 1992).

Among other factors, the rate of hydrolysis depends on the size of the particles, since larger particles are more prone to binding the endothelium and LPL (Martins *et al.*, 1996). A more efficient contact with LPL lowers the residence time of CM in plasma and their atherogenicity. It has been demostrated that CM are not completely hydrolysed and that they still contain about 50% of their initial TG load when they leave the bloodstream (Karpe *et al.*, 1997b). In the postprandial period, both CM and VLDL compete for a common lipolytic pathway (Björkegren *et al.*, 1996). However, the competence is unbalanced, since there is a preference of LPL for CM and an accumulation of VLDL occurs (Cohn *et al.*, 1988; Karpe *et al.*, 1993; Schneeman *et al.*, 1993; Björkegren *et al.*, 1996). Such preference might be due to the apolipoprotein composition or the size of the particles.

Once hydrolysed, CM present the necessary size to be taken up by the liver (Fraser *et al.*, 1995). The original Apo E, acquired from HDL, as well as CM-associated LPL, interact with proteoglycans on the hepatocyte membrane (Friedman and Cardell, 1972; Ji *et al.*, 1993, 1995). At that moment, the particles can be further hydrolysed by hepatic lipase, and further enriched in apo E. When they acquire enough apo E, CM remnants can be recognized by LDL receptor (LDLr) and LDLr-related protein (LRP) (Chappell, 1992, 1993; Nykjaer *et al.*, 1993). The VLDL receptor also contributes to the uptake of CM remnants by the liver (Hussain *et al.*, 1996). Plasma levels of both CM remnants and VLDL remnants are elevated because of defective clearance, which in 90% of cases is associated with the apo E2/E2 phenotype, since the apo E2 isoform has reduced affinity for the LDL receptor (Gotto and Pownall, 1999; Mahley *et al.*, 1999; Eichner *et al.*, 2002). The disorder occurs in about 1 in 5000 of the population, despite the fact that apo E2/E2 occurs at a rate of 1 in 100, and secondary factors such as obesity or diabetes mellitus are usually required for full clinical expression (Gotto and Pownall, 1999).

Once the CM remnant has entered the hepatocyte, it is degraded by lysosomes (Hussain *et al.*, 1996). This is the way for most of dietary cholesterol to reach the liver pool (Beisiegel *et al.*, 1989). The incorporated cholesterol and TG can be stored or released again into circulation in the form of VLDL (Bravo *et al.*, 1995).

There is considerable information indicating that dietary fatty acid composition affects CM clearance in the postprandial period (Zampelas *et al.*, 1994; Sato *et al.*, 1999). It is well established that n-3 PUFA reduce plasma levels of TG and VLDL-TG (Harris *et al.*, 1983; Nozaki *et al.*, 1991). Additionally, the intake of n-3 PUFA lowers the postprandrial concentrations of CM and VLDL, probably due to an enhancement in the lipolysis rate and uptake by the liver (Zampelas *et al.*, 1994; Bravo *et al.*, 1995; Lambert *et al.*, 1995; Botham *et al.*, 1997).

The intake of fats and oils rich in saturated fatty acids leads to the formation of small CM due to a low efficiency in absorption and a slow clearance (Sakr *et al.*, 1997). CM enriched in linoleic acid (18:2, n-6), formed after the intake of oils rich in that fatty acid, are cleared from plasma more rapidly (Groot *et al.*, 1988; Levy *et al.*, 1991; Botham *et al.*, 1997; Porsgaard and Hoy, 2000).

3. Effect of Olive Oil on the Clearance of Serum Remnants

3.1. Evidence of the beneficial effects of olive oil on health

The great number of epidemiological studies developed in different countries constitutes a firm and reliable experimental base supporting the beneficial effects of olive oil in regard to the reduction of cardiovascular diseases. The Seven Countries Study, developed by Ancel Keys and colleagues for 15 years (Keys *et al.*, 1986) revealed a low rate of cardiovascular diseases in the Mediterranean area, where the main oil ingested was olive oil. The work of Katan and Mensink provided essential knowledge, demonstrating that dietary saturated fatty acids raise LDL-cholesterol whereas oleic and linoleic acids raise HDL and slightly lower LDL (Katan *et al.*, 1994, 1995).

An enhanced antioxidant capacity in the blood of the volunteers fed olive oil has also been observed and this was accompanied by a lower rate of monocyte adhesion to endothelial cells (Mata *et al.*, 1996).

Moreover, virgin olive oil significantly reduces blood pressure in subjects with both normo- and hypercholesterolaemic essential hypertension (Ruiz-Gutierrez *et al.*, 1996; Perona *et al.*, 2004a) (Fig. 8.2). At the same time, changes have been found in the fatty acid composition of erythrocyte membrane of women with untreated essential hypertension after the intake of diets enriched with olive oil (Ruiz-Gutierrez *et al.*, 1996) and it is known that olive oil enhances the content of oleic acid in membranes of spontaneously hypertensive rats (SHR) (Mills *et al.*, 1989). These changes have been related to other membrane modifications, such as the activities of the Na+-Li+ countertransport, the Na+-K+ cotransport and the Na+-K+ ATPase (Muriana *et al.*, 1996), the membrane protein G activity (Escriba *et al.*, 2003) and membrane fluidity (Vazquez *et al.*, 1996).

However, recent studies have pointed out that the content of oleic acid alone cannot fully explain the impact on health of olive oil. This conclusion has been drawn from studies comparing the effects of diets enriched with virgin olive oil or HOSO (Perez-Jimenez *et al.*, 1995; Jeffrey *et al.*, 1996; Ruiz-Gutierrez *et al.*, 1996; Perona and Ruiz-Gutierrez, 1998, 2000). Unlike virgin olive oil, HOSO was not able to reduce the blood pressure in hypertensive patients (Ruiz-Gutierrez *et al.*, 1996). Furthermore, it has been reported that LDL from olive oil-fed rats was more resistant to oxidation *in vitro* than those separated from plasma of triolein-fed rats (Scaccini *et al.*, 1992). These data support the idea that the protective effects of olive oil against cardiovascular disease must not be attributed exclusively to oleic acid, but to some other components of the oil.

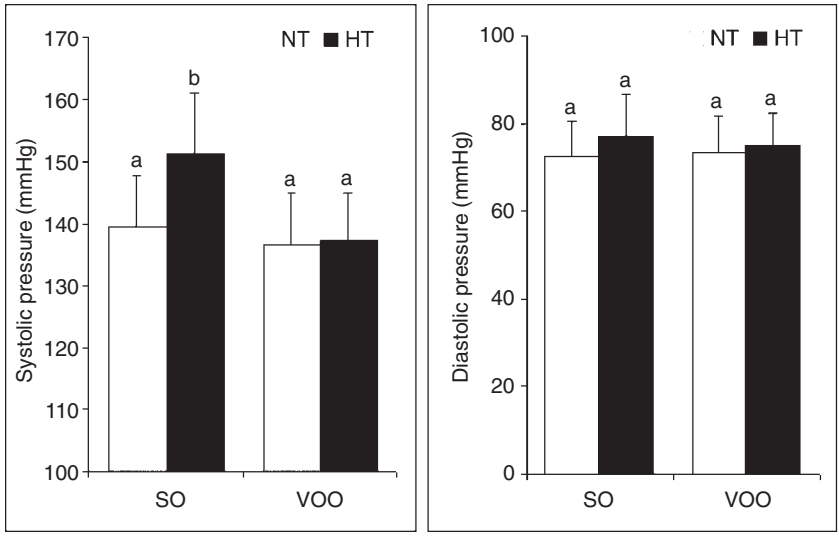


Fig. 8.2. Systolic and diastolic pressures of normotensive (NT) and hypertensive (HT) elderly subjects after consuming sunflower (SO) or virgin olive oil diets (VOO). Mean values within a row sharing the same letter are not significantly different ($p > 0.01$). Data from Perona *et al.* (2004a).

Among the differential characteristics of virgin olive oil, the quantitatively most important is the TG molecular species composition. Compared with HOSO, which contains mainly triolein, virgin olive oil contains also important amounts of dioleoyl-palmitoyl-glycerol. In much lower concentrations, but with growing evidence of important biological effects, minor components of olive oil can also be used to differentiate this oil from others. This suggestion has been corroborated by Nielsen *et al.* (2002) on the basis of experiments developed in fasting and postprandial conditions.

3.2. Olive oil in oxidative stress

Oxidation of lipoproteins, particularly LDL, is believed to play a major role in the etiology of atherosclerosis (Albertini *et al.*, 2002). Current evidence suggests that oxidation of LDL occurs within the artery wall by the action of cell-associated lipoxygenase and/myeloperoxidase (Staprans *et al.*, 1993; Martens and Holvoet, 2001). However, it has also been shown that oxidized lipids from the diet may play a significant role in generating oxidized lipoproteins in the circulation, through their absorption in CM and transport in CM remnants (Staprans *et al.*, 1996; Vine *et al.*, 1997, 1998).

There is evidence to indicate that both oxysterols, produced from cholesterol oxidation, and peroxidized fatty acids are absorbed in CM and are delivered to the liver in CM remnants, where they may be incorporated into endogenous lipoproteins. Feeding oxidized cholesterol in the diet has been shown to lead to the incorporation of oxysterols into CM and endogenous lipoproteins, including VLDL and LDL, in humans as well as in rats or rabbits (Vine *et al.*, 1997, 1998; Staprans *et al.*, 2003). Dietary oxidized fatty acids have also been reported to be absorbed via the lymph, transported in CM and delivered to the liver, where they are incorporated into VLDL (Aw *et al.*, 1992; Staprans *et al.*, 1994, 1996), although in one study lipid hydroperoxides were not detected in the lymph after intragastric administration (Mohr *et al.*, 1999). Moreover, the quantity of oxidized fatty acids in plasma has been shown to correlate directly with the level of oxidized CM in lymph in rats (Aw *et al.*, 1992; Staprans *et al.*, 1994, 1996) and with the oxidizability of the postprandial CM fraction in humans (Staprans *et al.*, 1994). In diabetic patients, a sustained increase in the levels of oxidized fats in CM has been reported, suggesting that the potentially increased atherogenicity of these lipoproteins may contribute to the accelerated atherosclerosis associated with diabetes (Staprans *et al.*, 1999).

3.3. The favourable effects of olive oil minor components

The low unsaturation of olive oil fatty acids, in addition to water soluble antioxidant protection in the form of phenolic compounds, favourably influences a reduced susceptibility to oxidation of olive oil-derived CM (Sutherland *et al.*, 2002). Weinbrenner *et al.* (2004) have recently reported that an olive oil intake of 25 ml in a single dose does not promote exacerbated hypertriglyceridaemia or hypergly-

caemia, which are linked to postprandial oxidative stress. These authors found a postprandial increase of both tyrosol and hydroxytyrosol, two phenolic compounds present in olive oil that are frequently used as markers of olive oil intake. The increased concentration of these phenols in plasma may be related to the reduced oxidative stress, since their increase was concomitant to a postprandial decrease in plasma oxidized LDL and 8-oxo-7,8-dihydro-2′-deoxyguanosine in mitochondrial DNA. Unfortunately, these authors did not measure CM oxidizability.

The unsaponifiable fraction of virgin olive oil is also rich in other minor components with antioxidant and anti-inflammatory properties, such as tocopherols, sterols or terpenic compounds (de la Puerta *et al.*, 2000; Rodriguez-Rodriguez *et al.*, 2004). We recently incubated endothelial cells with CM derived from a virgin olive oil enriched in its unsaponifiable fraction, finding a reduction in the production of prostaglandin E2 and thromboxane B2 compared with normal virgin olive oil and HOSO (Perona *et al.*, 2004b) (Fig. 8.3). These results suggest that minor components from virgin olive oil, transported postprandially in CM, may have a beneficial activity on endothelial cells.

3.4. The influence of olive oil on the clearance of CM from plasma

There are very few studies assessing the influence of olive oil on the rate of clearance of CM and therefore they are not very conclusive. Botham and collaborators have carried out a number of experiments in this regard. In one of their first studies they showed that the rates of clearance of CM produced following consumption of olive oil were lower than those of CM generated following consumption of PUFA-rich oils, but higher than those isolated after the intake of SFA-rich diets (Bravo *et al.*, 1995). However, later on they indicated that the rate of clearance was similar to CM formed after corn or fish oils (Lambert *et al.*, 1996; Botham *et al.*, 1997). Others have reported that substitution of MUFA, derived from olive oil, for saturated fat improved postprandial lipid metabolism and thrombosis and significantly reduced plasma cholesterol concentrations (Roche and Gibney, 2000).

Nielsen *et al.* (2002) recently compared two monounsaturated oils on TG postprandial response. Olive oil consumption resulted in higher postprandial plasma and lipoprotein TG concentration compared with rapeseed oil. Actually, these authors also found a positive correlation between fasting and postprandial TG concentrations, suggesting that the habitual intake affects also the postprandial response to olive oil. Lambert *et al.* (2001) reported that when virgin olive oil is chronically administrated to rats, CM uptake is enhanced, due to an increase in the expression of the LDL receptor. Therefore, the type of oil habitually consumed affects not only its absorption, but also CM formation and clearance. Zampelas *et al.* (1998) compared the postprandial response to SFA-rich and MUFA-rich meals of young males from Northern (Ireland and the United Kingdom) and Southern Europe (Crete, Greece). In response to both meals, subjects from Southern Europe showed a pronounced early increase in TG, with a rapid return to fasting conditions that was not seen in Northern Europeans. The sharp decrease in TG characteristic of the Southern European response was

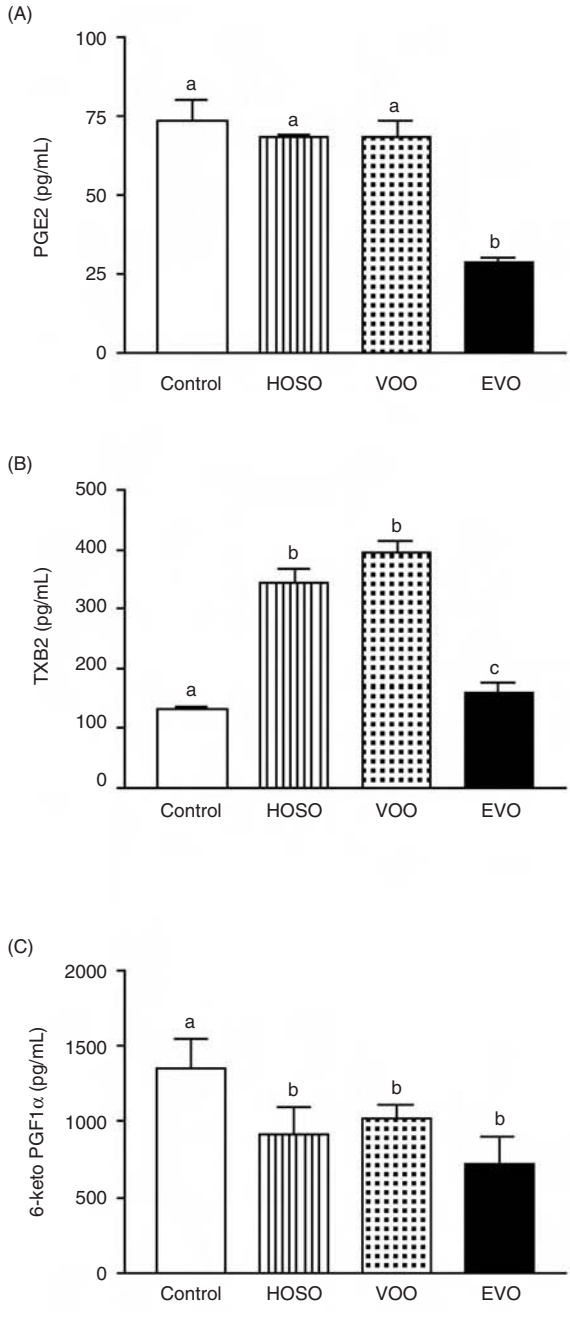


Fig. 8.3. Effect of chylomicrons (CM) on eicosanoid production. HUVEC were incubated (for 24 h) with CM obtained 2 h after the ingestion of high-oleic sunflower oil (HOSO), virgin olive (VOO) or enriched-virgin olive (EVO) oils. Eicosanoid released to the medium were determined by EIAs. Results corresponding to PGE2, TXB2 and PGI2 (as 6-keto-PGF1α) are shown in panels A, B and C respectively. a: $P < 0.05$, *vs* control; b: $P < 0.05$, *vs* HOSO; c: $P < 0.05$, *vs* VOO. Data from Perona *et al.* (2004b).

matched by a similar pattern of apo B-48 response. It was suggested that these differences might reflect greater hormonal activation of LPL and thereby faster clearance of circulating TG in Southern than Northern Europeans, although no differences in LPL concentrations were found. In agreement with previous observations, a larger increase in TG concentrations (but not apo B-48) was observed at time point 2 h in Southern Europeans, which was related to an earlier entry of larger CM. All these effects were attributed to habitual olive oil intake, but genetic differences could not be discarded.

In our study comparing the postprandial response to virgin olive oil and HOSO, data indicated that the predominant TG-molecular species in the CM fraction 2 h after the ingestion of the meals were similar to the TG composition of virgin olive oil; however, the proportions changed during the processes leading to their formation and clearance (Abia *et al.*, 2001) (Fig. 8.4). TG clearance from plasma was faster when subjects consumed virgin olive oil. In other words, intake of HOSO led to a delay in TG disappearance from plasma, which had already been described by Sakr *et al.* (1997). The lipid composition seems not to be a determinant factor in CM internalization by hepatocytes. In contrast, the function of the other pathways for TG clearance, i.e. the hydrolytic path, depends substantially on the TG structure and composition of CM (Botham *et al.*, 1997). Using artificial emulsions of monoacidic TG, Wang *et al.* (1992) found that LPL preferentially hydrolyzes fatty acids in the following order 18:1>18:3>18:2>14:0>16:0>18:0. However, more recently, Sato *et al.* (1999) observed that TRL enriched in palmitic acid were hydrolysed more efficiently than those enriched in oleic acid. These authors related the rate of hydrolysis with a lowering in particle fluidity, which enhanced the affinity between lipoproteins and LPL.

In our study we found very slight differences in the fatty acid composition of the CM after ingestion of the two high-oleic rich oils. However, relevant differences were found when we looked at the TG molecular species composition (Abia *et al.*, 2001). CM formed after virgin olive oil showed a higher content in TG species containing palmitic acid. In fact, these species were cleared more rapidly than those rich in oleic and/or linoleic acids, which is in agreement with the suggestion of Sato *et al.* (1999). Therefore, the fatty acid composition of the TG molecular species contained in CM would be determinant to assess the effect of dietary fat on the rate of clearance of postprandial CM. We have suggested that a preferential hydrolysis of palmitic acid-rich lipoproteins might be related with its storage in adipose tissue. In contrast, unsaturated fatty acids would remain in the lipoprotein particle and its final fate would be the liver. In the postabsorptive state the LPL activity is enhanced in the adipose tissue and suppressed in skeletal muscle (Cryer and Jones, 1978; Bergö *et al.*, 1996), in order to facilitate movement of fatty acids to adipose tissue, where they will form part of the storage TG.

Findings by de Bruin *et al.* (1993) and Brouwer *et al.* (1993) suggest that hepatic lipase plays a significant role in the removal of olive oil-derived lipoproteins. It has been proposed that CM remnants formed after virgin olive oil intake are taken up by the liver more slowly than those formed after corn or fish oil (Lambert *et al.*, 1995). This suggestion was made on the basis of a slower incorporation into rat liver of rat CM remnants containing labelled oleic acid and cholesterol. In that study the authors used CM obtained 1.45 h after the oil intake. At that time point,

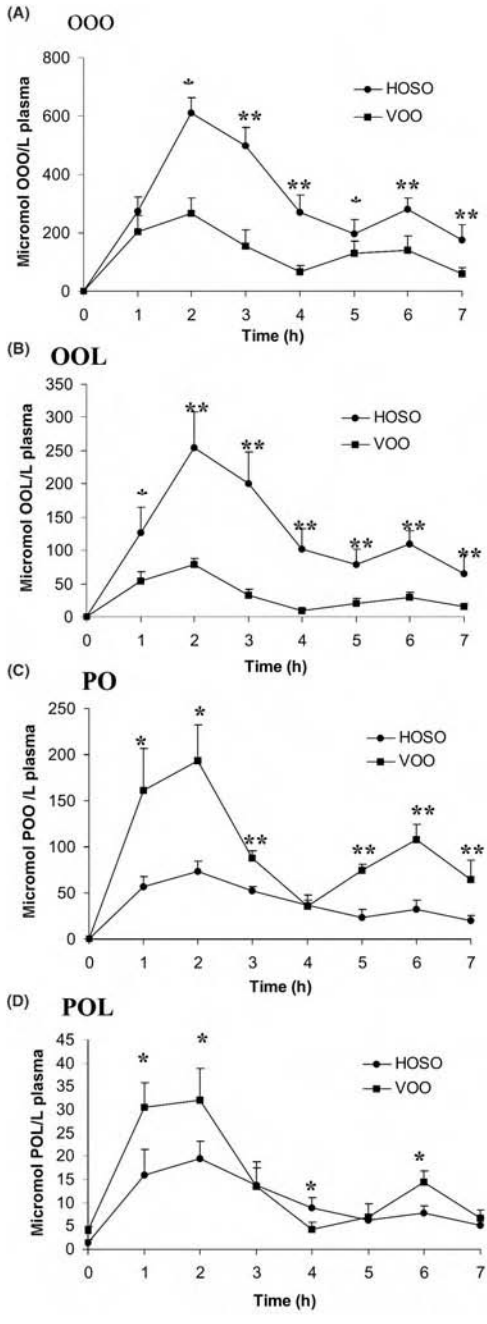


Fig. 8.4. Triacylglycerol molecular species (TG) composition of chylomicrons (CM) in men after the ingestion of virgin olive oil (VOO) or high-oleic sunflower oil (HOSO). Panels A–D show some of the most abundant TG detected. Values are means ± SD, $n = 8$. Means at a time without a common letter differ significantly, $P < 0.05$. Abbreviations used: OOO, triolein; OOL, dioleoyl-linoleoyglycerol; POO, palmitoyldioleoyl-glycerol; POL, palmitoyl-oleoyllinoleoyl-glycerol. Data from Abia *et al.* (2001).

CM might be too large or might not be sufficiently enriched in apo E to be taken up by the hepatocytes. The slower rate of uptake in the liver of virgin olive oil-derived CM remnants might be related to their bigger size (Karpe *et al.*, 1997a).

An abnormal metabolism of postprandial lipoproteins is a common finding in type 2 diabetes (Curtin *et al.*, 1994; De Man *et al.*, 1996). It has been shown that both fasting and postprandial CM are elevated in diabetes (Curtin *et al.*, 1996; Phillips *et al.*, 2000) and that diet modifies apo B-48 and apo B-100 secretion postprandially in diabetics (Madigan *et al.*, 2000). The postprandial glucose curves after ingestion of olive oil are lower when compared with butter or sunflower oil, as well as the postprandial response for TG and apo B-48 in type-2 diabetic subjects (Rasmussen *et al.*, 1996; Madigan *et al.*, 2000; Thomsen *et al.*, 2003). Therefore, in these patients olive oil has been demonstrated to provide a beneficial postprandial lipid and glucose profile and has proved to be the most suitable dietary option.

4. Conclusion

Elevated plasma TG concentration is now being considered as a risk factor for CHD, since TRL remnants are recognized to be atherogenic as they can enter the endothelial wall and interact with vascular cells like LDL. Dietary fatty acid composition influences the synthesis of postprandial TRL, such as CM, and their rate of clearance, therefore affecting their atherogenicity. Ingestion of oleic acid rich-oils, and especially olive oil, leads to formation of larger CM that are more rapidly cleared from plasma because they are more efficiently hydrolyzed by LPL. This might explain some of the favourable effects of olive oil on CHD, but does not explain the observed differences among oleic acid-rich oils. We have shown that olive oil and HOSO have many different effects on the processes related to the development of atherosclerosis, including those occurring in the postprandial state. The differences in the TG molecular species composition and minor components contained in the unsaponifiable fraction of the oil may be responsible for these different effects. These latter compounds present other interesting biological activities, such as antioxidant and anti-inflammatory activities that should be investigated.

5. References

Abia, R., Pacheco, Y.M., Perona, J.S., Montero, E., Muriana, F.J. and Ruiz-Gutierrez, V. (2001) The metabolic availability of dietary triacylglycerols from two high oleic oils during the postprandial period does not depend on the amount of oleic acid ingested by healthy men. *Journal of Nutrition* 131, 59–65.

Albertini, R., Moratti, R. and DeLuca, G. (2002) Oxidation of low-density lipoprotein in atherosclerosis from basic biochemistry to clinical studies. *Current Molecular Medicine* 2, 579–592.

Aw, T.Y., Williams, M.W. and Gray, L. (1992) Absorption and lymphatic transport of peroxidized lipids by rat small intestine in vivo: role of mucosal GSH. *American Journal of Physiology* 262, G99–G106.

Beisiegel, U., Weber, W., Ihrke, G., Herz, J. and Stanley, K.K. (1989) The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature* 341, 162–164.

Benlian, P., De Gennes, P.L., Foubert, L., Zhang, H., Gagne, S.E. and Hayden, M. (1996) Premature atherosclerosis in patients with familial chylomicronemia caused by mutations in the lipoprotein lipase gene. *New England Journal of Medicine* 335, 848–854.

Bergö, M., Olivecrona, G. and Olivecrona, T. (1996) Forms of lipoprotein lipase in rat tissues: in adipose tissue the proportion of inactive lipase increases on fasting. *Biochemical Journal* 313, 893–898.

Bergstedt, S.E., Hayashi, H., Kritchevsky, D. and Tso, P. (1990) A comparison of absorption of glycerol tristearate and glycerol trioleate by rat small intestine. *American Journal of Physiology* 259, G386–G393.

Björkegren, J., Hamsten, A., Milne, R.W. and Karpe, F. (1997) Alterations of VLDL composition during alimentary lipemia. *Journal of Lipid Research* 38, 301–314.

Björkegren, J., Packard, C.J., Hamsten, A., Bedford, D., Caslake, M., Foster, L., Shepherd, J., Stewart, P. and Karpe, F. (1996) Accumulation of large very low density lipoprotein in plasma during intravenous infusion of a chylomicron-like triglyceride emulsion reflects competition for a common lipolytic pathway. *Journal of Lipid Research* 37, 76–86.

Boren, J., Gustafsson, M., Skalen, K. and Flood, C. (2000) Innerarity TL. Role of extracellular retention of low density lipoproteins in atherosclerosis. *Current Opinion in Lipidology* 11, 451–456.

Botham, K.M., Avella, M., Cantafora, A. and Bravo, E. (1997) The lipolysis of chylomicrons derived from different dietary fats by lipoprotein lipase in vitro. *Biochimica et Biophysica Acta* 1349, 257–263.

Bravo, E., Ortu, G., Cantafora, A., Lambert, M.S., Avella, M., Mayes, P.A. and Bothman, K.M. (1995) Comparison of the uptake and processing of cholesterol from chylomicrons of different fatty acid composition in the rat in vivo. *Biochimica et Biophysica Acta* 1258, 328–336.

Brouwer, C.B., de Bruin, T.W., Jansen, H. and Erkelens, D.W. (1993) Different clearance of intravenously administered olive oil and soybean-oil emulsions: role of hepatic lipase. *American Journal of Clininical Nutrition* 57, 533–539.

Cartwright, I.J. and Higgins, J.A. (1999) Increased dietary triacylglycerol markedly enhances the ability of isolated rabbit enterocytes to secrete chylomicrons: an effect related to dietary fatty acid composition. *Journal of Lipid Research* 40, 1858–1866.

Cartwright, I.J. and Higgins, J.A. (2001) Direct evidence for a two-step assembly of ApoB48-containing lipoproteins in the lumen of the smooth endoplasmic reticulum of rabbit enterocytes. *Journal of Biological Chemistry* 276, 48048–48057.

Chappell, D.A., Fry, G.L., Waknitz, M.A., Iverius, P.-H., Williams, S.E. and Strickland, D.K. (1992) The low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor binds and mediates catabolism of bovine milk lipoprotein lipase. *Journal of Biological Chemistry* 267, 25764–25767.

Chappell, D.A., Fry, G.L., Waknitz, M.A., Muhonen, L.E., Pladet, M.W., Iverius, P.-H. and Strickland, D.K. (1993) Lipoprotein lipase induces catabolism of normal triglyceride-rich lipoproteins via the low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor in vitro. A process facilitated by cell-surface proteoglycans. *Journal of Biological Chemistry* 268, 14168–14175.

Christensen, M.S., Hoy, C.-E., Becker, C.C. and Redgrave, T. (1995) Intestinal absorption and lymphatic transport of eicosapentaenoic (EPA), docosahexaenoic (DHA) and decanoic acids: dependence of intramolecular triacylglycerol structure. *American Journal of Clinical Nutrition* 61, 56–61.

Cohn, J.S., McNamara, J.R., Cohn, S.D., Ordovás, J.M. and Schaefer, E.J. (1988) Plasma apolipoprotein changes in the triglyceride-rich lipoprotein fraction of human subjects fed a fat-rich meal. *Journal of Lipid Research* 29, 925–936.

Cryer, A. and Jones, H.M. (1978) Developmental changes in the activity of lipoprotein lipase (clearing-factor lipase) in rat lung, cardiac muscle, skeletal muscle and brown adipose tissue. *Biochemistry Journal* 174(2), 447–451.

Curtin, A., Deegan, P., Owens, D., Collins, P., Johnson, A. and Tomkin, G.H. (1994) Alterations in apolipoprotein B-48 in the postprandial state in NIDDM. *Diabetologia* 37, 1259–1264.

Curtin, A., Deegan, P., Owens, D., Collins, P., Johnson, A. and Tomkin, G.H. (1996) Elevated triglyceride-rich lipoproteins in diabetes. A study of apolipoprotein B-48. *Acta Diabetologica* 333, 205–210.

de Bruin, T.W., Brouwer, C.B., van Linde-Sibenius Trip, M., Jansen, H., Erkelens, D.W. (1993) Different postprandial metabolism of olive oil and soybean oil: a possible mechanism of the high-density lipoprotein conserving effect of olive oil. *American Journal of Clinical Nutrition* 58, 477–483.

de la Puerta, R., Martinez-Dominguez, E. and Ruiz-Gutierrez, V. (2000) Effect of minor components of virgin olive oil on topical anti-inflammatory assays. *Zeitschrift Fur Naturforschung C-A Journal of Bioscience* 55, 814–819.

De Man, F.H., Cabezas, M.C., Van Barlingen, H.H., Erkelens, D.W. and de Bruin, T.W. (1996) Triglyceride-rich lipoproteins in non-insulin-dependent diabetes mellitus: postprandial metabolism and relation to premature atherosclerosis. *European Journal of Clinical Investigation* 26, 89–108.

Durrington, P.N. (1998) Triglycerides are more important in atherosclerosis than epidemiology has suggested. *Atherosclerosis* 141, S57–S62.

Eichner, J.E., Dunn, S.T., Perveen, G., Thompson, D.M., Stewart, K.E. and Stroehla, B.C. (2002) Apolipoprotein E polymorphism and cardiovascular disease. *American Journal of Epidemiology* 155, 487–495.

El Boustani, S., Colete, C., Monnier, L., Descomps, B., Crastes de Pauet, A. and Mendy, F. (1987) Enteral absorption in man of eicosapentaenoic acid in different chemical forms. *Lipids* 22, 318–321.

Escriba, P.V., Sanchez-Dominguez, J.M., Alemany, R., Perona, J.S. and Ruiz-Gutierrez, V. (2003) Alteration of lipids, G proteins, and PKC in cell membranes of elderly hypertensives. *Hypertension* 41(1), 176–82.

Field, F.J., Albright, E. and Mathur, S.N. (1988) Regulation of triglyceride-rich lipoprotein secretion by fatty acids in CaCo-2 cells. *Journal of Lipid Research* 29, 1427–1437.

Fielding, B.A., Callow, J., Owen, R.M., Samra, J.S., Matthews, D.R. and Frayn, K.N. (1996) Postprandial lipemia: the origin of an early peak studied by specific dietary fatty acid intake during sequential meals. *American Journal of Clinical Nutrition* 63, 36–41.

Fraser, R., Dobbs, B.R. and Rogers, G.W. (1995) Lipoproteins and the liver sieve: the role of the fenestrated sinusoidal endothelium in lipoprotein metabolism, atherosclerosis, and cirrhosis. *Hepatology* 21, 863–874.

Friedman, H.I. and Cardell, R.R. (1972) Morphological evidence for the release of chylomicron from intestinal absorptive cells. *Experimental Cell Research* 75, 57–62.

Ghung, B.H., Talis, G., Yalamoori, V., Anantharamaiah, G.M. and Segrest, J.P. (1994) Liposome-like particles isolated from human atherosclerotic plaques are structurally similar to surface remnants of triglyceride-rich lipoproteins. *Arteriosclerosis Thrombosis and Vascular Biology* 14, 622–635.

Gotto, A. (1998) Triglyceride as a risk factor for coronary artery disease. *American Journal of Cardiology* 82, 22Q–25Q.

Gotto, A. and Pownall, H. (1999) *Manual of Lipid Disorders*, Williams and Wilkins, Baltimore.

Groot, P.H.E., De Boer, B.C., Haddman, E., Houstmuller, U.M.T. and Hülsmann, W.C. (1988) Effect of dietary fat composition on the metabolism of triacylglycerol-rich plasma lipoproteins in the postprandial phase in meal-fed rats. *Journal of Lipid Research* 29, 541–551.

Groot, P.H.E., van Stiphout, W.A.H.J., Krauss, X.H., Jansen, H., van Tol, A., van Ramshorst, E., Chin-On, S., Hofman, A., Cresswell, S.R. and Havekes, L. (1991) Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. *Arteriosclerosis Thrombosis and Vascular Biology* 11, 653–662.

Grundy, S.M. and Mok, H.Y. (1976) Chylomicron clearance in normal and hyperlipidemic man. *Metabolism* 25, 1225–1239.

Harris, W.S., Connor, W.E. and McMurry, M.P. (1983) The comparative reductions of the plasma lipids and lipoproteins by dietary polyunsaturated fats: salmon oil versus vegetable oils. *Metabolism* 32, 179–184.

Havel, R.J., Kane, J.P. and Kashyap, M.L. (1973) Interchange of apolipoproteins between chylomicrons and high density lipoproteins during alimentary lipemia in man. *Journal of Clinical Investigation* 52, 32–38.

Higashi, K., Ishikawa, T., Shige, H., Tomiyasu, K., Yoshida, H., Ito, T., Nakajima, K., Yonemura, A., Sawada, S. and Nakamura, H. (1997) Olive oil increases the magnitude of postprandial chylomicron remnants compared to milk fat and safflower oil. *Journal of the American College of Nutrition* 16, 429–434.

Hussain, M.M. (2000) A proposed model for the assembly of chylomicrons. *Atherosclerosis* 148, 1–15.

Hussain, M.M., Kancha, R.K., Zhou, Z., Luchoomun, J., Zu, H. and Bakillah, A. (1996) Chylomicron assembly and catabolism: role of apolipoproteins and receptors. *Biochimica et Biophysica Acta* 1300, 151–170.

Hussain, M.M., Kedees, M.H., Singh, K., Athar, H. and Jamali, N.Z. (2001) Signposts in the assembly of chylomicrons. *Frontiers in Bioscience* 6, D320–D331.

Jackson, K.G., Robertson, M.D., Fielding, B.A., Frayn, K.N. and Williams, C.M. (2002a) Measurement of apolipoprotein B-48 in the Svedberg flotation rate (S(f))>400, S(f) 60–400 and S(f) 20–60 lipoprotein fractions reveals novel findings with respect to the effects of dietary fatty acids on triacylglycerol-rich lipoproteins in postmenopausal women. *Clinical Science (London)* 103, 227–237.

Jackson, K.G., Robertson, M.D., Fielding, B.A., Frayn, K.N. and Williams, C.M. (2002b) Olive oil increases the number of triacylglycerol-rich chylomicron particles compared with other oils: an effect retained when a second standard meal is fed. *American Journal of Clinical Nutrition* 76, 942–949.

Jeffery, N.M., Yaqoob, P., Newsholme, E.A. and Calder, P.C. (1996) The effects of olive oil upon rat serum lipid levels and lymphocyte functions appear to be due to oleic acid. *Annals of Nutrition and Metabolism* 40, 71–80.

Ji, Z.S., Brecht, W.J., Miranda, R.D., Hussain, M.M., Innerarity, T.L. and Mahley, R.W. (1993) Role of heparan sulphate proteoglycans in the binding and uptake of apolipoprotein E-enriched remnant lipoproteins by cultured cells. *Journal of Biological Chemistry* 268, 10160–10167.

Ji, Z.S., Sanan, D.A. and Mahley, R.W. (1995) Intravenous heparinase inhibits remnant lipoprotein clearance from the plasma and uptake by the liver: in vivo role of heparan sulphate proteoglycans. *Journal of Lipid Research* 36, 583–592.

Kadar, A. and Glasz, T. (2001) Development of atherosclerosis and plaque biology. *Cardiovascular Surgery* 9, 109–121.

Karpe, F. (1999) Postprandial lipoprotein metabolism and atherosclerosis. *Journal of Internal Medicine* 246, 341–355.

Karpe, F., Steiner, G., Olivecrona, T., Carlson, A. and Hamsten, A. (1993) Metabolism of triglyceride-rich lipoproteins during alimentary lipemia. *Journal of Clinical Investigation* 91, 748–758.

Karpe, F., Olivecrona, T., Hamsten, A. and Hultin, M. (1997a) Chylomicron/chylomicron remnant turnover in humans: evidence for margination of chylomicrons and poor conversion of larger to smaller chylomicron remnants. *Journal of Lipid Research* 38, 949–961.

Karpe, F., Humphreys, S.M., Samra, J.S., Summers, L.K. and Frayn, K.N. (1997b) Clearance of lipoprotein remnant particles in adipose tissue and muscle in humans. *Journal of Lipid Research* 38, 2335–2343.

Katan, M.B., Zock, P.L. and Mensink, R.P. (1994) Effects of fats and fatty acids on blood lipids in humans: an overview. *American Journal of Clinical Nutrition* 60, 1017S–1022S.

Katan, M.B., Zock, P.L. and Mensink, R.P. (1995) Dietary oils, serum lipoproteins, and coronary heart disease. *American Journal of Clinical Nutrition* 61, 1368S–1373S.

Keys, A., Menotti, A., Karvonen, M.J., Aravanis, C., Blackburn, H., Buzina, R., Djordjevic, B.S., Dontas, A.S., Fidanza, F. and Keys, M.H. (1986) The diet and 15-year death rate in the seven countries study. *American Journal of Epidemiology* 124, 903–915.

Lambert, M.S., Botham, K.M. and Mayes, P.A. (1995) Variations in composition of dietary fats affect hepatic uptake and metabolism of

chylomicron remnants. *Biochemical Journal* 310, 845–852.

Lambert, M.S., Botham, K.M. and Mayes, P.A. (1996) Modification of the fatty acid composition of dietary oils and fats on incorporation into chylomicrons and chylomicron remnants. *British Journal of Nutrition* 76, 435–445.

Lambert, M.S., Avella, M.A., Berhane, Y., Shervill, E. and Botham, K.M. (2001) The fatty acid composition of chylomicron remnants influences their binding and internalization by isolated hepatocytes. *European Journal of Biochemistry* 268, 3983–3992.

Lawson, L.D. and Hughes, B.G. (1988) Human absorption of fish oil fatty acids as triacylglycerols, free acids, or ethyl esters. *Biochemical and Biophysical Research Communications* 152, 328–335.

Levy, E., Roy, C.C., Goldstein, R., Bar-On, H. and Ziv, E. (1991) Metabolic fate of chylomicrons obtained from rats maintained on diets varying in fatty acid composition. *Journal of the American College of Nutrition* 10, 69–78.

Lichtenstein, A.H., Ausman, L.M., Carrasco, W., Jenner, J.L., Gualtieri, L.J., Goldin, B.R., Ordovas, J.M. and Schaefer, E.J. (1993) Effects of canola, corn, and olive oils on fasting and postprandial plasma lipoproteins in humans as part of a National Cholesterol Education Program Step 2 diet. *Arteriosclerosis Thrombosis and Vascular Biology* 13, 1533–1542.

Lichtenstein, A.H., Hachey, D.L., Millar, J.S., Jenner, J.L., Booth, L., Ordovas, J. and Schaefer, E.J. (1992) Measurement of human apolipoprotein B-48 and B-100 kinetics in triglyceride-rich lipoproteins using [5,5,5-2H3]leucine. *Journal of Lipid Research* 33, 907–914.

Madigan, C., Ryan, M., Owens, D., Collins, P. and Tomkin, G.H. (2000) Dietary unsaturated fatty acids in type 2 diabetes: higher levels of postprandial lipoprotein on a linoleic acid-rich sunflower oil diet compared with an oleic acid-rich olive oil diet. *Diabetes Care* 23, 1472–1477.

Mahley, R.W., Huang, Y. and Rall, S.C. (1999) Pathogenesis of type III hyperlipoproteinemia (dysbetalipoproteinemia): questions, quandaries, and paradoxes. *Journal of Lipid Research* 40, 1933–1949.

Mamo, J.C. (1995) Atherosclerosis as a postprandial disease. *Endocrinology and Metabolism* 2, 229–244.

Martins, I.J., Mortimer, B.-C., Miller, J. and Redgrave, T.G. (1996) Effects of particle size and number on the plasma clearance of chylomicrons and remnants. *Journal of Lipid Research* 37, 2696–2705.

Mata, P., Alonso, R., Lopez-Farre, A., Ordovas, J.M., Lahoz, C., Garces, C., Caramelo, C., Codoceo, R., Blazquez, E. and de Oya, M. (1996) Effect of dietary fat saturation on LDL oxidation and monocyte adhesion to human endothelial cells in vitro. *Arteriosclerosis Thrombosis and Vascular Biology* 16, 1347–1355.

Mekki, N., Charbonnier, M., Borel, P., Leonardi, J., Juhel, C., Portugal, H. and Lairon, D. (2002) Butter differs from olive oil and sunflower oil in its effects on postprandial lipemia and triacylglycerol-rich lipoproteins after single mixed meals in healthy young men. *Journal of Nutrition* 132, 3642–3649.

Mills, D.E., Ward, R.P., Mah, M. and DeVette, L. (1989) Dietary N-6 and N-3 fatty acids and salt-induced hypertension in the borderline hypertensive rat. *Lipids* 24, 17–24.

Mohr, D., Umeda, Y., Redgrave, T.G. and Stocker, R. (1999) Antioxidant defenses in rat intestine and mesesnteric lymph. *Redox Reports* 4, 79–87.

Muriana, F.J., Villar, J. and Ruiz-Gutierrez, V. (1996) Erythrocyte membrane cholesterol distribution in patients with untreated essential hypertension: correlation with sodium-lithium countertransport. *Journal of Hypertension* 14, 443–446.

Nielsen, N.S., Pedersen, A., Sandstrom, B., Marckmann, P. and Hoy, C.E. (2002) Different effects of diets rich in olive oil, rapeseed oil and sunflower-seed oil on postprandial lipid and lipoprotein concentrations and on lipoprotein oxidation susceptibility. *British Journal of Nutrition* 87, 489–499.

NIH Consensus Conference (1993) Triglyceride, high-density lipoprotein, and coronary heart disease. NIH Consensus Development Panel on Triglyceride, High-Density Lipoprotein, and Coronary Heart Disease. *Journal of the American Medical Association* 269, 505–510.

Nordestgaard, B.G. and Ziversmit, D.B. (1988) Large lipoproteins are excluded from the arterial wall in diabetic cholesterol-fed rabbits. *Journal of Lipid Research* 29, 1491–1500.

Nozaki, S., Vega, G.L. and Grundy, S.M. (1991) Postheparin lipolytic activity and plasma lipoprotein response to ω-3 polyunsaturated fatty acids in patients with primary hypertriglyceridemia. *American Journal of Clinical Nutrition* 53, 638–643.

Nykjaer, A., Bengtsson-Olivecrona, G., Lookene, A., Moestrup, S.K., Petersen, C.M., Weber, W., Beisiegel, U. and Gliemann, J. (1993) The alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein binds lipoprotein lipase and beta-migrating very low density lipoprotein associated with the lipase. *Journal of Biological Chemistry* 268, 15048–15055

Ooi, T.C. and Ooi, D.S. (1998) The atherogenic significance of an elevated plasma triglyceride level. *Critical Reviews in Clinical Laboratory Sciences* 35, 489–516.

Pal, S., Semorine, K., Watts, G.F. and Mamo, J. (2003) Identification of lipoproteins of intestinal origin in human atherosclerotic plaque. *Clinical Chemistry and Laboratory Medicine* 41, 792–795.

Patsch, J.R. (1987) Postprandial lipaemia. *Baillieres Clinical Endocrinology and Metabolism* 1, 551–80.

Patsch, J.R., Miesenbock, G., Hopferwieser, T., Muhlberger, V., Knapp, E., Dunn, J.K., Gotto, A.M., Jr. and Patsch, W. (1992) Relation of triglyceride metabolism and cardiovascular disease. *Arteriosclerosis Thrombosis and Vascular Biology* 12, 1336–1345.

Peel, A.S., Zampelas, A., Williams, C.M. and Gould, B.J. (1993) A novel antiserum specific to apolipoprotein B-48: application in the investigation of postprandial lipemia in humans. *Clinical Science* 85, 521–524.

Perez-Jimenez, F., Espino, A., Lopez-Segura, F., Blanco, J., Ruiz-Gutierrez, V., Prada, J.L., Lopez-Miranda, J., Jimenez-Pereperez, J. and Ordovas, J.M. (1995) Lipoprotein concentrations in normolipidemic males consuming oleic acid-rich diets from two different sources: olive oil and oleic acid-rich sunflower oil. *American Journal of Clinical Nutrition* 62, 769–775.

Perona, J.S. and Ruiz-Gutiérrez, V. (1998) Two highly monounsaturated oils, olive oil and high-oleic sunflower oil, induce different triacylglycerol molecular species distribution in rat liver. *Nutrition Research* 18, 1723–1732.

Perona, J.S. and Ruiz-Gutierrez, V. (2000) Effect of two high-oleic oils on the liver lipid composition of spontaneously hypertensive rats. *Life Sciences* 66, 521–531.

Perona, J.S., Canizares, J., Montero, E., Sanchez-Dominguez, J.M., Catala, A. and Ruiz-Gutierrez, V. (2004a) Virgin olive oil reduces blood pressure in hypertensive elderly subjects. *Clinical Nutrition* 23, 1113–1121.

Perona, J.S., Martinez-Gonzalez, J., Sanchez-Dominguez, J.M., Badimon, L. and Ruiz-Gutierrez, V. (2004b) The unsaponifiable fraction of virgin olive oil in chylomicrons from men improves the balance between vasoprotective and prothrombotic factors released by endothelial cells. *Journal of Nutrition* 134, 3284–3289.

Phillips, C., Murugasu, G., Owens, D., Collins, P., Johnson, A. and Tomkin, G.H. (2000) Improved metabolic control reduces the number of postprandial apolipoprotein B-48-containing particles in type 2 diabetes. *Atherosclerosis* 148, 283–291.

Porsgaard, T. and Hoy, C.-E. (2000) Lymphatic transport in rats of several dietary fats differing in fatty acid profile and triacylglycerol structure. *Journal of Nutrition* 130, 1619–1624.

Proctor, S.D., Vine, D.F. and Mamo, J.C. (2002) Arterial retention of apolipoprotein B48- and B100-containing lipoproteins in atherogenesis. *Current Opinion in Lipidology* 13, 461–470.

Rapp, J.H., Harris, H.W., Hamilton, R.L., Krupski, W.C., Reilly, L.M., Ehrenfeld, W.K., Stoney, R.J., Goldstone, J. and Kane, J.P. (1989) Particle size distribution of lipoproteins from human atherosclerotic plaque: a preliminary report. *Journal of Vascular Surgery* 9, 81–88.

Rapp, J.H., Lespine, A., Hamilton, R.L., Colyvas, N., Chaumeton, A.H., Tweedie-Hardman, J., Kotite, L., Kunitake, S.T., Havel, R.J. and Kane, J.P. (1994) Triglyceride-rich lipoproteins isolated by selected-affinity anti-apolipoprotein B immunosorption from

human atherosclerotic plaque. *Arteriosclerosis Thrombosis and Vascular Biology* 14, 1767–1774.

Rapp, R.J. (2002) Hypertriglyceridemia: a review beyond low-density lipoprotein. *Cardiology in Review* 10, 163–172.

Rasmussen, O., Lauszus, F.F., Christiansen, C., Thomsen, C. and Hermansen, K. (1996) Differential effects of saturated and monounsaturated fat on blood glucose and insulin responses in subjects with non-insulin-dependent diabetes mellitus. *American Journal of Clinical Nutrition* 63, 249–253.

Roche, H.M. and Gibney, M.J. (2000) Effect of long-chain n-3 polyunsaturated fatty acids on fasting and postprandial triacylglycerol metabolism. *American Journal of Clinical Nutrition* 71, 232S–237S.

Roche, H.M. and Gibney, M.J. (1999) Long-chain n-3 polyunsaturated fatty acids and triacylglycerol metabolism in the postprandial state. *Lipids* 34, S259–256.

Roche, H.M., Zampelas, A., Knapper, J.M., Webb, D., Brooks, C., Jackson, K.G., Wright, J.W., Gould, B.J., Kafatos, A., Gibney, M.J. and Williams, C.M. (1998) Effect of long-term olive oil dietary intervention on postprandial triacylglycerol and factor VII metabolism. *American Journal of Clinical Nutrition* 68, 552–560.

Rodriguez-Rodriguez, R., Herrera, M.D., Perona, J.S. and Ruiz-Gutierrez, V. (2004) Potential vasorelaxant effects of oleanolic acid and erythrodiol, two triterpenoids contained in 'orujo' olive oil, on rat aorta. *British Journal of Nutrition* 92, 635–642.

Ross, R. (1993) The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362, 801–809.

Ruiz-Gutierrez, V., Muriana, F.J., Guerrero, A., Cert, A.M. and Villar, J. (1996) Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources. *Journal of Hypertension* 14, 1483–1490.

Sakr, S.W., Attia, N., Haourigui, M., Paul, J.L., Soni, T., Vacher, D. and Girard-Globa, A. (1997) Fatty acid composition of an oral load affects chylomicron size in human subjects. *British Journal of Nutrition* 77, 19–31.

Sato, K., Takahashi, T., Takahashi, Y., Shiono, H., Katoh, N. and Akiba, Y. (1999) Preparation of chylomicron and VLDL with monoacid-rich triacylglycerol and characterization of kinetic parameters in lipoprotein lipase-mediated hydrolysis in chickens. *Journal of Nutrition* 129, 126–131.

Scaccini, C., Nardini, M., D'Aquino, M., Gentili, V., Di Felice, M. and Tomassi, G. (1992) Effect of dietary oils on lipid peroxidation and on antioxidant parameters of rat plasma and lipoprotein fractions. *Journal of Lipid Research* 33, 627–633.

Schneeman, B.O., Kotite, L., Todd, K.M. and Havel, R.J. (1993) Relationship between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and b-100 to a fat-containing meal normolipidemic humans. *Proceedings of the National Academy of Sciences of USA* 90, 2069–2073.

Sharrett, A.R., Chambless, L.E., Heiss, G., Paton, C.C. and Patsch, W. (1995) Association of postprandial triglyceride and retinyl palmitate responses with asymptomatic carotid artery atherosclerosis in middle-aged men and women. The Atherosclerosis Risk in Communities (ARIC) Study. *Arteriosclerosis Thrombosis and Vascular Biology* 15, 2122–2129.

Silva, K.D.R.R., Jones, A.E., Smith, R.D., Kelly, C.N.M., Lovegrove, J.A., Wootton, S.A. and Williams, C.M. (2001) Different postprandial responses of larger and smaller chylomicrons and remnant particles. *Proceedings of the Nutrition Society* 60, 44A.

Staprans, I., Pan, X.M., Miller, M. and Rapp, J.H. (1993) The effect of oxidation on the chylomicron metabolism in rats. *American Journal of Physiology* 27, G561–G568.

Staprans, I., Pan, X.M., Rapp, J.H., Kim, K.Y. and Feingold, R. (1994) Oxidized lipids in the diet are a source of oxidized lipid in chylomicrons of human serum. *Arteriosclerosis Thrombosis and Vascular Biology* 14, 1090–1095.

Staprans, I., Pan, X.M., Rapp, J.H. and Feingold, R. (1996) Oxidized lipids in the diet are incorporated by the liver into very low density lipoprotein in rats. *Journal of Lipid Research* 37, 420–430.

Staprans, I., Hardman, D.A., Pan, X.-M. and Feingold, R. (1999) Effect of oxidized lipids in the diet on oxidized lipid levels in postprandial serum chylomicrons of diabetic patients. *Diabetes Care* 22, 300–306.

Staprans, I., Pan, X.M., Rapp, J.H. and Feingold, R. (2003) Oxidized cholesterol in the diet is a source of oxidized lipoproteins in human serum. *Journal of Lipid Research* 44, 705–715.

Stender, S. and Zilversmit, D.B. (1981) Transfer of plasma lipoprotein components and of plasma proteins into aortas of cholesterol-fed rabbits: molecular size as a determinant of plasma lipoprotein influx. *Arteriosclerosis* 1, 38–49.

Sutherland, W.H., de Jong, S.A., Walker, R.J., Williams, M.J., Murray Skeaff, C., Duncan, A. and Harper, M. (2002) Effect of meals rich in heated olive and safflower oils on oxidation of postprandial serum in healthy men. *Atherosclerosis* 160, 195–203.

Tholstrup, T., Sandstrom, B., Bysted, A. and Holmer, G. (2001) Effect of 6 dietary fatty acids on the postprandial lipid profile, plasma fatty acids, lipoprotein lipase, and cholesterol ester transfer activities in healthy young men. *American Journal of Clinical Nutrition* 73, 198–208.

Thomsen, C., Rasmussen, O., Lousen, T., Holst, J.J., Fenselau, S., Schrezenmeir, J. and Hermansen, K. (1999) Differential effects of saturated and monounsaturated fatty acids on postprandial lipemia and incretin responses in healthy subjects. *American Journal of Clinical Nutrition* 69, 1135–1143.

Thomsen, C., Storm, H., Holst, J.J. and Hermansen, K. (2003) Differential effects of saturated and monounsaturated fats on postprandial lipemia and glucagon-like peptide 1 responses in patients with type 2 diabetes. *American Journal of Clinical Nutrition* 77, 605–611.

Thomson, A.B.R. (1980) Influence of site and unstirred layers on the rate of uptake of cholesterol and fatty acids into rabbit intestine. *Journal of Lipid Research* 21, 1097–1107.

Tomkin, G.H. and Owens, D. (2001) Abnormalities in apoB-containing lipoproteins in diabetes and atherosclerosis. *Diabetes-Metabolism Research and Reviews* 17, 27–43.

Vallot, A., Bernard, A. and Carlier, H. (1985) Influence of the diet on the portal and lymph transport of decanoic acid in rats. Simultaneous study of its mucosal catabolism. *Comparative Biochemistry and Physiology* 82, 693–699.

van Greevenbroek, M.M., van Meer, G., Erkelens, D.W. and de Bruin, T.W. (1996) Effects of saturated, mono-, and polyunsaturated fatty acids on the secretion of apo B containing lipoproteins by Caco-2 cells. *Atherosclerosis* 121, 139–150.

van Greevenbroek, M.M., Robertus-Teunissen, M.G., Erkelens, D.W. and de Bruin, T.W. (1998) Participation of the microsomal triglyceride transfer protein in lipoprotein assembly in Caco-2 cells: interaction with saturated and unsaturated dietary fatty acids. *Journal of Lipid Research* 39, 173–185.

van Greevenbroek, M.M., Erkelens, D.W. and de Bruin, T.W. (2000) Caco-2 cells secrete two independent classes of lipoproteins with distinct density: effect of the ratio of unsaturated to saturated fatty acid. *Atherosclerosis*. 149, 25–31.

Vazquez, C.M., Zanetti, R. and Ruiz-Gutierrez, V. (1996) Lipid composition and fluidity in the jejunal brush-border membrane of spontaneously hypertensive rats. Effects on activities of membrane-bound proteins. *Bioscience Reports* 16, 217–226.

Vine, D.F., Croft, K.D., Beilin, L.J. and Mamo, J.C. (1997) Absorption of dietary cholesterol oxidation products and incorporation into rat lymph chylomicrons. *Lipids* 32, 887–893.

Vine, D.F., Mamo, J.C.L., Beilin, L.J., Mori, T.A. and Croft, K.D. (1998) Dietary oxysterols are incorporated in plasma triglyceride-rich lipoproteins, increase their susceptibility to oxidation and increase aortic cholesterol concentration of rabbits. *Journal of Lipid Research* 39, 1995–2004.

Wang, C.S., Hartsuck, J. and McConathy, W.J. (1992) Structure and functional properties of lipoprotein lipase. *Biochimica et Biophysica Acta* 1123, 1–17.

Weinbrenner, T., Fito, M., de la Torre, R., Saez, G.T., Rijken, P., Tormos, C., Coolen, S., Albaladejo, M.F., Abanades, S., Schroder, H., Marrugat, J. and Covas, M.I. (2004) Olive

oils high in phenolic compounds modulate oxidative/antioxidative status in men. *Journal of Nutrition* 134, 2314–2321.

Wilhelm, M.G. and Cooper, A.D. (2003) Induction of atherosclerosis by human chylomicron remnants: a hypothesis. *Journal of Atherosclerosis and Thrombosis* 10, 132–139.

Williams, C.M., Bateman, P.A., Jackson, K.G. and Yaqoob, P. (2004) Dietary fatty acids and chylomicron synthesis and secretion. *Biochemical Society Transactions* 32, 55–58.

Yang, L.Y., Kuksis, A. and Myher, J.J. (1990) Lipolysis of menhaden oil triacylglycerols and the corresponding fatty acid alkyl esters by pancreatic lipase in vitro: a reexamination. *Journal of Lipid Research* 31, 137–147.

Yu, K.C. and Cooper, A.D. (2001) Postprandial lipoproteins and atherosclerosis. *Frontiers in Bioscience* 6, D332–D354.

Zampelas, A., Peel, A.S., Gould, B.J., Wright, J. and Williams, C.M. (1994) Polyunsaturated fatty acids of the n-6 and n-3 series: effects on postprandial lipid and apolipoprotein levels in healthy men. *European Journal of Clinical Nutrition* 48, 842–848.

Zampelas, A., Roche, H., Knapper, J.M., Jackson, K.G., Tornaritis, M., Hatzis, C., Gibney, M.J., Kafatos, A., Gould, B.J., Wright, J. and Williams, C.M. (1998) Differences in postprandial lipaemic response between Northern and Southern Europeans. *Atherosclerosis* 139, 83–93.

Zilversmit, D.B. (1979) Atherogenesis: a postprandial phenomenon. *Circulation* 60, 473–485.

9 Effect of Olive Oil on Cardiovascular Risk Factor, LDL Oxidation and Atherosclerosis Development

M. Dolores Mesa,[1] Concepción M. Aguilera,[1] Cesar L. Ramirez-Tortosa,[2] Angel Gil[1] and M. Carmen Ramírez-Tortosa[1]

[1]*Institute of Nutrition and Food Technology, Department of Biochemistry and Molecular Biology, University of Granada, Granada, Spain*; [2]*Department of Pathology, Complejo Hospitalario de Jaen, Jaen, Spain*

1. Introduction

Cardiovascular diseases (CVD) are considered as a group of multifactorial conditions associated with atherosclerosis, hypertension and thrombosis. These pathologic entities are closely related to both genetic factors and environmental influences. Atherosclerosis is the underlying pathology in most CVD. This multifactorial disease is modifiable by dietary components and lifestyle practices. Main risk factors are involved, including elevated low and very low-density lipoproteins (LDL and VLDL), hypertension, smoking, overweight and obesity, diabetes mellitus and sedentary lifestyle.

The origin of the word atherosclerosis is in the Greek word *athera*, which means gruel, and *sclerosis*, which means hardening, suggesting a fatty, degenerating lesion in the vascular wall. The focus of the problem of atherosclerosis is the development of these *atheromas*, characterized by the gradual deposition of cholesterol and fatty materials into the inner endothelial lining of the blood vessel wall, leading to fatty streak formation, hardened plaques and thrombotic processes inside the intima. Eventually, these processes may cut off blood supply to surrounding tissues, underlying the pathology of CVD such as myocardial and cerebrovascular infarction. Factors implicated in these processes include oxidation, inflammation, proliferation, necrosis and thrombosis.

Atherosclerosis has a high incidence and prevalence in Western countries, but the origin of the disease is not clear. In Mediterranean countries, this incidence is far from occidental countries, differences that could be explained because of the dietary

 Olive Oil and Health (eds J.L. Quiles, M.C. Ramírez-Tortosa and P. Yaqoob)

pattern. It is widely accepted that lipids, especially cholesterol, are involved in the development of CVD. A number of studies have indicated an association between dietary fat, elevated serum cholesterol and lipid levels, and atherosclerosis. However, recent research has emphasized the importance of other macronutrients and micronutrients as modulators implicated in the development of CVD (Table 9.1).

For the treatment of atherosclerosis, the first line of intervention is dietary therapy to modulate weight, blood pressure, and the lipid profile, including LDL levels, specifically small dense LDL, triglycerides rich lipoproteins (TRL), lipoprotein a (Lp (a)), and high density lipoprotein (HDL). Nevertheless, more evidence is needed to specify the best dietary recommendations for the prevention of CVD. On the other hand, lifestyle recommendations are essential, including not smoking, exercising and the control of physiological stress.

Nutritional treatments are focused not only on lowering plasma cholesterol levels, but also on interfering with the cellular mechanisms implicated in the plaque development, endothelial dysfunction, inflammatory response and in the formation and stabilization of the thrombus.

Although dietary changes should be individualized, two main strategies have been effectively described: the modification of the pattern, and thus the quality, of dietary fats and carbohydrates. Substitution of saturated fats and *trans*-fatty acids with non-hydrogenated mono- and polyunsaturated fatty acids (MUFA and PUFA), particularly n-3 PUFA, is recommended. In addition, high intakes of cereal fibre and low glycaemic load, instead of refined grain products, are also recommended, as described in Table 9.2 (Cernea *et al.*, 2003).

Table 9.1. Main nutrients implicated in the modulation of CVD and their effects.

	Effects	
Nutrient	Increased	Decreased
Non-starch polysacharides	Insulin resistance	Glycaemia and insulinaemia
Saturated fatty acids	LDL and thrombosis	
Trans-unsaturated fatty acids	LDL, Lp(a) and triglycerides	HDL and endothelial function
Monounsaturated fatty acids	HDL	LDL
n-6 Polyunsaturated fatty acids		LDL, total:HDL cholesterol ratio and post-prandial hypertriglyceridaemia
n-3 Polyunsaturated fatty acids	Endothelial function	Fasting and post-prandial hypertriglycerides and remnant lipoproteins, platelet aggregation, and risk for thrombosis, blood pressure (slightly), inflammatory responses, risk for arrhythmias
Antioxidant nutrients		LDL oxidation
Folate and vitamin B_{12}		Homocysteinaemia
Sodium	Blood pressure	

Table 9.2. Summary of nutritional recommendation in cardiovascular disease prevention (Cernea *et al.*, 2003).

Decrease	
Carbohydrates	Refined grains
Fats	Saturated fats, dietary cholesterol and *trans*-fatty acids: red processes meat, fatty dairy products, eggs yolks, margarines, animal fat shortenings
Macronutrients	Sodium
	Alcohol
Increase	
Carbohydrates	Whole grain cereals, fruit, vegetables and legumes
Fats	Non-hydrogenated unsaturated fats: low-fat dairy products, poultry, fish, nuts and virgin olive oil
Proteins	Mainly from fish, soybeans, nuts, legumes and poultry
Macronutrients	Potassium, calcium and magnesium
Micronutrients	Traces of zinc, selenium, copper, manganese and vanadium
	Antioxidant vitamins, vitamins E and C, β-carotene
Phytonutrients	Isoflavonoids (soybeans)
	Carotenoids, licopene (tomato)
	Organosulphur compounds (from garlic, onion and leek)
	Plant sterol (edible oils such as virgin olive oil)

There is now growing evidence supporting the cardiovascular benefits of foods rich in bioactive compounds 'phytonutrients' present in all whole grains, vegetables, legumes, fruits, nuts and edible oils. Virgin olive oil is a rich source of monounsaturated fatty acids (MUFA) and is widely used in Mediterranean areas. Its composition depends on the crop and manufacturing characteristic; oleic acid is present between 60 and 80%, while the polar fraction contains a number of active antioxidant phenolic compounds.

Benefits of virgin olive oil include the modification of the plasma lipid profile, increasing HDL and lowering LDL, and the induction of antioxidant protection against lipoprotein oxidation. Moreover, a beneficial modulation of thrombosis and fibrinolysis has been observed and, finally, the phenolic components may exert some anti-inflammatory effects.

2. Pathophysiology of Atherosclerosis

Atherosclerosis is a multifactorial disease that starts in childhood and takes years to advance. It occurs in several stages, involving oxidative, inflammatory, proliferative, necrotic and thrombotic processes. It takes place in the vascular endothelium, around specific areas of certain vessels, such as aorta, and low calibre arteries. In fact, the susceptibility to develop atherosclerotic plaques in the aortic arch is greater than in the descending fragments. Coronary, cerebrovascular, pulmonary and renal arteries are all susceptible to the development of this pathology, depending on the permeability of arterial walls, on the blood pressure they support, and on LDL transport and metabolism.

Lipoproteins and blood cells are attracted into the damaged endothelium, where increased permeability favours the recruitment of these particles and cells, and initiates the development of the disease. Endothelial dysfunction contributes to plaque progression; modified lipids are phagocytosed and lead to foam cell and fatty streak formation. The next step is the proliferation of smooth muscle cells (SMC) into the intima, generating fibrous plaques called atheromas. Foam cells, damaged endothelial cells and proliferated SMC secrete metabolic products, inducing advanced lesions where inflammation, calcification, necrosis and thrombotic processes lead to fatal and non-fatal consequences.

2.1. Endothelial dysfunction

Endothelial cells form a physical barrier to the bloodstream on the artery wall and supply the necessary medium for the oxygen and nutrient transport. Endothelial dysfunction represents the first alteration in atherosclerosis, allowing the recruitment of blood lipids and cells (monocytes and leukocytes) into the arterial wall. Endothelial dysfunction includes abnormalities in vasomotor control (dilation *vs* constriction), adhesion molecule activation, inflammatory response (leukocyte and platelet adhesion) and proliferation of SMC (Patrick and Fletcher, 2001).

Active molecules generated inside the altered endothelium act as intermediates and activators for the ensuing processes. Nitric oxide (NO), a key molecule released by endothelial cells, is implicated in vasodilatation, platelet aggregation, SMC proliferation and monocyte adherence. Reduced levels of NO promote the development of atherosclerosis, and are considered to be induced by diabetes, hypertension and smoking.

2.1.1. Lipids infiltration and oxidation

Plasma lipoproteins transport lipids toward peripheral tissues, playing important roles in the development of atherosclerosis; but not all of them have the same pathogenic implication in disease. LDL, the main cholesterol carrier in the blood, is considered the essential atherogenic fraction. Likewise, growing evidence implicates triglyceride-rich lipoproteins (TRL) in atherosclerosis, since high triglyceride levels has been identified as an important risk factor for CVD and atherosclerosis. Atherosclerotic lesions contain foam cells that arise from the excessive uptake of lipoprotein by monocyte-macrophages and SMC, through many receptors, such as the LDL receptor, VLDL receptor, LDL receptor-related protein and scavenger receptors. However, little is known about the expression of these receptors in normal and atherosclerotic arteries.

Low-density lipoprotein is formed in the liver, and carries about two thirds or more of the plasma cholesterol (Table 9.3). High levels of this lipoprotein signify a higher risk for atherosclerosis, but pathogenic mechanisms remain unclear. Both genetic predisposition and dietary components play important roles in the modification of plasma levels of LDL. Moreover, LDL infiltration into the arterial wall is also increased by smaller particle size, mechanical and immunological alterations to the endothelium, blood pressure and by a decrease in the flow rate in specific places susceptible to the development of atheromatous plaques.

Table 9.3. Percentages of lipidic components of main plasma lipoproteins.

	Cholesterol	Triglycerides	Phospholipids	Apolipoprotein
Chylomicrons	45–50	10–30	15–22	20–25
VLDL	10–15	60–80	15–20	5–10
LDL	2–7	80–95	3–6	1–2
HDL	20	5–10	25–30	45–50

When lipoproteins are recruited to the arterial wall, the cells present in the intima, mainly macrophages, but also SMC and endothelial cells, attract and phagocytose LDL particles, leading to foam cell formation. Mechanisms for LDL uptake are still unclarified, but some investigations indicate that apo B100, also present in VLDL, is the responsible molecule. This apoprotein is recognized by the cellular LDL receptors, which can be regulated by free cholesterol concentration inside the cell. Moreover, LDL uptake is also regulated by some inflammatory mediators, cytokines and growth factors, such as tumour necrosis factor (TNF-α), interleukin 1 (IL-1), which may induce transcription of surface LDL receptors (Stopeck *et al.*, 1993). On the other hand, the uptake of LDL is significantly increased by noradrenaline and by adrenaline at pathophysiological blood concentrations, probably mediated by transcytosis through the endothelial cells (Born, 1994).

Once inside the intima, LDL undergoes oxidative modification to become oxidized LDL (oxLDL), and, together with changes in lipoprotein charge and the glycation of these particles, LDL becomes recognized by scavenger receptors, contributing to foam cell formation. The oxidizability of LDL depends on its fatty acid profile and on the antioxidant content, determined by dietary components. MUFAs have the advantage that they are unsaturated and more stable than polyunsaturated fatty acids (PUFAs). Many studies have demonstrated that diets rich in MUFAs, such as the Mediterranean diet, generate LDL which is less susceptible to oxidation, reflecting a protective mechanism of olive oil in the prevention of atherosclerosis.

Very low-density lipoprotein is the main source of triglycerides in the blood (Table 9.3), produced in the liver from endogenous fats. Growing evidence emphasizes the cardiovascular consequences of an excess in plasma triglycerides. Hiltunen *et al.* (1998) reported the presence of triglycerides and increased levels of VLDL-receptor mRNA in human atheromas, implicating these lipoprotein and receptors in the formation of both SMC and macrophage-derived foam cells. New investigations are needed to elucidate the true role of VLDL and triglycerides in the development of atherosclerosis.

Chylomicrons are postprandial TRL produced in the intestine, which transport dietary cholesterol and fats to the liver and periphery (Table 9.3). Chylomicrons and their remnants circulate in portal blood and are efficiently cleared by the specific enzyme lipoprotein lipase. However, evidence of their atherogenicity is increasing. In humans, chylomicrons have been demonstrated to accumulate in lesions in the arterial wall. Doi *et al.* (1998) described that chylomicrons alter vascular tone response to acetylcholine, inducing endothelial dysfunction. However, as VLDL, chylomicrons need to be investigated to find out more data about the atherogenic properties.

High-density lipoproteins are beneficial antiatherogenic particles implicated in reverse cholesterol transport. The main function is cholesterol transport from tissues to the liver for metabolism and elimination. HDL are synthesized in the liver and are composed of 45–50% protein, mainly apo A-I, and lower amounts of lipids (Table 9.3). High levels of HDL have been associated with low levels of chylomicrons, VLDL remnants and small, dense LDL. It is noteworthy to emphasize that olive oil induces an increase in serum HDL, which is one of the beneficial mechanisms of this fat source. The presence of paraoxonase in HDL surface protects LDL from oxidation (Lusis, 2000).

Lipoprotein (a) (Lp(a)) containing apo B has been demonstrated to have great atherogenic potential in epidemiological studies (Korpilahti *et al.*, 1998). Terres *et al.* (1995) observed high levels of this lipoprotein in 67% of patients with progressive coronary stenosis, and only in 33% of patient without lesions. The uptake mechanisms are not completely clear, but they seem to be similar to LDL in atheromas. Lp(a) leads to foam cell formation, having similar effect to LDL in aortic lesions. However, these effects are different in non-atherosclerotic normal aortas (Nielsen *et al.*, 1998). There are several hypotheses to explain this atherogenicity. Lp(a) oxidation induces an increase in monocyte adhesion to endothelial cells. Lp(a) lipids and apo B content may promote oxidation and SMC proliferation. Lp(a) may inhibit the fibrinolytic system, since apo a, present in these lipoproteins, has a similar structure to fibrinogen and may compete with fibrin, streptokinase, tPA, endothelial cells and platelets (Figs 9.1 and 9.2).

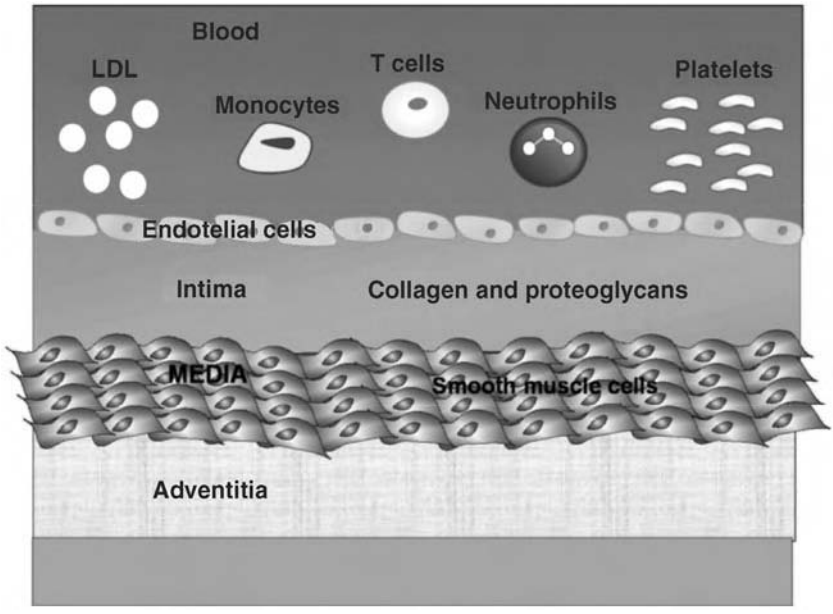


Fig. 9.1. Structure of normal artery that consist of three morphologically distinct layers: the *intima*, with a monolayer of endothelial cells, the *media*, the middle layer, consists of SMC; and the *adventitia*, the outer layer.

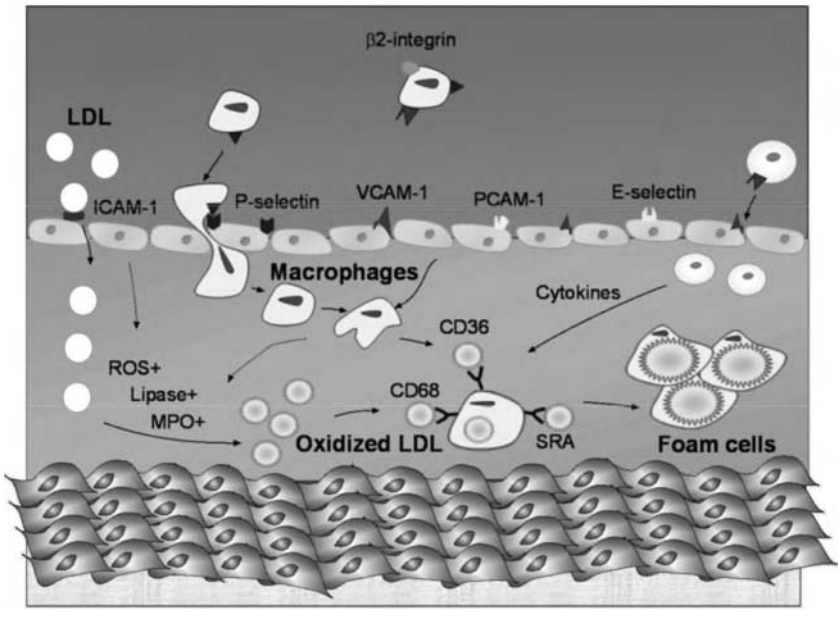


Fig. 9.2. Lesion initiation. Alteration in the endothelium and increased permeability to macromolecules such as LDL. The LDL undergoes oxidative modification as a result of interaction with reactive oxygen species and increases the expression of monocyte chemotactic protein (MCP-1) and the adhesion molecules (VCAM-1, ICAM-1, P-selectin, E-selectin) by endothelium.

2.1.2. Cell infiltration

Once recruited into the altered arterial wall, macrophages contribute to inflammatory responses. Many studies demonstrate oxLDL to be chemotactic for monocytes, and an activator of endothelial cells. Endothelial cells, SMC and monocytes release cytokines after interaction with oxLDL, inducing cell infiltration, macrophage proliferation, and foam cell formation. In the early stages of atherosclerosis, high levels of vascular adhesion molecules (VCAM-1) have been found in endothelial cells. These surface molecules establish a staunch adhesion between these cells and circulating monocytes, allowing their recruitment. On the other hand, many studies have demonstrated that oxLDL promotes monocyte and neutrophil adhesion into the endothelial wall. This union is mediated by some lipid-derivatived molecules, such as leukotrienes or P-selectin.

Kim *et al.* (1994) observed that the induction of E-selectin, VCAM-1, or intercellular adhesion molecule 1 (ICAM-1) allows the binding of monocytes to endothelial cells induced by incubation with minimally oxidized LDL (moxLDL). Indeed, these particles induce great adhesion of monocytes with endothelial cells through the production of monocyte activators, such as monocyte chemotactic protein (MCP-1) and monocyte colony stimulating factor (MCSF) (Liao *et al.*, 1995). MCP-1 release is also stimulated by moxLDL in SMC (Liao *et al.*, 1991). Navab *et al.* (2002) hypothesized that oxidized arachidonic acid present in the phospholipid fraction of LDL may be responsible for this stimulation.

NO also induces leukocyte adhesion on the vascular surface. Some studies have demonstrated low NO levels in hypercholesterolemic patients, contributing to monocyte recruitment. On the other hand, an elevated NO production decreases LDL susceptibility to oxidation, suggesting a protective effect of this molecule (Calo *et al.*, 1998).

2.2. Fatty streak formation

Lipids and recruited monocytes, foam cells, together with SMC proliferation, and T cell and platelet activation, characterize fatty streak formation. Accumulation of cholesterol in macrophages and SMC is an uncontrolled process, driven by cell receptors and modulated by lipoproteins oxidative status. OxLDL is recognized by scavenger receptors present on macrophage surfaces, through the modified apo B100, mainly due to the loss of lysine residues and the generation of peroxidative products, especially from arachidonic acid. Moreover, phagocytosis is another mechanism for oxLDL uptake by macrophages (Boullier *et al.*, 2001). On the other hand, TRL may be captured by macrophages after changes in the apoprotein fraction (Ooi and Ooi, 1998).

The accumulation of cholesterol inside SMC has been demonstrated, although the mechanisms are not clear since these cells do not express LDL-receptors on the surface. However, CD36 has been identified as an oxLDL receptor, expressed in SMC and macrophage surfaces. Some authors have suggested other mechanisms for lipid uptake by SMC, such as pinocytosis, endocytosis and probably the presence of another low specificity unidentified receptor.

2.3. Fibrous cap formation

As the atherosclerotic lesion develops, fibrous cap formation occurs as a result of proliferation and migration of SMC surrounding the lipid core and net matrix deposition. These steps result in a reduction of the thickness of the media and incrementation of the size and instability of the lesion. A number of cytokines and growth factors, from macrophage foam cells and from the endothelium, regulate these processes, mainly IL-1β, IL-6, TNF-α, platelet derived growth factor (PDGF), transforming growth factor (TGF-β) and heparin bound-epidermic growth factor (HB-EGF), which are increased inside the atherosclerotic lesions.

OxLDL and its derivatives are implicated in the modulation of cell proliferation through different mechanisms (Chatterjee *et al.*, 2004). Bassa *et al.* (1998) suggested that lysophosphatidylcholine may, in part, act as an active component in ox-LDL-mediated effects. It has been observed that a lysophosphatidylcholine oxidation-derived products induce an increase in PDGF and HB-EGF mRNA in macrophages and endothelial cells (Kita *et al.*, 1997, 1999) and SMC (Kohno *et al.*, 1998), while oxLDL and oxidized lipids increase IL-1β production by monocytes (Liu-Wu *et al.*, 1998). Furthermore, incubation of HUVEC with glycoxidized LDL and lysophosphatidylcholine-enriched lipoprotein resulted in upregulation of MCP-1 mRNA expression through increased NF-kappaB, under

diabetic conditions (Sonoki *et al.*, 2003). Sánchez-Quesada *et al.* (2003) have demonstrated that electronegative LDL enriched in esterified and free cholesterol and triglyceride and poor in apo B and phospholipids, from familial hypercolesterolemia patients, induces a 2-fold increase in the release of cytokines by endothelial cells, contributing to leukocyte recruitment and promoting atherogenesis in these subjects. However, more studies are needed to find the role of cytokines inside the lesion, since their presence does not guarantee their activity.

Connective tissue present in the extracellular matrix is involved in the adhesion and recruitment of lipoproteins. The interaction between apo B and apo E containing particles with some components of the extracellular matrix leads to the precipitation of insoluble components inside the plaque. Plaque extracellular matrix depends on the balance between SMC protein synthesis, modulated by TGF-β, TNF-α and IL-1, and protein degradation by matrix metalloproteinases (MMPs). In 1998, Baker *et al.* (1998) suggested the relevance of the proteolytic activity of extracellular MMPs on proliferation and migration processes and on the stability of the plaque. Joseph *et al.* (2003) suggested that LDL has a regulatory effect on collagen metabolism in fibroblasts, since the incubation of cultured human skin fibroblasts with LDL resulted in a time-dependent and dose-dependent increase in the MMP activity. However, Wilson *et al.* (2003) reported that oxLDL can reduce MMP-2 and MMP-9 activity and hence increase the deposition of extracellular matrix proteins within SMC-rich vascular lesions. High cholesterol intake can induce some MMPs in SMC, while lipid lowering reduced expression and activity of MMPs (Aikawa and Libby, 2000) (Fig. 9.3).

2.4. Formation of advanced lesions

The main characteristic of advanced atheromas is their fragility, which may lead to rupture of the plaque, internal haemorrhages and thrombosis. As SMC migrate into the intima, the destabilization of the plaque increases; necrosis and calcification contribute to the weakness of the lesion, because of the reduction of the flexibility of the plaque.

One feature of the atherosclerotic plaque is the deposition of calcium, which appears to result from the differentiation and calcification of vascular osteoblastic cells. Oxidative stress modulates differentiation of vascular cells and enhances osteoblastic differentiation (Mody *et al.*, 2001).

When analysing different models of experimental atherosclerosis, a number of studies have shown that ultrastructural changes in all arterial wall layers are associated with the proportion of cholesterol. Moreover, the instability of the plaque depends on the amount of cholesterol in the necrotic core, and may be regulated by lipid peroxidation. In addition, plaque fragility is greater at the edges of the plaque, where monocyte infiltration and metalloproteinase activity are enhanced.

Apoptotic processes and the toxicity of oxLDL may influence cellular death and tissue necrosis inside the plaque core. Cholesterol-derived oxidative products and hydroxynonenal are lethal for macrophages, SMC, fibroblasts, and endothelial cells. Moreover, oxysterols may induce cellular clones in the arterial wall leading to calcification processes that seem to be similar to bone formation by osteoblasts (Watson *et al.*, 1994).

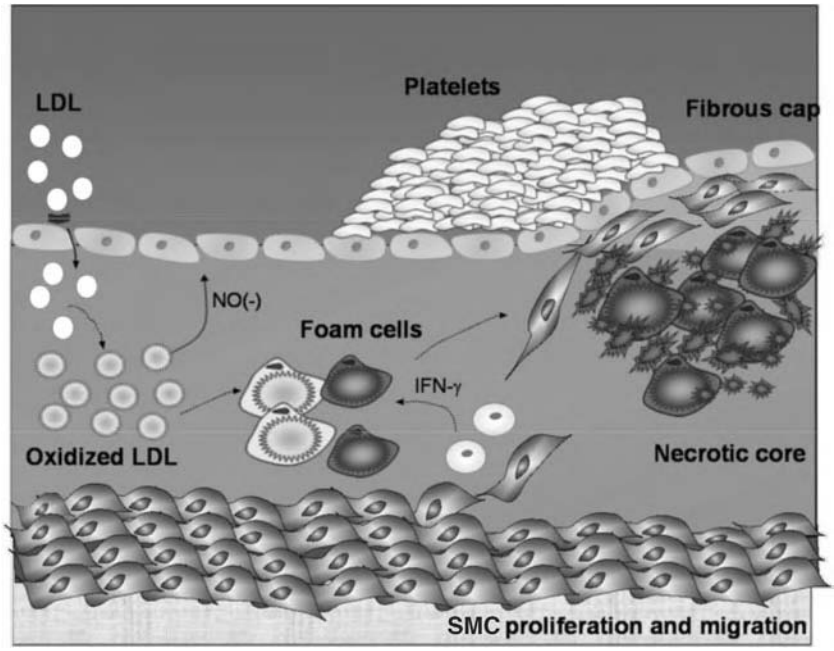


Fig. 9.3. Foam-cell formation and development of atheroma plaque. LDL is uptake by macrophage scavenger receptors (CD68, CD36 and SRA). The expression of these receptors is mediated by cytokines as tumour necrosis factor (TNF-alfa). Finally, the fibrous plaque is formatted. It is characterized by a growing mass of extracellular lipid and by the accumulation of SMC. Cytokines and growth factors secreted by macrophages and T cells are important for SMC migration and proliferation.

To summarize, all these data suggest that the necrotic core is an apoptotic core, that oxidized lipids may induce apoptosis and that apoptotic residues and oxidized lipids may contribute to calcification. However, more investigations are needed to elucidate the mechanisms.

2.5. Plaque regression

Some authors have indicated that vascular lesions may regress when plasma cholesterol levels are normalized by dietary or pharmacological treatment, or by changes in lifestyle (Deedwania, 1995). In the Familiar Atherosclerotic Treatment Study (FATS) Brown *et al.* (1993) observed that a low lipid diet stabilized the disease, and diminished the number of myocardial infarctions, angina pectoris and cardiac deaths in these patients. Our research group has demonstrated that the modification of dietary fatty acid profile may help to promote plaque regression since dietary supplementation with extra virgin olive oil and, to a lesser extent, fish oil, stabilize and could regress the size of the lesion, in rabbits with experimental atherosclerosis induced by a high intake of cholesterol and lard (Aguilera *et al.*, 2002). However, more studies are needed to reach definitive conclusions.

3. Effects of Virgin Olive Oil on Cardiovascular Risk Factors

Many studies have demonstrated that olive oil and especially virgin olive oil may modulate, directly or indirectly, a number of cardiovascular risk factors and contribute to the prevention of atherosclerosis. It is important to mention that all these factors exert a direct effect on the development of atheromas, and indeed, some of them are interrelated. Table 9.4 shows the main atherogenic risk factors; some of them are modifiable by dietary changes, while others are not.

Different studies support the idea of the beneficial effect of MUFAs on the blood lipid profile and oxidation, insulin resistance, haemostasis and other cardiovascular risk factors (Kelly *et al.*, 2001; Vessby *et al.*, 2001; Rivellese *et al.*, 2003). However, investigations about the mechanism of action of oleic acid and minor compounds from virgin olive oil on the prevention of atherosclerosis and on the modulation of healthy risk factors need to be developed.

3.1. Genetic predisposition

Atherosclerosis can be regarded as a complex combination of lipid transport and metabolism disorder, with chronic inflammation and thrombosis. These processes can be regulated by a number of gene polymorphisms related to lipid transport, including apo E encoding genes, lipoprotein receptor genes, like LDL receptor, and polymorphic genes encoding lipid and lipoprotein processing enzymes, as lipoprotein lipase. Moreover, the regulation of the genetic aspect of inflammation, such as TNFα and interleukins production, represents an important modulator of atherosclerosis.

On the other hand, polymorphic genes related to hypertension, obesity and homocystein metabolism and those controlling the haemostatic system are involved, indirectly, in the regulation of atherosclerosis, since these processes represent independent coronary risk factors. The repercussions of all these factors on the development of atherosclerosis is detailed hereafter.

The real understanding of the relationship between multiple polymorphic gene variants, dietary factors and atherosclerosis remains unclear, because of the number of factors implicated in these processes and the inconsistency of the studies. Dietary components cannot induce genetic modification, but it is obvious that they could modulate the phenotypic expression of a genetic encoding atherosclerotic pattern.

Table 9.4. Dietary modulation of cardiovascular risk factor.

Unmodifiable	Modifiable	Modifiable symptoms
Genetic predisposition	Hypertension	Tobacco
Infecting agents	Obesity	Insulin resistance
	Homocystein	Insulin-independent diabetes mellitus
	Fibrinogen	Menopause
	PAI-1	

3.2. Tobacco

Epidemiological studies confirm that tobacco is one of the main risk factors for CVD, but mechanisms remain not well understood. On one hand, it is well known that tobacco increases oxidative stress in the body, and decreases antioxidative potential. On the other hand, tobacco promotes thrombosis, and thus contributes to plaque evolution. Fibrinogen levels are higher in smokers, and this increases the tendency to thrombogenesis and its complications.

The relationship between tobacco, atherosclerosis and olive oil intake has not been investigated. However, virgin olive oil contains many antioxidant compounds that diminish oxidative stress (Visioli *et al.*, 2002), and olive oil appears to convey protection against some tobacco-induced diseases, such as pancreatic cancer (Soler *et al.*, 1998) and carcinomas of the oral cavity (Bosetti *et al.*, 2002; Petridou *et al.*, 2002). Therefore, we can speculate that the antioxidant capacity of this edible oil could be useful for the inhibition of tobacco-induced oxidative stress, avoiding oxidation processes not only in LDL but also in surrounding cells, and inhibiting inflammatory and aggregation processes induced by oxLDL-stimulated cells. Nevertheless, this hypothesis needs to be investigated.

3.3. Hypertension

Hypertension is a common disease in developed countries, and an important risk factor for atherosclerosis. Hypertension is commonly called 'the silent killer' because of its influence in many diseases such as cerebrovascular, cardiac isquemia, cardiac and renal insufficiency and fatal or non-fatal heart attack. Mechanisms implicated in this hypertension-induced atherogenesis include damage to the vascular endothelium, increased lipid and cell permeability, and a contribution in the development of acute episodes. Regarding the influence of diet on blood pressure-related CHD complications, data from the Seven Countries Study revealed that diastolic blood pressure was three times lower in Mediterranean populations compared with USA and Northern Europe populations (Van den Hoogen *et al.*, 2000).

Blood pressure is regulated by a number of genetic factors; indeed, some genetic patterns can be responsible for essential hypertension (Luft, 2002). Furthermore, obesity and nutritional factors, such as dietary salt and alcohol intake, modulate blood pressure, and these effects are under investigation. The effect of dietary lipids on the modulation of hypertension is not clear. Recent research shows that dietary lipid modifications may lower blood pressure substantially and strongly affect other cardiovascular risk factors, such as obesity and diabetes, that influence hypertension.

A Spanish study with 20 healthy men evaluated the effects of two high-fat, MUFA-rich diets (40% fat, 22% as MUFA), one with virgin olive oil and the other with high-oleic sunflower oil. The MUFA diet led to a significant reduction in systolic and diastolic blood pressure. Both MUFA oils produced similar changes (Espino *et al.*, 1996). However, another study with healthy men and women was carried out to evaluate the effects of a MUFA-rich diet with virgin olive oil and a

PUFA-rich diet compared with a saturated fat-rich diet. The MUFA-enriched olive oil diet produced a significant decrease in systolic blood pressure as compared with SFA and PUFA (Lahoz *et al.*, 1997). These results indicate that there may be a direct effect of MUFA on blood pressure but at the moment there is not enough evidence to confirm it.

Costa (2002) observed that olive oil seems to be particularly helpful in the regulation of hypertension, since it is able to produce significant blood pressure reductions in women. Moreover, it has been observed that a slight reduction in saturated fat, along with the use of extra virgin olive oil, lowers the daily antihypertensive dosage requirement (−48%) in hypertensive USA patients (Ferrara *et al.*, 2000). However, the separate effects of virgin olive oil, oleic acid, and its minor components on hypertension remain to be investigated.

3.4. Obesity

Obesity is a serious independent health problem, and a core element of a number of metabolic abnormalities, such as insulin resistance, diabetes mellitus, hypertension and atherosclerosis. Since the 1990s, overweight and obesity have been considered determinants for CVD; many studies show that body mass index (BMI) is a good predictor for these diseases. Body fat, and moreover the distribution of this fat, determines the development of atherosclerosis. A number of studies have associated abdominal fat with a higher cardiovascular risk, even in people not overweight.

Individual susceptibility to obesity depends strongly on the genetic pattern. However, diet, in combination with low physical activity, is the crucial environmental factor implied in this problem. It is well known that the quality but not the quantity of dietary fats is responsible for overweight and obesity. Moreover, either high carbohydrate or high MUFA diets were effective in decreasing body weight, albeit, at steady weight conditions; only the MUFA diet improved LDL and HDL subclass distribution abnormalities present in mildly obese normolipidemic women (Zambon *et al.*, 1999). A recent study has demonstrated that substitution of dietary SFA with MUFA can induce a small but significant loss of body weight and fat mass in obese men, without a significant change in total energy or fat intake (Piers *et al.*, 2003).

3.5. Insulin resistance and insulin-independent diabetes mellitus

A low response to plasma insulin increases blood glucose, the production of pancreatic insulin and, consequently, levels of plasma free fatty acids. The elevation of these parameters constitutes risk factors for atherosclerosis, since it may modify, indirectly, other cardiovascular risk factors (Egan *et al.*, 2001). Insulin mediates lipoprotein profile alterations, increasing plasma triglycerides and decreasing HDL. On the other hand, epidemiological studies associate high levels of insulin with hypertension. Finally, insulin is the major regulator of PAI-1, which modulates fibrinolysis and thus the development of atherosclerosis.

Modification of dietary fat in the diet of diabetic patients is of interest with respect to metabolic and other consequences. Garg (1998) observed that high-monounsaturated-fat diets improve lipoprotein profiles as well as glycaemic control in patients with type 2 diabetes, and improves glycaemic tolerance when compared with a high SFA oil (Rocca *et al.*, 2001).

A high-carbohydrate, low-fat diet has been recommended for diabetes mellitus patients because of the reduction of lipid levels; however, this kind of diet appears to aggravate hypertriglyceridaemia. MUFA-rich diets improve lipid profiles and may also have antioxidant properties. A recent study has shown that replacement of SFA with MUFA may be more effective in lowering CVD risk in women with type 2 diabetes than the replacement with carbohydrates (Tanasescu *et al.*, 2004). Indeed, MUFA exert a beneficial effect on HDL levels, which is not produced by the classical diet recommended for these patients.

The only study that has investigated the effect of virgin olive oil in free-living individuals with Type 2 diabetes mellitus has shown similarities between a high-carbohydrate and a high-MUFA diet when comparing glycaemic control; however, the latter also has a beneficial effect on the lipid profile and superior patient acceptance (Rodríguez-Villar *et al.*, 2004).

3.6. Infecting agents

Some viruses and microorganisms, such as *chlamydia*, are commonly found in atherosclerotic plaques of the carotid arteries, and their presence may predispose to a greater risk of thrombosis in the plaques (Chiu *et al.*, 1997). Berggson *et al.* (1998) found that oleic acid did not reduce the infectivity titre of *Chlamydia trachomatis*, but no studies have investigated the effect of olive oil or their components as an antimicrobial agent.

Infecting diseases are often associated with abnormal lipid metabolism and with the reduction of plasma cholesterol levels; the state of metabolic insufficiency is reflected in the pattern of cardiorespiratory, vascular and physiological compensation (Siegel *et al.*, 1979). Virgin olive oil may have a beneficial effect as activator of the immune system. Therefore, it might be speculated that virgin olive oil could prevent the evolution of infections that contribute to the development of atherosclerosis. However, further studies are needed to confirm this hypothesis.

3.7. Menopause

Resulting from the loss of ovarian follicular function, menopause leads to menstrual cycle disorders, vasomotor changes, urogenital disorders together with some other atypical symptoms. The incidence of CVD in premenopausal women is lower than in men, while, in postmenopausal women the incidence of hypertension and atherosclerosis increases. It could be partially explained because of the high levels of HDL in premenopausal women; moreover, it seems that oestrogen may have a direct effect on arterial wall. On the other hand,

homocysteinemia may contribute to the explanation of differences in the incidence of vascular disease in both sexes and the increased vascular risk in postmenopausal women, since these levels increase significantly in postmenopausal condition (Reis *et al.*, 1999).

Oestrogens protect against CVD, since they decrease LDL and Lp(a), increase HDL levels, promote vasodilatation, enhance blood flow, decrease oxidative stress and exert antioxidant activity, and increase NO release. On the other hand, oestrogens increase heart rate variability, which constitutes a good predictor of mortality after myocardial infarction; thus, the rate of cardiovascular mortality decreases in premenopausal stages (Medina *et al.*, 2003).

Under these conditions, a Mediterranean diet could have a positive effect with regard to cardiovascular complications, even in secondary prevention. The effect of dietary fat saturation on plasma lipoproteins was independent of menopause status. MUFA decrease total cholesterol and LDL-c, but increase HDL-c and apolipoprotein A-I (Mata *et al.*, 1992). Many studies have investigated the effect of olive oil in different diseases in postmenopausal women. Minor compounds from virgin olive oil also exert beneficial effects in these situations. Oubina *et al.* (2001) suggested that differences in the type of minor compounds, as well as in the concentration of linoleic acid, influence the modulation of lipoprotein peroxidation and eicosanoid production when different sources of high-oleic oils constitute a large proportion of the diet of postmenopausal women.

3.8. HDL

HDL accomplishes cholesterol reverse transport, taking cholesterol from tissues to the liver for elimination. This action confers antiatherogenic properties to these particles. On the other hand, HDL exerts antioxidant activity against LDL oxidation, because of the presence of paraoxonase, an antioxidant enzyme associated to this lipoprotein (Kaplan and Aviram, 1999; Canales and Sánchez-Muniz, 2003). In addition, HDL can stop monocyte recruitment and promote fibrinolysis (Spieker *et al.*, 2004).

Supplementation with extra virgin olive oil influences positively the lipoprotein profile, since it declines the total-to-HDL and LDL-to-HDL cholesterol ratios in elderly men (Haban *et al.*, 2004). Moreover, Marrugat *et al.* (2004) described that the content of phenolic compounds in virgin olive oil determines the effectiveness in rising HDL levels. These minor compounds may be incorporated into the particle, contributing to the natural antioxidant properties of this lipoprotein.

3.9. Homocysteine

Homocysteine is an intermediate amino acid in the metabolism of methionine and cysteine, and a modulator of atherosclerosis in different ways. The elevation of homocysteine levels is considered as an independent cardiovascular risk

factor, since it has been associated to premature atherosclerosis, myocardial infarction and arterial and venous thrombosis (Stanger *et al.*, 2003). It induces the thickening of the intima, muscle hypertrophy and platelet aggregation. Furthermore, hyperhomocysteinaemia is correlated with other cardiovascular risk factors, renal function, smoking and increased levels of fibrinogen and C reactive protein.

Elevation of plasma homocysteine levels may be determined genetically or may be originated by some pathologies, pharmacological treatments or nutritional deficiencies, as well as in determined physiological situations. Folic acid, vitamin B_{12} and B_6 and piridoxal-5-phosphate are implicated in homocysteine metabolism. There is a negative correlation between the ingestion and plasma concentration of these vitamins with homocysteine levels (Stanger *et al.*, 2003). Hence, supplementation with folic acid and vitamins B_{12} and B_6 could normalize these levels; however, results are still controversial.

There are multiple consequences of homocysteine-induced vascular damage. Homocysteine can violate LDL free amino groups, leading to particle aggregation, macrophage uptake and an increase in the antifibrinolytic potential of Lp(a) (Harpel and Borth, 1992). Moreover, oxidation of homocysteine generates H_2O_2, inducing endothelial damage, platelet aggregation and coagulation (Hultberg *et al.*, 1995). Prothrombotic effects of homocysteine include tissue plasminogen attenuation, activation of factor V and C protein, reduction of endothelial antithrombotic activity and the alteration of thrombomodulin function.

One study has shown no effect of olive oil supplementation on serum homocysteine (Olszewski and McCully, 1993). However, no other investigations have described the effect of olive oil, or any of its minor compounds, on the modulation of homocysteine levels.

4. Mechanisms of Action of Olive Oil in the Prevention of Atherosclerosis

Many investigations are providing a better understanding of the metabolism of cholesterol and its relationship with atherosclerosis. This knowledge contributes to the development of new dietary therapeutic and preventive tools. Advanced research indicates that the reduction of total dietary fat, but more importantly the substitution of SFA rich animal foods with unsaturated fats, lowers blood cholesterol levels. Nevertheless, new strategies are needed to facilitate the development of heart-healthy dietary patterns that maximally reduce CVD risk.

The beneficial effect of virgin olive oil in the prevention of chronic diseases such as atherosclerosis is widely accepted. In 1952, Keys *et al.* (1952) observed a low incidence of coronary diseases in Italy, where olive oil was the main fat source. Later, epidemiological research reported that the risk of CVD in Mediterranean areas was lower than in Anglo-Saxon countries (Mancini *et al.*, 1995). In both areas, the consumption of fat is high, differing primarily in the quality of the fat source. Later, in 1999, Kris-Etherton *et al.* (1999) estimated a 25% decrease in CVD risk in healthy subjects from the USA fed a high fat diet

(34%), with 21% of MUFA from olive oil. Recently, Moreno and Mitjavila (2003) reviewed the advantages of the Mediterranean diet rich in olive oil on atherosclerosis, describing the possible mechanisms implicated in the modulation of the cellular oxidative stress/antioxidant status, the modification of lipoproteins and the down-regulation of inflammatory mediators. These effects have been attributed mainly to oleic acid, but recent research has considered the key role of other minor antioxidant substances present in the edible oil.

'Bioactive compounds' are extranutritional constituents, found mainly in plants, that typically occur in small quantities in foods. They are being intensively studied to evaluate their effects on health, and many epidemiologic studies have shown protective effects of plant-based diets on CVD. Virgin olive oil is an important source of phenolic compounds, but the composition depends on the bioavailability and the agrobiological properties.

Some authors have described the incorporation of these compounds in plasma and LDL particles, in a dose-dependent manner, stimulating interest in the potential for therapeutic effects of this edible oil (Caruso *et al.*, 2001; Visioli *et al.*, 2001; Miró-Casas *et al.*, 2003a). Furthermore, it has been shown that after the administration of 25 ml of virgin olive oil, a dose close to the daily intake in Mediterranean countries, approximately 98% of hydroxytyrosol appears to be present in plasma and urine in conjugated forms, mainly glucuronoconjugates, suggesting extensive first-pass intestinal/hepatic metabolism of the ingested hydroxytyrosol (Miró-Casas *et al.*, 2003b).

More investigations are needed to elucidate the composition of the different biovarieties of olive oils, and to find out which components – oleic acid or minority compounds – are responsible for the biological effects of olive oil. The characterization of the agrobiological properties of all olive oil varieties will help to achieve this.

4.1. Resistance of LDL against oxidation

Antioxidants are chemical compounds capable of interfering with oxidative reactions, scavenging free radicals and, thus, reducing the oxidative stress induced in many diseases. They contribute to the prevention of atherosclerosis by inhibiting LDL oxidation, promoting plaque stability, impairing vascular endothelial function and decreasing thrombotic tendency.

Although it is well known that oxidative stress is a risk factor for CVD, the causative relation between oxidative stress and arterial lesion progression remains unclear, probably due to the variety of mechanisms implicated in oxidative processes. The oxidation hypothesis born 20 years ago through the work of Steinberg *et al.* (1989), and its viability has been reviewed (Steinberg and Witztum, 2002). Since there are no conclusive human studies demonstrating a specific antioxidant and a specific dosage necessary for the prevention of CVD, these authors concluded that appropriately designed clinical trials are needed in order to test the extent of the importance of oxidation and antioxidant therapy in human atherogenesis. On the other hand, Khatri *et al.* (2004) have demonstrated a novel pathway, related to human atheroma progression and destabiliza-

tion, by which oxidative stress can trigger *in vivo* experimental plaque progression and angiogenesis. These authors studied the effect of ebselen, an anti-inflammatory, neuroprotective and antioxidant agent that prevents free radical induced apoptosis and inhibits *in vitro* SMC activities and *in vivo* intra-lesion angiogenesis and lesion progression.

Many *in vitro* and *in vivo* studies indicate that lipoprotein oxidation can help in the recruitment of the monocytes in the arterial wall, inducing their activation and adhesion. When cells from the vascular endothelium are exposed to minimally oxidized LDL (LDLmox) *in vitro*, the interaction between the monocytes and the endothelial cells is enhanced (Kim *et al.*, 1994; Berliner and Heinecke, 1995); in addition, there is greater production of monocyte activators, such as monocyte chemotactic protein (MCP-1) (Cushing *et al.*, 1990), monocyte colony stimulating factor (MCSF) (Rajavasisth *et al.*, 1990) and protein G (Schwartz *et al.*, 1994). The moxLDL also induces MCP-1 synthesis by smooth-muscle cells. Moreover, moxLDL lipids responsible for these effects appear to be the oxidized arachidonic acid present in these particles (Watson *et al.*, 1995). The metabolite of this fatty acid, epoxyeicosatrienoic acid, also promotes monocyte adhesion. In fatty streaks, high levels of mRNA and MCSF and MCP-1 have been observed (Yla-Herttuala *et al.*, 1991; Clinton *et al.*, 1992). MCP-1 has been demonstrated to induce the activity of tissue factor (TF) on the surface of the SMC, which could prompt an advance in the development of the disease (Schecter *et al.*, 1997).

Similarly, highly oxidized LDL (oxLDL) also mediates the attachment of monocytes and neutrophiles to the vessels (Lehr *et al.*, 1993; Rong *et al.*, 1998; Staprans *et al.*, 1998). On the other hand, increased levels of P-selectin have been found in atherosclerotic lesions (Johnson *et al.*, 1994). However, the presence of neutrophiles in the lesions are scarce, perhaps due to the absence of neutrophil-activating molecules.

oxLDL alone can attract monocytes and inhibit the migration of the macrophages that are located in specific areas of the artery wall (Quinn *et al.*, 1988). In this way, glycosylated LDL alone could attract monocytes and activate the vascular endothelial cells *in vitro*, causing the attachment of the monocytes (Schmidt *et al.*, 1993).

It is known that resistance to lipid oxidation within lipoproteins can be altered by the dietary fatty-acid profile and antioxidant content (Ramirez-Tortosa *et al.*, 1999a). Parthasarathy *et al.* (1990) observed that VLDL and LDL fractions isolated from animals fed olive oil were much more resistant to oxidation than were those fed with sunflower oil. In addition, the generation of conjugated dienes was significantly lower in the LDL of the olive-oil group. In turn, this LDL incubated with endothelial cells was degraded to a lesser extent by the macrophages. Since then, numerous research teams have studied whether a MUFA-rich diet truly prevents oxidative alteration of lipoproteins more than does a PUFA-rich diet, generating lipoproteins with marked resistance to oxidation.

In fact, Aviram *et al.* (1993) noted that LDL incubated with oleic acid was less oxidized than that with linoleic or arachidonic acid. In some studies on hamsters, a prolonged lag phase was observed in addition to lower formation of conjugated dienes in the LDL isolated from animals subjected to a diet supplemented

with *cis* and *trans* 18:1 acid, together with a greater concentration of α-tocopherol in these particles (Nicolosi *et al.*, 1998). Our research team has demonstrated that in rabbits with experimental atherosclerosis a diet rich in virgin or refined olive oil protects the LDL particles from oxidation (Ramirez-Tortosa *et al.*, 1998). Similarly, studies performed in hypercholesterolemic patients have concluded that LDL particles rich in oleic acid and poor in PUFA, after ingesting an olive-oil-rich diet, become more resistant to oxidation (Baroni *et al.*, 1999). Moreover our group has reported that the intake of a diet rich in MUFA by rabbits with experimental atherosclerosis leads to a decrease in the plasma-lipid content and a lower susceptibility of LDL to oxidation, whereas diets rich in PUFA also decrease the plasma-lipid fraction but increase LDL susceptibility to oxidation (Ramirez-Tortosa *et al.*, 1998). We reported the same results in patients with peripheral vascular disease fed a diet rich in olive oil (Ramirez-Tortosa *et al.*, 1999b). Thus, oleic acid itself protects LDL against oxidation.

Many studies suggest that when dietary saturated fats are replaced by n-3 PUFA, LDL becomes susceptible to oxidation. In humans, the susceptibility to LDL oxidation is related to the degree of coronary stenosis (Suzukawa *et al.*, 1995). In addition, a study on the impact of different fatty acids on endothelial cell cultures and their possible influence on the lipid peroxidation of LDL reported significant increases in LDL peroxidation mediated by cells supplemented with PUFA.

The studies by our research group using a model of experimental atherosclerosis in rabbits coincide with the above results. Thus, LDL enrichment with n-3 PUFA increases the susceptibility to oxidation, in comparison with n-6 PUFA and MUFA (Ramirez-Tortosa *et al.*, 1998). However, we have also found that the oxidative alterations of LDL with a high percentage of n-3 PUFA patients with peripheral vascular pathology can be reduced by the simultaneous intake of extra virgin olive oil, since lower uptake of LDL by macrophages and lower electrophoretic mobility of LDL was demonstrated in patients who ingested 40 g of extra virgin olive oil daily together with a 16-g supplement of fish oil for 3 months, in comparison with a control group of patients without dietary treatment (Ramirez-Tortosa *et al.*, 1999a).

Some studies (Quiles *et al.*, 1999; Ramirez-Tortosa *et al.*, 1999c) highlight the importance of the presence of antioxidant compounds in the unsaponified fraction of virgin olive oils. Nicolaiew *et al.* (1998) found a lower susceptibility to LDL oxidation in normolipemic patients, on comparing the consumption of extra virgin olive oil to high oleic sunflower oil. Virgin olive oil protects against oxidation of LDL and prevents the development of atherosclerosis more efficiently than refined olive oil (Fito *et al.*, 2000; Aguilera *et al.*, 2002).

Fito *et al.* (2002) demonstrated no changes in LDL lipid peroxidation or resistance to oxidation postprandially, but increased resistant to oxidation and plasma oleic acid 24 h after the ingestion of 50 ml of virgin olive oil in a single dose. Moreover, these authors observed higher levels of plasma oleic acid, resistance of LDL to oxidation, and plasma glutathione reductase activity after one week of virgin olive oil consumption (25 ml per day). Likewise, Bonanome *et al.* (2000) described that virgin olive oil consumption reduced postprandial, rather than fasting, LDL susceptibility to oxidation.

In 2002, Ochoa *et al.* described that olive oils from different biovariabilities exert different protection against LDL oxidation, depending on the proportion of different phenolic compounds (Ochoa *et al.*, 2002). It seems that hydroxytyrosol, as a free molecule or belonging to complexes such as oleuropein, is the most important constituent, with an important antioxidant activity (Coni *et al.*, 2000). *In vitro* studies provide further evidence that phenolic compounds bound to LDL are likely to protect the lipoprotein from oxidation, and showed the capacity of virgin olive oil phenolics to protect other phenolic compounds previously bound to LDL, especially the ability of tyrosol to bind LDL (Visioli *et al.*, 1995; Covas *et al.*, 2000). In 2001, Oubina *et al.* suggested that differences in the type of minor compounds may play an important role in the modulation of eicosanoid production and lipoprotein peroxidation when they constitute a large proportion of the diet (Oubina *et al.*, 2001).

On the other hand, Nicolaiew *et al.* (1998) suggested a mild effect of minor components of virgin olive oil related to a decrease of LDL susceptibility to oxidation compared with a MUFA-rich sunflower oil diet. However, in a similar type of experiment, Castro *et al.* (2000) observed significant differences in oxidative lag times only after the consumption of a MUFA-rich sunflower oil diet but not after a virgin olive oil-rich diet, probably related to the relative enrichment of plasma LDL particles in α-tocopherol from the MUFA-rich sunflower oil. These differences in both experiments could be due to the dietary background of volunteers implicated in the study, although further analyses are necessary to give clear conclusions about these results.

Natural antioxidants present in virgin olive oil may be essential for the prevention of cytotoxic products formation, such as lipid peroxides and, thus, for the observed healthful cardiovascular effects (Visioli *et al.*, 2002). Our research group has observed that supplementation with virgin olive oil decreases LDL oxidation in rabbits with experimental atherosclerosis induced by a high intake of cholesterol and lard (Ramirez-Tortosa *et al.*, 1998) and leads to lower atherosclerotic lesions in all aortic fragment isolated from these rabbits (Aguilera *et al.*, 2002). These studies concluded that the replacement of a high cholesterol-saturated fat diet by another cholesterol free-unsaturated fat, using extra virgin olive, sunflower or fish oil, does not regress atherosclerosis in rabbit. However, sunflower oil provokes a significant progression of the lesion development, whereas the enrichment of the diet with extra virgin olive oil and, to a lesser extent, fish oil stops this progression (Fig. 9.4).

Compounds such as tyrosol, cumarin, oleuropeins and biphenols, which protect LDL against oxidation have been studied (Coni *et al.*, 2000; Masella *et al.*, 2004). Furthermore, Vissers *et al.* (2004) have reviewed the antioxidant effects of olive oil polyphenols on markers of oxidative processes in the body. These authors conclude that, although the absorption of phenols from olive oil seems to be good, the content of olive oil phenols with antioxidant potential in the Mediterranean diet is probably too low to produce a measurable effect on LDL oxidizability or other oxidation markers in humans. Thus the available evidence does not suggest that consumption of these amounts of phenols provided by dietary olive oil will protect LDL against oxidative modification to any significant extent. Furthermore, the explanation for the fact that *in vitro* studies show

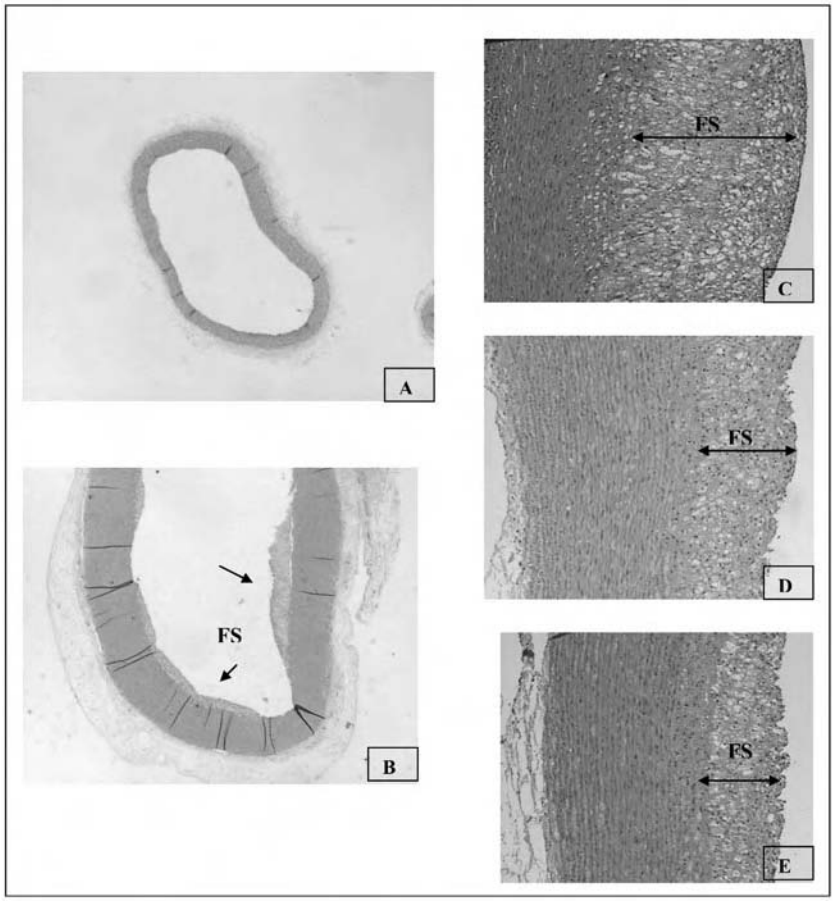


Fig. 9.4. Presence of fatty streak in sections from atherosclerotic rabbits in comparison with control animals. A (hematoxylin and eosin [H&E] × 20): aortic arch, control group; B (H&E × 20): aortic arch, aterosclerotic group; C (H&E × 100): toracic aorta, sunflower oil group; D (H&E × 100): toracic aorta, fish oil group; E (H&E × 100): abdominal aorta, virgin olive oil group.

protective effects in contrast to the lack of effect in the majority of human studies may be that the dose of the phenols and thus their plasma concentration in humans was too low to influence *ex vivo* LDL oxidizability.

During the oxidation of LDL, F_2 isoprostanes (8-iso-$PGF_{2\alpha}$) are generated and excreted. Thus, the evaluation of urinary 8-iso-$PGF_{2\alpha}$ represents a good biomarker of *in vivo* lipid peroxidation (Patrono and FitzGerald, 1997). In 2000, Visioli *et al.* demonstrated that the administration of catecholic phenolic from olive oil, hydroxityrosol and oleuropein, decrease, in a dose-dependent manner, the urinary excretion of 8-iso- $PGF_{2\alpha}$, indicating lower *in vivo* lipid peroxidation processes in supplemented volunteers (Visioli *et al.*, 2000).

On the other hand, the interaction of atherogenic lipoproteins with arterial wall matrix molecules can influence the genesis and progression of atherosclerosis and its complications. Newby and Zaltsman (1999) suggested the importance

of extracellular matrix, surrounding SMC, on proliferative and migration processes and in the stability of the plaque. This content depends on the proteolitic activity of MMPs, the matrix degrading enzymes present in vascular tissue, enzymes that contribute to the destabilization of the plaque. Oxidized lipoproteins also can modulate the expression of MMPs, thereby influencing plaque stability (Chait and Wight, 2000). Hence, antioxidant protection prevents, directly and indirectly, the development of atherosclerosis. Song *et al*. (2001) described that the incubation with quercetin, an antioxidant flavonoid present in virgin olive oil, inhibited basal and oxLDL-stimulated MMP-1 expression, suggesting that quercetin might contribute to plaque stabilization. This fact has increased the interest about the beneficial effect of antioxidant compounds from edible oils.

5. References

Aguilera, C.M., Ramirez-Tortosa, M.C., Mesa, M.D., Ramirez-Tortosa, C.L. and Gil, A. (2002) Sunflower, virgin-olive and fish oils differentially affect the progression of aortic lesions in rabbits with experimental atherosclerosis. *Atherosclerosis* 162, 335–344.

Aikawa, M. and Libby, P. (2000) Lipid lowering reduces proteolytic and prothrombotic potential in rabbit atheroma. *Annals of the New York Academy of Sciences* 902, 140–152.

Aviram, M. and Eias, K. (1993) Dietary olive oil reduces low density lipoprotein uptake by macrophages and decreases the susceptibility of the lipoprotein to undergo lipid peroxidation. *Annals Nutrition Metabolism* 37, 75–84.

Baker, A.H., Zaltsman, A.B., George, S.J. and Newby, A.C. (1998) Divergent effects of tissue inhibitor of metalloproteinase-1, -2, or -3 overexpression on rat vascular smooth muscle cell invasion, proliferation, and death in vitro. TIMP-3 promotes apoptosis. *The Journal of Clinical Investigation* 15;(101), 1478–1487

Baroni, S.S., Amelio, M., Sangiorfi, Z., Gaddi, A. and Battino, M. (1999) Solid monounsaturated diet lowers LDL unsaturation trait and oxidisability in hypercholesterolemic (type II) patients. *Free Radical Research* 30, 275–285.

Bassa, B.V., Roh, D.D., Kirschenbaum, M.A. and Kamanna, V.S. (1998) Atherogenic lipoproteins stimulate mesangial cell p42 mitogen-activated protein kinase. *Journal of the American Society of Nephrology* 9, 488–496.

Bergsson, G., Arnfinnsson, J., Karlsson, S.M., Steingrimsson, O. and Thormar, H. (1998) In vitro inactivation of Chlamydia trachomatis by fatty acids and monoglycerides. *Antimicrobial. Agents and Chemotherapy* 42, 2290–2294.

Berliner, J.A. and Heinecke, J.W. (1995) The role of oxidized lipoproteins in atherogenesis. *Free Radical in Biology and Medicine* 20, 707–727.

Bonanome, A., Pagnan, A., Caruso, D., Toia, A., Xamin, A., Fedeli, E., Berra, B., Zamburlini, A., Ursini, F. and Galli, G. (2000) Evidence of postprandial absorption of olive oil phenols in humans. *Nutrition Metabolism and Cardiovascular Disease* 10(3), 111–120.

Born, G.V. (1994) New determinants of the uptake of atherogenic plasma proteins by arteries. *Basic Research in Cardiology* 89(S1), 103–106.

Bosetti, C., La Vecchia, C., Talamini, R., Negri, E., Levi, F., Dal Maso, L. and Franceschi, S. (2002) Food groups and laryngeal cancer risk: a case-control study from Italy and Switzerland. *International Journal of Cancer* 20, 100(3), 355–360.

Boullier, A., Bird, D.A., Chang, M.K., Dennis, E.A., Friedman, P., Gillotre-Taylor, K., Horkko, S., Palinski, W., Quehenberger, O., Shaw, P., Steinberg, D., Terpstra, V. and Witztum, J.L. (2001) Scavenger receptors, oxidized LDL, and atherosclerosis. *Annals of the New York Academy of Sciences* 947, 214–222.

Brown, B.G., Zhao, X.Q., Sacco, D.E. and Albers, J.J. (1993) Lipid lowering and plaque regression. New insights into prevention of plaque disruption and clinical events in coronary disease. *Circulation* 87(6), 1781–1791.

Calo, L., Sartore, G., Bassi, A., Basso, C., Bertocco, S., Marin, R., Zambon, S., Cantaro, S., D'Angelo, A., Davis, P.A., Manzato, E. and Crepaldi, G. (1998) Reduced susceptibility to oxidation of low-density lipoprotein in patients with overproduction of nitric oxide (Bartter's and Gitelman's syndrome). *Journal of Hypertension* 1, 16(7), 1001–1008.

Canales, A. and Sánchez-Muniz, F.J. (2003) Paraoxonase, something more than an enzyme? *Medicina Clinica* 25, 121(14), 537–548.

Caruso, D., Visioli, F., Patelli, R., Galli, C. and Galli, G. (2001) Urinary excretion of olive oil phenols and their metabolites in humans. *Metabolism* 50(12), 1426–1428.

Castro, P., Miranda, J.L., Gomez, P., Escalante, D.M., Segura, F.L., Martin, A., Fuentes, F., Blanco, A., Ordovas, J.M. and Jimenez, F.P. (2000) Comparison of an oleic acid enriched-diet vs NCEP-I diet on LDL susceptibility to oxidative modifications. *European Journal of Clinical Nutrition* 54(1), 61–67.

Cernea, S., Hâncu, M. and Razm, I. (2003) Diet and coronary heart disease in diabetes. *Acta Diabetologica* 40, S389–S400.

Chait, A. and Wight, T.N. (2000) Interaction of native and modified low-density lipoproteins with extracellular matrix. *Current Opinion in Lipidology* 11(5), 457–463.

Chatterjee, S., Berliner, J.A., Subbanagounder, G.G., Bhunia, A.K. and Koh, S. (2004) Identification of a biologically active component in minimally oxidized low density lipoprotein (MM-LDL) responsible for aortic smooth muscle cell proliferation. *Glycoconjugate Journal* 20(5), 331–338.

Chiu, B., Viira, E., Tucker, W. and Fong, I.W. (1997) Chlamydia pneumoniae, cytomegalovirus, and herpes simplex virus in atherosclerosis of the carotid artery. *Circulation* 7, 96(7), 2144–2148.

Clinton, S.K., Underwood, R., Hayes, I., Sherman, M.L., Kufe, D.W. and Libby, P. (1992) Macrophage colony stimulating factor gene expression in vascular cells and in experimental and human atherosclerosis. *American Journal of Pathology* 140, 301–316.

Coni, E., Di Benedetto, R., Di Pasquale, M., Masella, R., Modesti, D., Mattei, R. and Carlini, E.A. (2000) Protective effect of oleuperine, an olive oil biophenol, on low density lipoprotein oxidizability in rabbits. *Lipids* 35, 45–54.

Costa, F.V. (2002) Non-pharmacological treatment of hypertension in women. *Journal of Hypertension* 20 Suppl. 2, S57–61.

Covas, M.I., Fito, M., Lamuela-Raventos, R.M., Sebastia, N., de la Torre-Boronat, C. and Marrugat, J. (2000) Virgin olive oil phenolic compounds: binding to human low density lipoprotein (LDL) and effect on LDL oxidation. *International Journal of Clinical Pharmacology Research* 20(3–4), 49–54.

Cushing, S.D., Berliner, J.A., Valente, A.J., Territo, M.C., Navab, M., Parhami, F., Gerrity, R., Schwartz, C.J. and Fogelman, A.M. (1990) Minimally modified LDL induces monocyte chemotactic proteins in human endothelial and smooth muscle cells. *Proceedings in Natural Academy of Science USA* 87, 5134–5138.

Deedwania, P.C. (1995) Clinical perspectives on primary and secondary prevention of coronary atherosclerosis. *Medical Clinics of North America* 79(5), 973–998.

Doi, H., Kugiyama, K., Ohgushi, M., Sugiyama, S., Matsumura, T., Ohta, Y., Nakano, T., Nakajima, K. and Yasue, H. (1998) Remnants of chylomicron and very low density lipoprotein impair endothelium-dependent vasorelaxation. *Atherosclerosis* 137(2), 341–349.

Egan, B.M., Greene, E.L. and Goodfriend, T.L. (2001) Insulin resistance and cardiovascular disease. *American Journal of Hypertension* 14(6 Pt 2), 116S–125S.

Espino, A., Lopez-Miranda, J. and Castro, P. (1996) Monounsaturated fatty acids enriched diets lower plasma insulin levels and blood pressure in healthy young men. *Nutrition Metabolism of Cardiovascular Disease* 6, 147–154.

Ferrara, L.A., Raimondi, A.S., d'Episcopo, L., Guida, L., Dello Russo, A. and Marotta, T. (2000) Olive oil and reduced need for antihypertensive medications. *Archives of Internal Medicine* 27, 160(6), 837–842.

Fito, M., Gimeno, E., Covas, M.I., Miro, E., Lopez-Sabater, M.C., Farre, M., de la Torre, R. and Marrugat, J. (2002) Postprandial and short-term effects of dietary virgin olive oil on oxidant/antioxidant status. *Lipids* 37(3), 245–251.

Garg, A. (1998) High-monounsaturated-fat diets for patients with diabetes mellitus: a meta-analysis. *The American Journal of Clinical Nutrition* 67(3 Suppl.), 577S–582S.

Haban, P., Klvanova, J., Zidekova, E. and Nagyova, A. (2004) Dietary supplementation with olive oil leads to improved lipoprotein spectrum and lower n-6 PUFAs in elderly subjects. *Medical Sciences Monitor* 23, 10(4), PI49–PI54.

Harpel, P.C. and Borth, W. (1992) Identification of mechanisms that may modulate the role of lipoprotein(a) in thrombosis and atherogenesis. *Annals of Epidemiology* 2(4), 413–417.

Hiltunen, T.P., Luoma, J.S., Nikkari, T. and Yla-Herttuala, S. (1998) Expression of LDL receptor, VLDL receptor, LDL receptor-related protein, and scavenger receptor in rabbit atherosclerotic lesions: marked induction of scavenger receptor and VLDL receptor expression during lesion development. *Circulation* 24, 97(11), 1079–1086.

Hultberg, B., Andersson, A. and Isaksson, A. (1995) Metabolism of homocysteine, its relation to the other cellular thiols and its mechanism of cell damage in a cell culture line (human histiocytic cell line U-937). *Biochimica et Biophysica Acta* 19, 1269(1), 6–12.

Johnson, R.R.L., Mcgregor, P.R., Taylor, R.N. and Poston, R.N. (1994) Increase in the adhesion molecule P-selectin in endothelium overlying atherosclerosis plaques-coexpression with intercellular adhesion molecule-1. *American Journal of Pathology* 144, 952–961.

Joseph, J., Ranganathan, S. and Mehta, J.L. (2003) Low density lipoproteins modulate collagen metabolism in fibroblasts. *Journal of Cardiovascular Pharmacology and Therapeutics* 8(2), 161–166.

Kaplan, M. and Aviram, M. (1999) Oxidized low density lipoprotein: atherogenic and proinflammatory characteristics during macrophage foam cell formation. An inhibitory role for nutritional antioxidants and serum paraoxonase. *Clinical Chemistry and Laboratory Medicine* 37(8), 777–787.

Kelly, C.M., Smith, R.D. and Williams, C.M. (2001) Dietary monounsaturated fatty acids and haemostasis. *Proceedings of the Nutrition Society* 60(2), 161–170.

Keys, A., Fidanza, F., Scardi, V. and Bergami, G. (1952). The trend of serum-cholesterol levels with age. *Lancet* 2, 2(5), 209–210.

Khatri, J.J., Johnson, C., Magid, R., Lessner, S.M., Laude, K.M., Dikalov, S.I., Harrison, D.G., Sung, H.J., Rong, Y. and Galis, Z.S. (2004) Vascular oxidant stress enhances progression and angiogenesis of experimental atheroma. *Circulation* 3, 109(4), 52052–52055.

Kim, J.A., Territo, M.C., Wayner, E., Carlos, T.M., Parhami, F., Smith, C.W., Haberland, M.E., Fogelman, A.M. and Berliner, J.A. (1994) Partial characterization of leukocyte binding molecules on endothelial cells induced by minimally oxidized LDL. *Arteriosclerosis and Thrombosis* 14(3), 427–433.

Kita, T., Kume, N., Ochi, H., Nishi, E., Sakai, A., Ishii, K., Nagano, Y. and Yokode, M. (1997) Induction of endothelial platelet-derived growth factor-B-chain and intercellular adhesion molecule-1 by lysophosphatidylcholine. *Annals of the New York Academy of Sciences* 15, 811, 70–75.

Kita, T., Kume, N., Ishii, K., Horiuchi, H., Arai, H. and Yokode, M. (1999) Oxidized LDL and expression of monocyte adhesion molecules. *Diabetes Research and Clinical Practises* 45(2–3), 123–126.

Kohno, M., Yokokawa, K., Yasunari, K., Minami, M., Kano, H., Hanehira, T. and Yoshikawa, J. (1998) Induction by lysophosphatidylcholine, a major phospholipid component of atherogenic lipoproteins, of human coronary artery smooth muscle cell migration. *Circulation* 28, 98(4), 353–359.

Korpilahti, K., Engblom, E., Syvanne, M., Hamalainen, H., Puukka, P., Vanttinen, E. and Ronnemaa, T. (1998) Angiographic changes in saphenous vein grafts and atherosclerosis risk factors. A 5-year study with serial measurements of serum lipids and lipoproteins. *Scandinavan Cardiovascular Journal* 32(6), 343–351.

Kris-Etherton, P.M., Pearson, T.A., Wan, Y.,

Hargrove, R.L., Moriarty, K., Fishell, V. and Etherton, T.D. (1999) High-monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. *The American Journal of Clinical Nutrition* 70(6), 1009–1015.

Lahoz, C., Alonso, R., Ordovas, J.M., Lopez-Farre, A., De Oya, M. and Mata, P. (1997) Effects of dietary fat saturation on eicosanoid production, platelet aggregation and blood pressure. *European Journal of Clinical Investigation* 27, 780–787.

Lehr, H.A., Seemuller, J., Hubner, C., Menger, M.D. and Mesmer, K. (1993) Oxidized LDL induced leucocyte-endothelium interaction in vivo involves the receptor for platelet-activating factor. *Arteriosclerosis Thrombosis and Vascular Biology* 13, 1013–1018.

Liao, F., Berliner, J.A., Mehrabian, M., Navab, M., Demer, L.L., Lusis, A.J. and Fogelman, A.M. (1991) Minimally modified low density lipoprotein is biologically active in vivo in mice. *The Journal of Clinical Investigation* 87(6), 2253–2257.

Liao, F., Andalibi, A., Lusis, A.J. and Fogelman, A.M. (1995) Genetic control of the inflammatory response induced by oxidized lipids. *American Journal of Cardiology* 23, 75(6), 65B–66B.

Liu-Wu, Y., Hurt-Camejo, E. and Wiklund, O. (1998) Lysophosphatidylcholine induces the production of IL-1beta by human monocytes. *Atherosclerosis* 137(2), 351–357.

Luft, F.C. (2002) Hypertension as a complex genetic trait. *Seminar of Nephrology* 22(2), 115–126.

Mancini, M., Parillo, M., Rivellese, A. and Riccardi, G. (1995) Nutrition and cardiovascular risk: the Mediterranean experience. *Acta of Cardiology* 44(6), 466–467.

Marrugat, J., Covas, M.I., Fito, M., Schroder, H., Miro-Casas, E., Gimeno, E., Lopez-Sabater, M.C., De La Torre, R. and Farre, M. (2004) Effects of differing phenolic content in dietary olive oils on lipids and LDL oxidation. A randomized controlled trial. *European Journal of Nutrition* 43(3), 140–147.

Masella, R., Vari, R., D'Archivio, M., Di Benedetto, R., Matarrese, P., Malorni, W., Scazzocchio, B. and Giovannini, C. (2004) Extra virgin olive oil biophenols inhibit cell-mediated oxidation of LDL by increasing the mRNA transcription of glutathione-related enzymes. *The Journal of Nutrition* 134(4), 785–791.

Mata, P., Garrido, J.A., Ordovas, J.M., Blazquez, E., Alvarez-Sala, L.A., Rubio, M.J., Alonso, R. and de Oya, M. (1992) Effect of dietary monounsaturated fatty acids on plasma lipoproteins and apolipoproteins in women. *The American Journal of Clinical Nutrition* 56(1), 77–83.

Medina, R.A., Aranda, E., Verdugo, C., Kato, S. and Owen, G.I. (2003) The action of ovarian hormones in cardiovascular disease. *Biological Research* 36(3–4), 325–341.

Miró-Casas, E., Covas, M.I., Fito, M., Farre-Albadalejo, M., Marrugat, J. and de la Torre, R. (2003a) Tyrosol and hydroxytyrosol are absorbed from moderate and sustained doses of virgin olive oil in humans. *European Journal of Clinical Nutrition* 57(1), 186–190.

Miró-Casas, E., Covas, M.I., Farre, M., Fito, M., Ortuno, J., Weinbrenner, T., Roset, P. and de la Torre, R. (2003b) Hydroxytyrosol disposition in humans. *Clinical Chemistry* 49(6 Pt 1), 945–952.

Mody, N., Parhami, F., Sarafian, T.A. and Demer, L.L. (2001) Oxidative stress modulates osteoblastic differentiation of vascular and bone cells. *Free Radical Biology and Medicine* 15, 31(4), 509–519.

Moreno, J.J. and Mitjavila, M.T. (2003) The degree of unsaturation of dietary fatty acids and the development of atherosclerosis (review). *The Journal of Nutritional Biochemistry* 14(4), 182–195.

Navab, M., Hama, S.Y., Reddy, S.T., Ng, C.J., Van Lenten, B.J., Laks, H., Fogelman, A.M. and Ready, S.T. (2002) Oxidized lipids as mediators of coronary heart disease. *Current Opinion in Lipidology* 13(4), 363–372.

Newby, A.C. and Zaltsman, A.B. (1999) Fibrous cap formation or destruction – the critical importance of vascular smooth muscle cell proliferation, migration and matrix formation. *Cardiovascular Research* 41(2), 345–360.

Nicolaiew, N., Lemort, N., Adorni, L., Berra, B., Montorfano, G., Rapelli, S., Cortesi, N. and Jacotot, B. (1998) Comparison between extra virgin olive oil and oleic acid rich sunflower oil: effects on postprandial lipemia and LDL susceptibility to oxidation. *Annals of Nutrition and Metabolism* 42(5), 251–260.

Nicolosi, R.J., Wilson, T.A., Rogers, E.J. and Kritchevsky, D. (1998) Effects of specific fatty acids (8:0, 14:0, cis-18:1, trans-18:1) on plasma lipoproteins, early atherogenic potential, and LDL oxidative properties in the hamster. *Journal of Lipid Research* 39, 1972–1980.

Nielsen, L.B., Juul, K. and Nordestgaard, B.G. (1998) Increased degradation of lipoprotein(a) in atherosclerotic compared with nonlesioned aortic intima-inner media of rabbits: in vivo evidence that lipoprotein(a) may contribute to foam cell formation. *Arteriosclerosis Thrombosis and Vascular Biology* 18(4), 64164–64169.

Ochoa, J.J., Quiles, J.L., Ramirez-Tortosa, M.C., Mataix, J. and Huertas, J.R. (2002) Dietary oils high in oleic acid but with different unsaponifiable fraction contents have different effects in fatty acid composition and peroxidation in rabbit LDL. *Nutrition* 18(1), 60–65.

Olszewski, A.J. and McCully, K.S. (1993) Fish oil decreases serum homocysteine in hyperlipemic men. *Coronary and Artery Disease* 4(1), 53–60.

Ooi, T.C. and Ooi, D.S. (1998) The atherogenic significance of an elevated plasma triglyceride level. *Critical Reviews in Clinical Laboratory Science* 35(6), 489–516.

Oubina, P., Sanchez-Muniz, F.J., Rodenas, S. and Cuesta, C. (2001) Eicosanoid production, thrombogenic ratio, and serum and LDL peroxides in normo- and hypercholesterolaemic post-menopausal women consuming two oleic acid-rich diets with different content of minor components. *British Journal of Nutrition* 85(1), 41–47.

Parthasarathy, S., Khoo, J.C., Miller, E., Barnett, J., Witztum, J.L. and Steinberg, D. (1990) Low density lipoprotein rich in oleic acid is protected against oxidative modification: implication for dietary prevention of atherosclerosis. *Proceedings Natural Academy of Science* 87, 3894–3898.

Patrick, T.E. and Fletcher, G.F. (2001) Endothelial function and cardiovascular prevention: role of blood lipids, exercise, and other risk factors. *Cardiology in Review* 9(5), 282–286.

Patrono, C. and FitzGerald, G.A. (1997) Isoprostanes: potential markers of oxidant stress in atherothrombotic disease. *Arteriosclerosis Thrombosis and Vascular Biology* 17(11), 2309–2315.

Petridou, E., Zavras, A.I., Lefatzis, D., Dessypris, N., Laskaris, G., Dokianakis, G., Segas, J., Douglas, C.W., Diehl, S.R. and Trichopoulos, D. (2002) The role of diet and specific micronutrients in the etiology of oral carcinoma. *Cancer* 1, 94(11), 2981–2988.

Piers, L.S., Walker, K.Z., Stoney, R.M., Soares, M.J. and O'Dea, K. (2003) Substitution of saturated with monounsaturated fat in a 4-week diet affects body weight and composition of overweight and obese men. *British Journal of Nutrition* 90(3), 717–727.

Quiles, J.L., Ramirez-Tortosa, M.C., Huertas, J.R., Ibañez, S., Gomez, J.A., Battino, M. and Mataix, J. (1999) Olive oil supplemented with vitamin E affects mitochondrial coenzyme Q levels in liver of rats after an oxidative stress induced by adriamycin. *Biofactors* 9, 331–336.

Quinn, M.T., Parthasartathy, S. and Steinberg, D. (1988) Lysophosphatidyl choline: A chemotactic factor for human monocytes and its potential role in atherogenesis. *Proceedings Natural Academy of Science USA* 85, 2805–2809.

Rajavasisth, T.B., Andalibi, A., Territo, M.C., Berliner, J.A., Navab, M., Fogelman, A.M. and Lusis, A.J. (1990) Modified LDL induce endothelial cell expression of granulocyte and macrophage colony stimulation factors. *Nature* 344, 254–257.

Ramirez-Tortosa, M.C., Aguilera, C.M., Quiles, J.L. and Gil, A. (1998) Influence of dietary lipids on lipoprotein composition and LDL Cu(2+)-induced oxidation in rabbits with experimental atherosclerosis. *BioFactors* 8(1–2), 79–85.

Ramirez-Tortosa, M.C., Suarez, A., Gomez, M.C., Mir, A., Ros, E., Mataix, J. and Gil, A. (1999a) Effect of extra-virgin olive oil and fish-oil supplementation on plasma lipids and susceptibility of low-density lipoprotein to oxidative alteration in free-living Spanish male patients with peripheral vascular disease. *Clinical Nutrition* 18(3), 167–174.

Ramírez-Tortosa, M.C., López-Pedrosa, J.M., Suarez, A., Ros, E., Mataix, J. and Gil, A.

(1999b) Olive oil and fish oil enriched diets modify plasma lipids and susceptibility of low density lipoprotein to oxidative modification in free-living male patients with peripheral vascular disease: the Spanish Nutrition Study. *British Journal of Nutrition* 82, 31–39.

Ramirez-Tortosa, M.C., Urbano, G., Lopez-Jurado, M., Nestares, T., Gomez, M.C., Mir, A., Ros, E., Mataix, J. and Gil, A. (1999c) Extra-virgin olive oil increases the resistance of LDL to oxidation more than refined olive oil in free-living men with peripheral vascular disease. *The Journal of Nutrition* 129(12), 2177–2183.

Reis, R.P., Azinheira, J., Reis, H.P., Pina, J.E., Correia, J.M. and Luis, A.S. (1999) The effect of sex and menopause on basal blood levels of homocysteine and after methionine loading. *Revista Portuguesa de Cardiologia* 18(2), 155–159.

Rivellese, A.A., Maffettone, A., Vessby, B., Uusitupa, M., Hermansen, K., Berglund, L., Louheranta, A., Meyer, B.J. and Riccardi, G. (2003) Effects of dietary saturated, monounsaturated and n-3 fatty acids on fasting lipoproteins, LDL size and post-prandial lipid metabolism in healthy subjects. *Atherosclerosis* 167(1), 149–158.

Rocca, A.S., LaGreca, J., Kalitsky, J. and Brubaker, P.L. (2001) Monounsaturated fatty acid diets improve glycemic tolerance through increased secretion of glucagon-like peptide-1. *Endocrinology* 142(3), 1148–1155.

Rodriguez-Villar, C., Perez-Heras, A., Mercade, I., Casals, E. and Ros, E. (2004) Comparison of a high-carbohydrate and a high-monounsaturated fat, olive oil-rich diet on the susceptibility of LDL to oxidative modification in subjects with Type 2 diabetes mellitus. *Diabetic Medicine* 21(2), 142–149.

Rong, J.X., Raugaswamy, S., Shen, L., Dave, R., Chang, Y.H., Peterson, H., Hodis, H.N., Chilsom, G.M. and Sevaniam, A. (1998) Arterial injury by cholesterol oxidation products acuses endothelial dysfunction and arterial wall cholesterol accumulation. *Arteriosclerosis Thrombosis and Vascular Biology* 18, 1885–1894.

Schecter, A.D., Rollins, B.J., Zhabg, Y.J., Charo, I.F., Fallon, J.T., Rossikhina, M., Giesen, P.L., Nemerson, Y. and Taubman, M.B. (1997) Tissue factor is induced by monocyte chemoattract protein-1 in human aortic smooth muscle and THP-1 cells. *Journal in Biology and Chemistry* 272, 28568–28573.

Schmidt, A.M., Yan, S.D., Brett, J., Mora, R., Nowygord, R. and Stern, D. (1993) Regulation of mononuclear phagocyte migration by cell surface bonding proteins for advanced glycosylation end products. *Journal of Clinical Investigation* 92, 2155–2168.

Schwartz, D., Andalibi, A., Chaverri-Almada, L., Berliner, J.A., Kirchgessner, T., Fang, Z.T., Tekamp-Olson, P., Lusis, A.J., Gallegos, C. and Fogelman, A.M. (1994) Role of the GRO family of chemokines in monocyte adhesion to MM-LDL-stimulated endothelium. *Journal of Clinical Investigation* 94, 1068–1973.

Siegel, J.H., Cerra, F.B., Coleman, B., Giovannini, I., Shetye, M., Border, J.R. and McMenamy, R.H. (1979) Physiological and metabolic correlations in human sepsis. Invited commentary. *Surgery* 86(2), 163–193.

Soler, M., Chatenoud, L., La Vecchia, C., Franceschi, S. and Negri, E. (1998) Diet, alcohol, coffee and pancreatic cancer: final results from an Italian study. *European Journal of Cancer Prevention* 7(6), 455–460.

Song, L., Xu, M., Lopes-Virella, M.F. and Huang, Y. (2001) Quercetin inhibits matrix metalloproteinase-1 expression in human vascular endothelial cells through extracellular signal-regulated kinase. *Archives of Biochemistry and Biophysics* 1, 391(1), 72–78.

Sonoki, K., Iwase, M., Iino, K., Ichikawa, K., Ohdo, S., Higuchi, S., Yoshinari, M. and Iida, M. (2003) Atherogenic role of lysophosphatidylcholine in low-density lipoprotein modified by phospholipase A2 and in diabetic patients: protection by nitric oxide donor. *Metabolism* 52(3), 308–314.

Spieker, L.E., Ruschitzka, F., Luscher, T.F. and Noll, G. (2004) HDL and inflammation in atherosclerosis. *Current Drug Targets. Immune, Endocrine and Metabolic Disorders* 4(1), 51–57.

Stanger, O., Herrmann, W., Pietrzik, K., Fowler, B., Geisel, J., Dierkes J., Weger, M.; DACH-LIGA. Homocystein e.V. (2003) DACH-LIGA homocystein (German, Austrian and Swiss

homocysteine society): consensus paper on the rational clinical use of homocysteine, folic acid and B-vitamins in cardiovascular and thrombotic diseases: guidelines and recommendations. *Clinical Chemistry and Laboratory Medicine* 41(11), 1392–1403.

Staprans, I., Pan, X.M., Rapp, J.H. and Feingold, K.R. (1998) Oxidized cholesterol in the diet accelerates the development of aortic atherosclerosis in cholesterol-fed rabbits. *Arteriosclerosis Thrombosis and Vascular Biology* 18, 977–983.

Steinberg, D. and Witztum, J.L. (2002) Is the oxidative modification hypothesis relevant to human atherosclerosis? Do the antioxidant trials conducted to date refute the hypothesis? *Circulation* 30, 105(17), 2107–2111.

Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C. and Witztum, J.L. (1989) Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *New England Journal of Medicine* 6, 320(14), 915–924.

Stopeck, A.T., Nicholson, A.C., Mancini, F.P. and Hajjar, D.P. (1993) Cytokine regulation of low density lipoprotein receptor gene transcription in HepG2 cells. *The Journal of Biological Chemistry* 15, 268(23), 17489–17494.

Suzukawa, M., Abbey, M., Howe, P.R.C. and Nestel, P.J. (1995) Effects of fish oil fatty acids on low density lipoprotein size, oxidizability and uptake by macrophages. *Journal Lipid Research* 36, 473–484.

Tanasescu, M., Cho, E., Manson, J.E. and Hu, F.B. (2004) Dietary fat and cholesterol and the risk of cardiovascular disease among women with type 2 diabetes. *The American Journal of Clinical Nutrition* 79(6), 999–1005.

Terres, W., Tatsis, E., Pfalzer, B., Beil, F.U., Beisiegel, U. and Hamm, C.W. (1995) Rapid angiographic progression of coronary artery disease in patients with elevated lipoprotein(a). *Circulation* 15, 91(4), 948–950.

Van den Hoogen, P.C.W., Feskens, E.J.M., Nagelkerke, N.J.D., Menotti, A., Nissinen, A. and Kromhourt, D. (2000) The relation between blood pressure and mortality due to coronary heart disease among men in different parts of the world. *New England Journal of Medicine* 342, 1–8.

Vessby, B., Unsitupa, M., Hermansen, K., Riccardi, G., Rivellese, A.A., Tapsell, L.C., Nalsen, C., Berglund, L., Louheranta, A., Rasmussen, B.M., Calvert, G.D., Maffetone, A., Pedersen, E., Gustafsson, I.B. and Storlein, L.H. (2001) Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. *Diabetologia* 44(3), 312–319.

Visioli, F., Bellomo, G., Montedoro, G. and Galli, C. (1995) Low density lipoprotein oxidation is inhibited in vitro by olive oil constituents. *Atherosclerosis* 117(1), 25–32.

Visioli, F., Caruso, D., Galli, C., Viappiani, S., Galli, G. and Sala, A. (2000) Olive oils rich in natural catecholic phenols decrease isoprostane excretion in humans. *Biochemical and Biophysical Research Communications* 30, 278(3), 797–799.

Visioli, F., Caruso, D., Plasmati, E., Patelli, R., Mulinacci, N., Romani, A., Galli, G. and Galli, C. (2001) Hydroxytyrosol, as a component of olive mill waste water, is dose-dependently absorbed and increases the antioxidant capacity of rat plasma. *Free Radical Research* 34(3), 301–305.

Visioli, F., Poli, A. and Gall, C. (2002) Antioxidant and other biological activities of phenols from olives and olive oil. *Medicinal Research Reviews* 22(1), 65–75.

Vissers, M.N., Zock, P.L. and Katan, M.B. (2004) Bioavailability and antioxidant effects of olive oil phenols in humans: a review. *European Journal of Clinical Nutrition* 58(6), 955–965.

Watson, A.D., Navab, M., Hama, S.Y., Sevanian, A., Prescott, S.M., Stafforini, D.M., McIntyre, T.M., La Du, B.N., Fogelman, A.M. and Berliner, J.A. (1995) Effect of platelet activating factor acetylhydrolase on the formation and action of minimally oxidized LDL. *Journal of Clinical Investigation* 95, 774–782.

Watson, K.E., Bostrom, K., Ravindranath, R., Lam, T., Norton, B. and Demer, L.L. (1994) TGF-beta 1 and 25-hydroxycholesterol stimulate osteoblast-like vascular cells to calcify. *Journal of Clinical Investigation* 93(5), 2106–2113.

Wilson, D., Massaeli, H., Pierce, G.N. and Zahradka, P. (2003) Native and minimally oxidized low density lipoprotein depress smooth muscle matrix metalloproteinase

levels. *Molecular and Cellular Biochemistry* 249(1–2), 141–149.

Yla-Herttuala, S., Lipton, B.A., Rosenfeld, M.E., Sarkioja, T., Yoshimura, T., Leonard, E.J., Witztum, J.L. and Steinberg, D. (1991) Macrophage express monocyte chemotactic protein (MCP-1) in human and rabbit atherosclerotic lesions. *Proceedings in National Academy of Science USA* 1991, 88, 5252–5256.

Zambon, A., Sartore, G., Passera, D., Francini-Pesenti, F., Bassi, A., Basso, C., Zambon, S., Manzato, E. and Crepaldi, G. (1999) Effects of hypocaloric dietary treatment enriched in oleic acid on LDL and HDL subclass distribution in mildly obese women. *Journal of Internal Medicine* 246(2), 191–201.

10 Olive Oil and Haemostasis

José López Miranda, Angeles Blanco Molina and Francisco Peréz Jiménez

Department of Medicine, University of Córdoba, Lipid and Arteriosclerosis Unit, Reina Sofia University Hospital, Córdoba, Spain

1. Introduction

Atherosclerosis is a complex pathogenic process. In recent years we have seen important developments in the study of the role of thrombotic phenomena in the biology of atheroma plaque. Research into other important factors is advancing towards defining the roles of inflammation and endothelial dysfunction at the onset and in later stages of the disease, as well as providing local and systemic evidence showing its pathogenic importance. These phenomena contribute to the development of the lesion, which is activated by a variety of local or systemic factors. The former include oxidative stress and shear stress; among the latter are typical risk factors such as diabetes, cigarette smoking, hypercholesterolaemia and hypertension (Ross, 1999; Libby, 2002). One of the most interesting new lines of study proposes the formation of the occlusive thrombus on a biologically disposed plaque, the vulnerable or high-risk plaque, which brings about a sudden interruption of arterial flow, triggering the primary signs of vascular disease: acute myocardial infarction, unstable angina and sudden cardiac death. Sudden occlusion occurs when a thrombus forms on a ruptured or fissured plaque, and this is a key factor in setting off the clotting cascade. Meanwhile, research is also being carried out into the role of thrombotic phenomena during the chronic phase of formation and growth of atherosclerotic lesions. Consequently, it is now recognized that there are two processes involved in coronary disease: atherosclerosis and thrombosis, which is why atherothrombosis is becoming generally accepted as the term which most accurately denotes the biological reality of the disease (Fuster *et al*., 1992).

Endothelial dysfunction plays a prominent role in the pathogenesis and progression of these phenomena, as it is the first pathological symptom of anatomical lesions. This dysfunction implies a breakdown in the defence mechanism of the endothelial wall, which in turn induces a prothrombotic state, activates the inflammatory process and alters the vasomotor regulation of the vascular wall.

 Olive Oil and Health
(eds J.L. Quiles, M.C. Ramírez-Tortosa and P. Yaqoob)

This is why the endothelium and the factors which can damage it are now considered so important, and it is also the reason why the traditional approach to atherosclerosis, which used to focus prevention and treatment on attacking risk factors, has been broadened to a more global approach. The new approach aims to treat the biological conditions which activate or advance the vascular lesion: preventing LDL oxidation, reducing the inflammatory process, increasing plaque stability and preventing thrombus formation (Badimon *et al.*, 1993). The traditional dietary approach, which sought to reduce the presence of risk factors, has changed direction. Recent studies show that diet may have an effect on atherogenesis mechanisms, because it could be a potential factor related not only to cholesterol, but also to thrombosis and the endothelial inflammation and dysfunction (Kris-Etherton, 1999; Mustad and Kris-Etherton, 2000). These studies have also shown that diet can lower cardiovascular risk to an even greater extent than drugs. In this chapter we will analyse the effect of olive oil on thrombosis mechanisms, as it can reduce cardiovascular risk as well as exerting a positive effect on other well-known risk factors. First of all, we shall briefly revise thrombosis mechanisms, and then study the specific effect of the main nutritional components in olive oil.

2. Main Factors in Blood Thrombogenicity

It has been established that plaque composition, rather than the degree of stenosis, is determinant in its vulnerability and possible rupture. The most vulnerable plaques have a thin cap, a lipid-rich core and numerous macrophages; they are rich in Tissue Factor (TF), which makes them very prone to thrombosis. The lesion rupture exposes the thrombogenic core to the components of the haemostatic system present in blood flow, which activates thrombus formation (Toschi *et al.*, 1997). This response depends on two processes: platelet activation and aggregation, and the coagulant potential of TF. This product is derived from the macrophages present in the plaque bed, and is activated by the apoptotic endothelial cells. When in contact with flowing blood it triggers the clotting cascade that leads to thrombin generation; this in turn propagates the cascade and aids platelet aggregation, which is essential for stabilizing the wall thrombus. Once the thrombus has formed, it releases growth factors and vasoconstrictor substances of platelet origin, which contribute to vessels narrowing and tissue ischemia. However, we should note that one third of acute coronary syndromes, especially those causing sudden cardiac death by acute myocardial infarction, do not occur due to the rupture of a lipid-rich plaque situated in a limited stenosis area. On the contrary, the thrombotic event is activated by the surface erosion of a fibrotic plaque with a high stenotic component. These cases may be caused by a systemic thrombogenic condition, activated by smoking, hypercholesterolaemia or a propensity to thrombus formation. Increased plasma TF levels have been observed in the presence of these risk factors. However, thrombosis does not always progress adversely, for fibrinolytic responses are capable of curbing the growth of the newly formed thrombus. In fact, 70% of stenotic plaques show previous rupture and repair, even when occlusion or other clinical symptoms are

absent. This means that repeated silent rupture sequences have taken place, which, in the long run, will bring about irregular and heterogeneous plaque growth. Consequently, in order to understand thrombotic events, it is essential to understand the connection between coagulation mechanisms and fibrinolysis (Burke *et al.*, 1997; Fuster *et al.*, 1999).

2.1. Coagulation

As in other mechanisms related to atherogenesis, haemostasis regulation depends on endothelial integrity. This is capital, as the possibility of acting on the endothelium through certain food nutrients sheds a new light on the prevention of vascular diseases. As previously stated, thrombus formation depends on the platelet system and the activation of the coagulation system; it starts with the exposure of the lipid core to flowing blood. Plaque determinants, which favour thrombotic events, are the quality and quantity of the thrombogenic material exposed and the degree of vessel stenosis, the surface irregularity and the balance between coagulation and fibrinolysis. Lipid-rich atheroma is a highly thrombogenic substrate, as is demonstrated by the fact that platelet deposition and thrombus formation on a lipid-rich core is up to sixfold greater than on other substrates (Lutgens *et al.*, 2003). After plaque disruption the proteins in the sub-endothelium are exposed to circulating platelets. Some of them are known as adhesive proteins, as they mediate platelet-endothelium interaction; these include fibronectin, laminin, vitronectin and the von Willebrand Factor (vWF). The latter binds to the platelet lb receptor and induces the platelet binding to the lesion area. Subsequently, platelets change their shape, from flat discs to spherical forms with pseudopods and the processes of platelet activation are set off: the contents of its granules are released, initiating aggregation, which is the irreversible binding of platelets by means of fibrinogen molecules as a binding activator. Therefore, the vWF plays a major role in clot formation and in atherosclerosis complications (Wagner and Burger, 2003).

The second main mechanism depends on the coagulation system (Fig. 10.1). This process develops in a complex series of stages and is triggered when the blood flow comes into contact with a negatively charged surface (intrinsic pathway), or with substances released following a vascular wall injury (extrinsic pathway). In the first case, all the requisite factors are present in the bloodstream. Thus, when Factor XII comes into contact with a negatively charged surface, such as collagen or the basal membrane exposed after endothelium denudation, it undergoes conformational changes, which makes it more sensitive to proteolysis. Another factor also absorbed by negatively charged surfaces, is the circulating high-molecular-weight prekallikrein-kininogen complex, which leads to a mutual activation between kallikrein and Factor XII, which then activates Factor XI, which in turn activates Factor IX. Subsequently, Factor IX and Factor VIII, acting as a non-enzymatic cofactor, will activate Factor X.

However, the most important thrombogenic mechanism acting in plaque rupture is the extrinsic pathway, triggered by the substances released from the

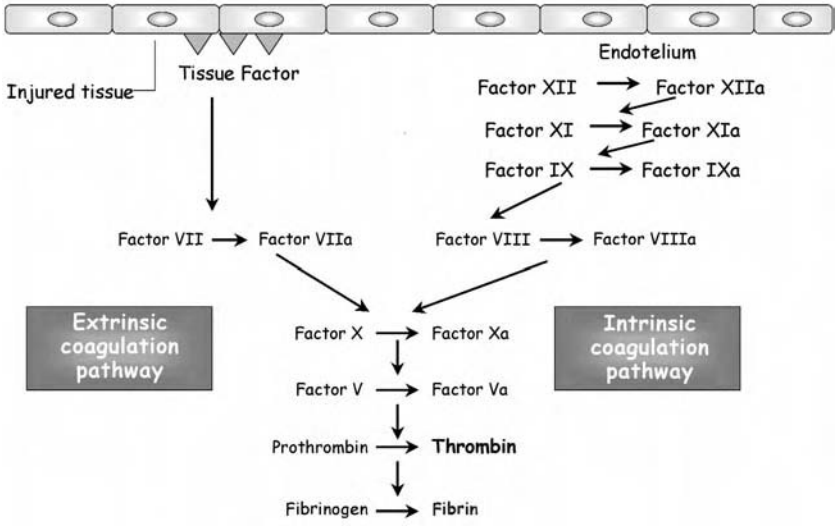


Fig. 10.1. Coagulation mechanism.

vascular wall. Only two blood components are found in it: the TF and Factor VII (FVII). The first is a low-molecular-weight transmembrane glucoprotein which is considered the principal regulator of haemostasis, and, as such, is regarded as the main determinant in thrombosis brought on by atheroma plaque rupture. The distribution of TF seems designed to prevent coagulation activation in the extravascular space, but also to prevent haemorrhage after physical injury to the vessels. Generally, it is not exposed to blood flow, but when tissues or membranes are damaged, it forms a high-affinity complex with FVII, leading to its activation (activated FVIIa). The TF-FVII complex entails a conformational change in TF, which facilitates its active core exposure and leads to activation of Factors IX and X. Thus, thrombin is generated, ultimately aiding the conversion of fibrinogen to fibrin. In humans, the intrinsic and extrinsic coagulation pathways converge, as is shown by the fact that in the presence of calcium, the TF/FVII complex can activate Factor IX, while Factor XII can activate FVII. The two pathways converge into one, beginning with the formation of Factor X, which converts prothrombin into thrombin, and, as stated earlier, the free thrombin then acts on the fibrinogen, converting it into fibrin (Broze, 1995; Sambola *et al.*, 2003b). When fibrin molecules are generated, they interact to form polymers that precipitate, forming mesh-like structure that can anchor the platelet thrombus. Finally, Factor XIII (the fibrin stabilizing factor) forms cross-links between neighbouring chains, which increases the clot's firmness and makes it less sensitive to plasmin action. TF/VIIa complex activity is regulated by an endogenous inhibitor known as Tissue Factor Pathway Inhibitor (TFPI), capable of forming a complex with the components of TF, FVIIa and Factor Xa, and inhibiting their activation in the coagulation cascade. The presence of TFPI in lipid-rich plaques reduces thrombotic capacity in those lesions, which is considered relevant in postangioplastic restenosis and in thrombogenicity mediated by TF expression (Roque *et al.*, 2000).

Generally, TF is present in the tunica adventitia of healthy arteries, but it has been demonstrated that it abounds in atherosclerotic plaques. It gathers in the lipid core and in the underlying luminal region, where it is found mainly in macrophages. This presence justifies the importance that it has been given in the thrombotic process of the atheroma plaque, being higher in the plaques of patients suffering from unstable angina than in those suffering from stable angina. This is also associated with a higher risk of developing arterial thrombotic episodes in this type of patient. Overexpression in mononuclear cells is directly or indirectly favoured by oxidized LDL particles, when they stimulate the production of Tumour Necrosis Factor (TNF) or interleukin-1 (IL-1) (Ardissino *et al.*, 1997; Soejima *et al.*, 1999).

Recent studies also demonstrate that the importance of TF is not limited to its local presence in plaques, but to its systemic presence in circulating monocytes, which suggests it may contribute to the thrombogenic state in patients suffering from acute coronary syndromes. In fact, patients suffering from unstable angina present higher levels of circulating TF and TFPI, and circulating microparticles with procoagulant activity have also been observed. It is believed that these particles are originated by the apoptotic destruction of the macrophages contained in the plaque and that they may be a key factor in both the propagation of the thrombotic process and the progression of other biological systemic phenomena, such as endothelial dysfunction and the dissemination of the prothrombotic and proinflammatory processes. It has recently been proposed that cardiovascular risk factors such as diabetes mellitus, hypercholesterolaemia and cigarette smoking may activate mononuclear cells and trigger the circulation of procoagulant microparticles. In line with this idea, the notion of vulnerable or high-risk plaque would develop into a broader concept: vulnerable or high-risk blood capable of inducing plaque pathogenic events, with the associated prothrombotic state, endothelial dysfunction and activation of the inflammatory process (Sambola *et al.*, 2003a).

2.2. Fibrinolysis

Coagulation relies on the efficiency of the intrinsic and extrinsic pathways and their feedback and interaction mechanisms. Likewise, a physiological mechanism of modulation works to prevent any overactivation that might develop disproportionate thrombotic responses exceeding biological needs, at least in normal circumstances. The balance is ensured by the fibrinolytic system, which eliminates the fibrin molecules deposited on the vascular bed (Fig. 10.2). If this does not function properly the fibrin mesh will take longer to loosen and will remain within the vessels, facilitating the thrombotic process. Plasmin is the key component of the fibrinolytic system. It is a proteolytic enzyme that degrades newly formed fibrin molecules. It is present in plasma as plasminogen, an inactive precursor that can be activated through two pathways: the tissue plasminogen activator (t-PA) and the urokinase plasminogen activator (u-PA). While t-PA can instantly convert plasminogen into plasmin, the urokinase-type must previously undergo modification from its standard single-chain form to a two-chain

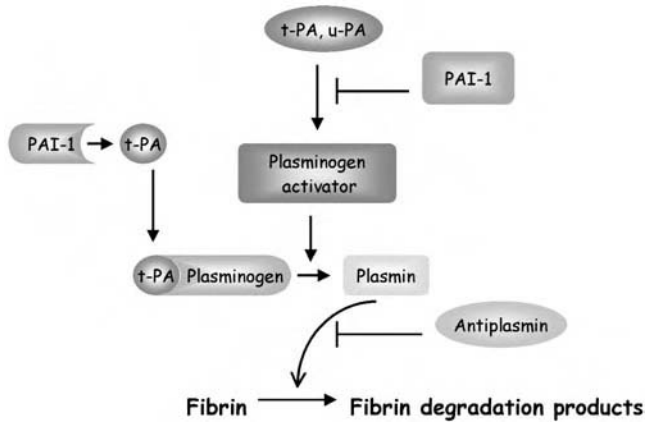


Fig. 10.2. Fibrinolytic system.

form. Plasminogen activators are synthesized in the vascular endothelium by various stimuli, and thrombus lysis is triggered primarily by t-PA release. However, the t-PA must be activated previously by thrombus fibrin in order to turn plasminogen into plasmin, the enzyme in charge of absorbing the fibrin present in the vascular wall (El-Hazmi, 2002).

The fibrinolytic system has a control mechanism limiting its overactivation, and is largely regulated by two types of inhibitors: the plasminogen activator inhibitor-1 (PAI-1) and the plasmin inhibitors (antiplasmins). The level of PAI-1 in blood is normally low and acts as the main inhibitor for the fibrinolytic pathways, both the t-PA dependent and the two-chain u-PA dependent. Approximately 75% of the PAI-1 in the circulation is found in plasma, in active form together with a stabilizing protein, and the rest is stored in platelets and released when these are activated. It is synthesized in endothelial cells, hepatocytes and other types of cells, and it is augmented by stimuli such as IL-1, TNF-α, endotoxins and insulin. Over stimulation of PAI-1 synthesis (as occurs in the insulin resistance syndrome) would inhibit fibrinolysis, which would in turn be responsible for thrombus propagation, the risk of atherothrombosis progression and the development of the acute coronary syndrome (Gensini *et al.*, 1998; Thogersen *et al.*, 1998).

3. Dietary Monounsaturated Fat and Haemostasis

As we have discussed, the formation of thrombi is a key factor in the development of clinical manifestations of coronary disease. Both platelets and coagulation as well as fibrinolysis mechanisms, influence their development. The latter are a series of enzyme sequences which, in the case of coagulation, cause the formation of thrombin, and in the case of fibrinolysis lead to plasmin generation. Their importance in the development of coronary disease has been demonstrated in observational studies to be a useful tool in predicting the risk of coronary heart attacks (Thompson *et al.*, 1995). The influence of dietary fatty acids on these mechanisms has long been known.

3.1. Effect on platelet function

The ingestion of n-3 fatty acids could influence haemostasis, especially by prolonging template bleeding time, but also by exerting some beneficial effects on erythrocyte flexibility and reducing platelet aggregation. However, it appears unlikely that n-3 fatty acids lower fibrinogen or interact with the fibrinolytic system directly (O'Brien *et al.*, 1976; Schmidt *et al.*, 1990; Knapp, 1997). The beneficial effects of dietary n-3 fatty acids on coagulation have been related to the substitution of these fatty acids for n-6 linoleic acid, with a reduction in the synthesis of n-6 arachidonic acid, as a substrate for cyclooxygenase enzyme. Consequently, thromboxane B_2 (TXB_2) synthesis is reduced and TXB_3, a molecule with a lower aggregatory activity towards platelets, is produced. On the other hand, the production of proinflammatory eicosanoids prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4), derived from the n-6 fatty arachidonic acid, decreases, while an increase is noted in the analogues derived from dietary n-3 fatty acids, α-linolenic and eicosapentaenoic and docosahexaenoic acids (James *et al.*, 2000). However, the influence of other dietary unsaturated fatty acids such as n-6 polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) on thrombosis is less well understood. Sirtori and co-workers described a change in platelet function characterized by reduced sensitivity to collagen during the consumption of an olive oil-enriched diet, while sensitivity to arachidonic acid decreased during a corn oil-enriched diet. The experiment was conducted in a free-living population, which was fed diets enriched with both vegetable oils over two consecutive periods (Sirtori *et al.*, 1986). These findings were confirmed by Smith *et al.* (2003), who compared two MUFA-rich diets, a moderate-MUFA and a high-MUFA diet in which 16 g and 32 g of dietary saturated fatty acids (SFA)/100 g total fatty acids were substituted with MUFA respectively. Platelet responses to ADP and arachidonic acid differed with time on the two diets; at 16 weeks, platelet aggregatory response to ADP was significantly lower on the high-MUFA than on the moderate-MUFA ($P < 0.01$) diet; ADP responses were also significantly lower within this group at 8 ($P < 0.05$) and 16 ($P < 0.01$) weeks compared with high-SFA diet. In another study, total lipids of Greek olive oils and seed oils of four kinds, namely, soybean, corn, sunflower and sesame oil, were separated into total polar lipids and total neutral lipids via a novel extraction procedure. Each lipid fraction from oils was tested *in vitro* for the capacity to induce or to inhibit washed rabbit platelet aggregation. Comparison between olive and seed oils supports the superiority of olive oil, as high levels of platelet activating factor antagonists were detected, mainly in total polar lipid (Karantonis *et al.*, 2002). In addition, the structure of the most active fraction from olive oil was elucidated as a glycerolglycolipid. In an experimental study on rats, animals fed an olive oil-enriched diet showed a significant delay in the thrombotic occlusion of the 'aortic loop', a lower incidence of venous thrombosis and a prolonged bleeding time in comparison with the control group fed the usual diet (Brzosko *et al.*, 2002). Furthermore, a recent study evaluates the effect of dietary supplementation with virgin olive oil in an experimental model with rabbits fed an atherogenic diet. Animals fed the virgin olive oil diet, compared with the saturated fatty acid-enriched diet group, showed decreased platelet hyperactivity and subendothelial thrombogenicity (De La Cruz *et al.*, 2000) (Table 10.1).

Table 10.1. Effects of high monounsaturated fat intake in humans on platelet function and eicosanoid pathway.

Study	Subjects and design	Outcome
(Sirtori *et al.*, 1986)	N = 23. Low-fat diets with olive oil or corn oil for 8 weeks	Reduced platelet sensitivity to arachidonic acid (particularly with corn oil) and to collagen (particularly with olive). Decrease of *in vitro* TXB_2 production in platelet samples obtained during the ingestion of olive oil
(Smith *et al.*, 2003)	N = 51. Randomized to a moderate-MUFA diet or a high-MUFA diet	A high-MUFA diet sustains potentially beneficial effects on platelet aggregation
(Lahoz *et al.*, 1997)	N = 42. Four consecutive diets SFA; MUFA; PUFA n-6; and PUFA n-3	Decrease in the urinary excretion of 11-dehydro-thromboxane B_2, during the MUFA diet compared with the n-6 PUFA
(Oubina *et al.*, 2001)	N = 14. Compares two monounsaturated oils, extra virgin olive oil (EVOO) and high-oleic acid sunflower oil (HOSO)	TXB2 levels in stimulated platelet-rich plasma were significantly higher after the HOSO diet than after the EVOO diet
(Rasmussen *et al.*, 1994)	N = 15. Compares a high-MUFA diet and on an isocaloric High CHO diet	The H-MUFA diet caused a decrease in vWF, whereas an unchanged level was observed after a H-CHO diet
(Perez-Jimenez *et al.*, 1999)	N = 25. Compares three consecutive diets: a low fat, high MUFA and a high SFA diet.	The high MUFA diet caused a decrease in vWF as compared to a low fat and a high SFA diet

Unlike the 18-carbon n-3 fatty acid α-linolenic acid, oleic acid is an 18-carbon fatty acid consumed in substantial amounts in the typical Western diet, and especially in the Mediterranean diet. Compared with α-linolenic and linoleic acid, it is not an essential fatty acid. However, eicosatrienoic acid is potentially synthesized from oleic-acid and it has been suggested that this fatty acid may act as a precursor for eicosanoids of a less inflammatory mixture, although this is speculative. Although the effect of eicosatrienoic acid on cyclooxygenase is poorly understood, as is the effect on lipoxygenase, this fatty acid has been suggested to inhibit TXB_2 and PGE_2 synthesis, resulting in a situation similar to that produced by an increased intake of long chain n-3 fatty acids from fish oil (Lerner *et al.*, 1995; James *et al.*, 2000). This could explain Sirtori's findings in the study mentioned above, where they observed a decrease of *in vitro* TXB_2 production in platelet samples obtained during the ingestion of olive oil, but not during the corn oil-enriched diet (Sirtori *et al.*, 1986). Likewise, in a study conducted on rats, the administration of olive oil was seen to induce a decrease in TXB_2 plasma levels (Navarro *et al.*, 1992). Furthermore, in the comparative dietary trial with different fatty acid compositions mentioned above, we observed

a decrease in the urinary excretion of 11-dehydro-thromboxane B_2, a TXB_2 metabolite, during the MUFA phase compared with the n-6 fatty acid dietary period, suggesting that dietary MUFA has an effect on this eicosanoid pathway (Lahoz *et al.*, 1997) (Table 10.1).

Olive oil is composed not only of fatty acids but numerous minor components of which the polyphenols are a significant category. A recent study compared the effects of two MUFA enriched oils, virgin olive oil and high-oleic acid sunflower oil, on eicosanoid production and TXB_2 in 14 non-obese postmenopausal women. Serum peroxides, and TXB_2 levels in stimulated platelet-rich plasma were significantly higher after the high-oleic acid sunflower oil diet than after the extra virgin olive oil diet (Oubina *et al.*, 2001). These findings suggest that differences in the type of minor compounds, as well as in the concentration of linoleic acid, in both these MUFA enriched oils may play an important role in modulating eicosanoid production. Some studies have clearly demonstrated that certain polyphenols, such as quercetin and resveratrol, inhibit platelet aggregation (Bertelli *et al.*, 1996; Ruf, 1999). Other new studies have shown that the polyphenols present in olive oil induce inhibition of platelet aggregation to a degree comparable to that observed with resveratrol and quercetin (Togna *et al.*, 2003). These compounds were effective free radical scavengers and inhibited platelet aggregation and thromboxane release evoked by agonists that induce reactive oxygen species-mediated platelet activation, including sodium arachidonate and collagen, but not ADP. These polyphenolic compounds inhibited arachidonic acid mobilization from platelet membrane phospholipids induced by thrombin and to a greater degree by collagen, suggesting a direct inhibition of phospholipase A2 (PLA2). Thrombin induces release of arachidonic acid from membrane phospholipids by directly stimulating PLA2 without (unlike collagen) ROS production. Therefore, the greater level of inhibition exhibited when using collagen could be the result of an additional indirect effect of the compounds on PLA2 mediated by their scavenging activity.

The combined intake of foods rich in MUFA fat (olive oil) and n-3 PUFA fat from fish and vegetable sources is characteristic of the Mediterranean diet, and this combination may have beneficial effects which go beyond the consumption of each in isolation, as have been noted by Vognild *et al.*, (1998). In this study the influence of various dietary marine oils and olive oil on platelets was investigated in 266 healthy volunteers who consumed 15 ml/d of cod liver oil, whale blubber oil, mixtures of seal blubber oil and cod liver oil, or olive oil and cod liver oil for 12 weeks. Lipopolysaccharide-stimulated blood tended to generate less TXB_2 in the cod liver oil and olive oil groups. The combination of cod liver oil and olive oil may produce better effects than these oils given separately. The changes in platelet function were directly associated with modifications of fatty acid composition in platelet membranes. In addition, a combined high MUFA and marine n-3 PUFA diet induced a significant lowering of Factor IIc, Factor IXc, Factor Xc, Factor VIIc, Factor VIIa, Factor XIIa, PAI-1, plasma viscosity and platelet activity, but led to an increase in fibrinogen, as compared with a low-fat complex carbohydrates and dietary fibre-rich diet (Junker *et al.*, 2001b).

A coagulation component associated with the formation of platelet thrombus is vWF. It is generally assumed to interact with components of the subendothelium

and also with platelet membrane receptors. In this case, vWF could participate in arterial thrombosis, binding to platelet membrane glycoproteins in both adhesion and aggregation, leading to thrombus formation in high shear stress rates (Fuster *et al.*, 1992). In a study conducted on subjects with type 2 diabetes mellitus, vWF was seen to decrease during the consumption of a very high-fat diet, containing 50% of total calories as fat, and with a high content of MUFA (30% of total calories) (Rasmussen *et al.*, 1994). Furthermore, a similar finding was observed by us in healthy normolipemic young males, during the consumption of a highly palatable Mediterranean diet (38% of calories as fat and 22% of calories as MUFA) commonly consumed in Mediterranean countries, while no changes in vWF were observed during a low-fat, high-carbohydrates enriched diet (Perez-Jimenez *et al.*, 1999). The per cent changes in vWF plasma levels correlated with per cent changes in total cholesterol, LDL cholesterol and apo B plasma levels. However, vWF decreased only with the Mediterranean diet and not with a low-fat diet, despite the fact that both diets induced similar changes in plasma lipid levels (Table 10.1). Since vWF is a product derived principally from the endothelium, these data suggest that a MUFA diet could perhaps have an effect on endothelial cell function.

3.2. Effect on coagulation factors

With regard to the effect of dietary fatty acids on other thrombogenic factors, several dietary intervention studies have shown that FVII, a key protein in thrombosis and a risk factor for coronary heart disease (Meade *et al.*, 1993), is indeed influenced by diet. An increase in FVII was observed during the chronic intake of a high SFA diet, as was the postprandial activation of coagulant FVIIc during acute intake (Mennen *et al.*, 1996; Marckmann *et al.*, 1998). These changes suggest that SFA may shift the haemostatic steady state toward hypercoagulability, with adverse consequences in individual risk of acute ischemic events. However, the effects of different kinds of fatty acids may be different (Sanders, 1996). In a 4-week intervention trial on 38 healthy volunteers, a decrease in FVIIc was observed during the consumption of an oleic acid-enriched diet compared with an SFA diet and an n-6 PUFA diet (Turpeinen and Mutanen, 1999). The same effect was evidenced in the comparison of specific dietary fatty acids (lauric acid, palmitic acid and oleic acid) in women and men fed with three different diets for 6 weeks (Temme *et al.*, 1999). The reducing effect of the MUFA-enriched diet on FVII was also explored by Larsen *et al.* in a comparative crossover intervention trial for a period of 3 weeks in 18 healthy men (Larsen *et al.*, 1999). The trial was divided into three different dietary periods: a virgin olive oil-enriched diet, a sunflower oil-enriched diet and a rapeseed oil-enriched diet. There were significant differences between the diets regarding fasting plasma values of FVII protein, FVIIc and activated FVIIa, compared with baseline diet. These data suggest that MUFA diets favour a less thrombogenic environment (Table 10.2).

In a recent study 58 healthy students received either a 4-week rapeseed oil, an olive oil or a sunflower oil diet. With the olive oil diet, a reduction of coagulation factors VIIc, XIIc, XIIa and Xc was found, whereas sunflower oil led to lower values of coagulation factors XIIc, XIIa and IXc (Junker *et al.*, 2001a).

Table 10.2. Effects of high monounsaturated fat intake in humans on coagulation factors.

Study	Subjects and design	Outcome
(Turpeinen and Mutanen, 1999)	N = 38. Saturated fat baseline diet and were then switched to either a high Linoleic Acid (LA) diet or a high Oleic Acid (OA) diet	Factor FVII coagulant activity was significantly lower after the Oleic Acid diet. No differences between the OA and LA diets were found in the plasma levels of fibrinogen, plasminogen activator inhibitor, antithrombin III, von Willebrand factor antigen or D-dimers
(Temme *et al.*, 1999)	N = 32. Compared the effects of specific saturated fatty acids (lauric acid and palmitic acid) with those of oleic acid	Diets rich in lauric or palmitic acid, compared with a diet rich in oleic acid, unfavourably influence factor VIIa and PAI-1 activity
(Larsen *et al.*, 1999)	N = 18. Randomized and crossover study, diets enriched with olive oil, sunflower oil, or rapeseed oil	Postprandial peak concentrations of FVIIa were lower after olive oil than after sunflower oil and rapeseed oil
(Junker *et al.*, 2001a)	N = 58. Received a rapeseed oil, an olive oil or a sunflower oil diet	With the olive oil diet, a reduction of coagulation factors VIIc, XIIc, XIIa, and Xc was found, whereas sunflower oil led to lower values of coagulation factors XIIc, XIIa and IXc
(Smith *et al.*, 2003)	N = 51. Randomized to a moderate-MUFA diet or a high-MUFA diet	Postprandial factor VIIc response was lower than on the high-MUFA diet compared with baseline
(Silva *et al.*, 2003)	N = 51. Compared two MUFA-rich diets, a moderate MUFA and a high MUFA diet	The postprandial increases in FVIIc and FVIIa were lower on the moderate and high MUFA diet
(Lopez-Segura *et al.*, 1996)	N = 21. Compared a low fat with a high MUFA diet	Consumption of diets rich in MUFAs decreases PAI-1 plasma activity
(Perez-Jimenez *et al.*, 1999)	N = 25. Compared three consecutive diets: a low fat, a high SFA and a high MUFA diet	Consumption of a high MUFA diet decrease vWF, PAI-1 and TFPI plasma levels
(Niskanen *et al.*, 1997)	N = 28. Compared a low-fat with a high MUFA diet	Fibrinogen levels or PAI-1 activities did not change with either of the diets

3.3. Effect on postprandial coagulation factors

The selective effect of MUFA on postprandial activation of FVII appears to be less evident. A postprandial increase of FVIIa and FVIIc has been observed during the intake of fat with different high-fat meals in normolipemic and in hypertrygliceridemic subjects (Sanders, 1996; Mennen *et al.*, 1998; Oakley *et al.*, 1998; Sanders *et al.*, 1999). However, certain data suggest that the background diet could influence this FVII postprandial activation. In a fat test meal conducted on the final day of the three-diet period of the trial, the mean

postprandial peak concentrations of FVIIa showed an 18% reduction after a 3-week olive oil dietary period versus a sunflower oil diet and a 15% reduction versus a rapeseed oil diet, with no change in FVIIc (Larsen *et al.*, 1999). However, in a recent study previously described (Smith *et al.*, 2003), the postprandial factor VIIc response was lower than on the high-MUFA diet compared with an SFA rich diet. These findings were confirmed by Silva *et al.* (2003), who compared two MUFA-rich diets, a moderate-MUFA and a high-MUFA diet, in which 16 g and 32 g of dietary SFA/100 g total fatty acids were substituted with MUFA respectively. The postprandial increases in FVIIc and FVIIa were18% and 17% lower respectively on the moderate MUFA diet. Postprandial increases in FVIIc and FVIIa were 50% and 29% lower on the high MUFA diet, and the area under the postprandial FVIIc response curve was also lower on the high MUFA diet (Table 10.2).

3.4. Effect on Tissue Factor

As we have shown, the activation and expression of TF in macrophages has been related to the coagulant activity of the lesion, which favours the development of acute coronary syndrome (Edgington *et al.*, 1991). *In vitro* studies have revealed that fatty acids can influence LPS-induced TF expression in monocytes while PUFA inhibits LPS-induced TF expression in monocytes and macrophages (Lale and Herbert, 1994). This has been confirmed in monocytes of hypertryglyceridemic patients during the ingestion of n-3 fatty acids (Tremoli *et al.*, 1994). Moreover, the isocaloric replacement of a SFA diet by a MUFA or a low-fat, high-carbohydrate diet in healthy subjects reduced TF expression in monocytes obtained during the different dietary phases (Bravo-Herrera *et al.*, 2004). TFPI is an activated factor X-dependent inhibitor of TF-induced coagulation (Lindahl, 1994). The main role of TFPI seems to be to inhibit small amounts of TF, which are probably essential for maintaining a normal haemostatic balance. It has been speculated that TFPI in endothelial cells, *in vivo*, plays a role in the regulation of thrombosis (Grabowski *et al.*, 1993). A previous study has shown that TFPI in the plasma of crab-eating monkeys increases notably in response to a high cholesterol diet (Abumiya *et al.*, 1994). The effect of diets enriched with fat from different sources was explored by Larsen *et al.* in a randomized crossover study with three dietary periods: an olive oil-enriched diet, a sunflower oil diet and a rapeseed oil diet, as discussed previously (Larsen *et al.*, 1999). However, the influence of dietary intervention on TFPI plasma levels was null. In contrast, we have shown more recently that the isocaloric replacement of a palm oil-enriched diet or a low-fat diet by a Mediterranean diet had a reducing effect on plasma TFPI and correlated with an increased resistance to LDL oxidation (Perez-Jimenez *et al.*, 1999). Although the decrease in TFPI plasma levels is difficult to interpret, it has previously been suggested that it may reflect an increase in the protease on the endothelial surface, which would have a regulatory effect on thrombogenesis. Therefore, the decrease in TFPI plasma levels would be interpreted as a change in the protective effect against thrombogenesis.

3.5. Effect on fibrinolysis

Few studies have been conducted to determine the influence of dietary factors on fibrinolysis, although certain data suggest its importance in the development of atherothrombosis. Marckmann *et al.* (1993) showed that administration of a carbohydrate and fibre-rich diet for a period of 2 weeks increased t-PA dependent fibrinolytic activity when compared with a diet rich in SFA, although changes in t-PA and PAI-1 plasma levels by antigenic methods were not observed. Administration of a diet rich in n-3 fatty acids caused an increase in plasma PAI-1 both in healthy individuals and in diabetics, suggesting an impairment of fibrinolytic activity (Fumeron *et al.*, 1991; Marckmann *et al.*, 1991). Studies to investigate the effect of MUFA on fibrinolysis are scarce. We carried out a study on 21 young, healthy male volunteers, who followed two low-fat diets with 115 or 280 mg of dietary cholesterol per 1000 kcal per day, and two oleic acid-enriched diets from virgin olive oil with the same dietary cholesterol as the low-fat diets. The study demonstrated a decrease in PAI-1 plasma levels with both oleic acid-enriched diets. Plasma levels of fibrinogen, thrombin-antithrombin complexes, plasminogen, α_2 antiplasmin and t-PA did not differ significantly among the experimental diets used in this study. The beneficial effects of both oleic acid-enriched diets were evident despite the addition of cholesterol to the diet. Moreover, changes in insulin levels and PAI-1 activity were positively correlated, suggesting a possible relationship between the decrease of PAI-1 and an improvement in insulin sensitivity during high-MUFA olive oil diets (Lopez-Segura *et al.*, 1996) (Table 2). This beneficial effect contrasts with the effect of diets enriched with n-3 fatty acids from fish oils, as previously discussed (Fumeron *et al.*, 1991; Marckmann *et al.*, 1991). The effect of a high-MUFA olive oil rich diet on PAI-1 has been explored more recently by us with a different experimental design. In an intervention study trial comparing the isocaloric substitution of a palmitic acid-enriched diet for a low-fat diet or a Mediterranean diet enriched with olive oil, both cardioprotective diets decreased PAI-1 plasma levels. The reducing effect was higher during the Mediterranean diet in this healthy population of 25 young males (Perez-Jimenez *et al.*, 1999). The positive findings of the Mediterranean diet were confirmed in another intervention trial with a free population in Italy, which compared the effects of substituting an urban diet for a rural Mediterranean diet. The urban diet was significantly higher in protein, cholesterol and fat (in particular SFA) and lower in carbohydrates and fibre. Moreover, the PUFA/SFA and MUFA/SFA ratios were significantly lower in the urban sample than in the rural sample. The rural population adopted the urban diet and the urban population followed the rural diet. Eight weeks after the intervention period and 8 weeks after returning to the original diet, different haemostatic parameters were measured at baseline. Consumption of the Mediterranean diet induced a decrease in both PAI-1 and FVII plasma levels in both males and females. Both groups returned to previous plasma levels when changing back to their original diets, suggesting the positive effects of the Mediterranean diet on thrombogenic risk (Avellone, 1998). In contrast to the beneficial effects of replacing an SFA diet with a MUFA diet, the comparative effects of two unsaturated fats (one enriched with linoleic fat and the other with

oleic fat) demonstrated a similar effect on PAI-1 plasma levels in a two-phase intervention trial on 38 healthy humans (Turpeinen and Mutanen, 1999). Furthermore, Niskanen *et al.* (1997) also failed to demonstrate a decrease in PAI-1 plasma levels with either a MUFA rich-diet or a low fat diet in impaired glucose tolerance subjects. On the other hand, contrary to the data indicated on the effects of chronic consumption of different diets on PAI-1 plasma levels, neither the amount nor type of fat during the postprandial state influenced PAI-1, t-PA or D-dimer concentration (Oakley *et al.*, 1998).

In summary, olive oil based diets might decrease a prothrombotic environment, modifying different haemostatic components and reducing platelet aggregation. Furthermore, this kind of diet reduces Von Willebrand Factor plasma levels, TF expression in mononuclear cells FVII and PAI-1 plasma levels in some studies and induces a lower postprandial activation of FVII.

4. References

Abumiya, T., Nakamura, S., Takenaka, A., Takenaka, O., Yoshikuni, Y., Miyamoto, S., Kimura, T., Enjyoji, K. and Kato, H. (1994) Response of plasma tissue factor pathway inhibitor to diet-induced hypercholesterolemia in crab-eating monkeys. *Arteriosclerosis and Thrombosis* 14, 483–488.

Ardissino, D., Merlini, P.A., Ariens, R., Coppola, R., Bramucci, E. and Mannucci, P.M. (1997) Tissue-factor antigen and activity in human coronary atherosclerotic plaques. *Lancet* 349, 769–771.

Avellone, G.D.G.V., Cordova, R., Scalffidi, L. and Bompiani, G.D. (1998) Effects of Mediterranean diet on lipid, coagulative and fibrinolytic parameters in two randomly selected population samples in Western Sicily. *Nutrition, Metabolism and Cardiovascular Disease* 8, 287–296.

Badimon, J.J., Fuster, V., Chesebro, J.H. and Badimon, L. (1993) Coronary atherosclerosis. A multifactorial disease. *Circulation* 87, II3–16.

Bertelli, A.A., Giovannini, L., Bernini, W., Migliori, M., Fregoni, M., Bavaresco, L. and Bertelli, A. (1996) Antiplatelet activity of cis-resveratrol. *Drugs Experimental Clinical Research* 22, 61–63.

Bravo-Herrera, M.D., Lopez-Miranda, J., Marin, C., Gomez, P., Gomez, M.J., Moreno, J.A., Perez-Martinez, P., Blanco, A., Jimenez-Gomez, Y. and Perez-Jimenez, F. (2004) Tissue factor expression is decreased in monocytes obtained from blood during Mediterranean or high carbohydrate diets. *Nutrition, Metabolism and Cardiovascular Disease* 14, 128–132.

Broze, G.J., Jr. (1995) Tissue factor pathway inhibitor. *Thrombosis and Haemostasis* 74, 90–93.

Brzosko, S., De Curtis, A., Murzilli, S., de Gaetano, G., Donati, M.B. and Iacoviello, L. (2002) Effect of extra virgin olive oil on experimental thrombosis and primary hemostasis in rats. *Nutrition, Metabolism and Cardiovascular Disease* 12, 337–342.

Burke, A.P., Farb, A., Malcom, G.T., Liang, Y.H., Smialek, J. and Virmani, R. (1997) Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. *The New England Journal of Medicine* 336, 1276–1282.

De La Cruz, J.P., Villalobos, M.A., Carmona, J.A., Martin-Romero, M., Smith-Agreda, J.M. and de la Cuesta, F.S. (2000) Antithrombotic potential of olive oil administration in rabbits with elevated cholesterol. *Thrombosis Research* 100, 305–315.

Edgington, T.S., Mackman, N., Brand, K. and Ruf, W. (1991) The structural biology of expression and function of tissue factor. *Thrombosis and Haemostasis* 66, 67–79.

El-Hazmi, M.A. (2002) Hematological risk factors for coronary heart disease. *Medicine Principles and Practice* 11S, 56–62.

Fumeron, F., Brigant, L., Ollivier, V., de Prost, D., Driss, F., Darcet, P., Bard, J.M., Parra, H.J., Fruchart, J.C. and Apfelbaum, M. (1991) n-3 polyunsaturated fatty acids raise low-density lipoproteins, high-density lipoprotein 2, and plasminogen-activator inhibitor in healthy young men. *American Journal of Clinical Nutrition* 54, 118–122.

Fuster, V., Badimon, L., Badimon, J.J. and Chesebro, J.H. (1992) The pathogenesis of coronary artery disease and the acute coronary syndromes (1). *The New England Journal of Medicine* 326, 242–250.

Fuster, V., Fayad, Z.A. and Badimon, J.J. (1999) Acute coronary syndromes: biology. *Lancet* 353, SII5–9.

Gensini, G.F., Comeglio, M. and Colella, A. (1998) Classical risk factors and emerging elements in the risk profile for coronary artery disease. *European Heart Journal* 19, A53-A61.

Grabowski, E.F., Zuckerman, D.B. and Nemerson, Y. (1993) The functional expression of tissue factor by fibroblasts and endothelial cells under flow conditions. *Blood* 81, 3265–3270.

James, M.J., Gibson, R.A. and Cleland, L.G. (2000) Dietary polyunsaturated fatty acids and inflammatory mediator production. *American Journal of Clinical Nutrition* 71, 343S–348S.

Junker, R., Kratz, M., Neufeld, M., Erren, M., Nofer, J.R., Schulte, H., Nowak-Gottl, U., Assmann, G. and Wahrburg, U. (2001a) Effects of diets containing olive oil, sunflower oil, or rapeseed oil on the hemostatic system. *Thrombosis and Haemostasis* 85, 280–286.

Junker, R., Pieke, B., Schulte, H., Nofer, R., Neufeld, M., Assmann, G. and Wahrburg, U. (2001b) Changes in hemostasis during treatment of hypertriglyceridemia with a diet rich in monounsaturated and n-3 polyunsaturated fatty acids in comparison with a low-fat diet. *Thrombosis Research* 101, 355–366.

Karantonis, H.C., Antonopoulou, S. and Demopoulos, C.A. (2002) Antithrombotic lipid minor constituents from vegetable oils. Comparison between olive oils and others. *Journal of Agriculture and Food Chemistry* 50, 1150–1160.

Knapp, H.R. (1997) Dietary fatty acids in human thrombosis and hemostasis. *American Journal of Clinical Nutrition* 65, 1687S–1698S.

Kris-Etherton, P.M. (1999) A new role for diet in reducing the incidence of cardiovascular disease: evidence from recent studies. *Current Atherosclerosis Reports* 1, 185–187.

Lahoz, C., Alonso, R., Ordovas, J.M., Lopez-Farre, A., de Oya, M. and Mata, P. (1997) Effects of dietary fat saturation on eicosanoid production, platelet aggregation and blood pressure. *European Journal of Clinical Investigation* 27, 780–787.

Lale, A. and Herbert, J.M. (1994) Polyunsaturated fatty acids reduce pyrogen-induced tissue factor expression in human monocytes. *Biochemistry and Pharmacology* 48, 429–431.

Larsen, L.F., Jespersen, J. and Marckmann, P. (1999) Are olive oil diets antithrombotic? Diets enriched with olive, rapeseed, or sunflower oil affect postprandial factor VII differently. *American Journal of Clinical Nutrition* 70, 976–982.

Lerner, R., Lindstrom, P., Berg, A., Johansson, E., Rosendahl, K. and Palmblad, J. (1995) Development and characterization of essential fatty acid deficiency in human endothelial cells in culture. *Proceedings of the Natural Academy of Sciences of USA* 92, 1147–1151.

Libby, P. (2002) Inflammation in atherosclerosis. *Nature*, 420, 868–874.

Lindahl, A.K. (1994) Tissue factor pathway inhibitor: a potent inhibitor of in-vitro coagulation and in-vivo thrombus formation. *Current Opinion in Lipidology* 5, 434–439.

Lopez-Segura, F., Velasco, F., Lopez-Miranda, J., Castro, P., Lopez-Pedrera, R., Blanco, A., Jimenez-Pereperez, J., Torres, A., Trujillo, J., Ordovas, J.M. and Perez-Jimenez, F. (1996) Monounsaturated fatty acid-enriched diet decreases plasma plasminogen activator inhibitor type 1. *Arteriosclerosis, Thrombosis and Vascular Biology* 16, 82–88.

Lutgens, E., van Suylen, R.J., Faber, B.C., Gijbels, M.J., Eurlings, P.M., Bijnens, A.P., Cleutjens, K.B., Heeneman, S. and Daemen, M.J. (2003) Atherosclerotic plaque rupture: local or systemic process? *Arteriosclerosis, Thrombosis and Vascular Biology* 23, 2123–2130.

Marckmann, P., Jespersen, J., Leth, T. and Sandstrom, B. (1991) Effect of fish diet versus meat diet on blood lipids, coagulation and fibrinolysis in healthy young men. *Journal of Intern Medicine* 229, 317–323.

Marckmann, P., Sandstrom, B. and Jespersen, J. (1993) Favorable long-term effect of a low-fat/high-fiber diet on human blood coagulation and fibrinolysis. *Arteriosclerosis and Thrombosis* 13, 505–511.

Marckmann, P., Bladbjerg, E.M. and Jespersen, J. (1998) Diet and blood coagulation factor VII – a key protein in arterial thrombosis. *European Journal of Clinical Nutrition* 52, 75–84.

Meade, T.W., Ruddock, V., Stirling, Y., Chakrabarti, R. and Miller, G.J. (1993) Fibrinolytic activity, clotting factors, and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study. *Lancet*, 342, 1076–1079.

Mennen, L.I., Schouten, E.G., Grobbee, D.E. and Kluft, C. (1996) Coagulation factor VII, dietary fat and blood lipids: a review. *Thrombosis and Haemostasis* 76, 492–499.

Mennen, L., de Maat, M., Meijer, G., Zock, P., Grobbee, D., Kok, F., Kluft, C. and Schouten, E. (1998) Factor VIIa response to a fat-rich meal does not depend on fatty acid composition: a randomized controlled trial. *Arteriosclerosis, Thrombosis and Vascular Biology* 18, 599–603.

Mustad, V.A. and Kris-Etherton, P.M. (2000) Beyond cholesterol lowering: deciphering the benefits of dietary intervention on cardiovascular diseases. *Current Atherosclerosis Reports* 2, 461–466.

Navarro, M.D., Hortelano, P., Periago, J.L. and Pita, M.L. (1992) Effect of dietary olive and sunflower oils on the lipid composition of the aorta and platelets and on blood eicosanoids in rats. *Arteriosclerosis and Thrombosis* 12, 830–835.

Niskanen, L., Schwab, U.S., Sarkkinen, E.S., Krusius, T., Vahtera, E. and Uusitupa, M.I. (1997) Effects of dietary fat modification on fibrinogen, factor VII, and plasminogen activator inhibitor-1 activity in subjects with impaired glucose tolerance. *Metabolism* 46, 666–672.

Oakley, F.R., Sanders, T.A. and Miller, G.J. (1998) Postprandial effects of an oleic acid-rich oil compared with butter on clotting factor VII and fibrinolysis in healthy men. *American Journal of Clinical Nutrition* 68, 1202–1207.

O'Brien, J.R., Etherington, M.D. and Jamieson, S. (1976) Effect of a diet of polyunsaturated fats on some platelet-function tests. *Lancet* 2, 995–996.

Oubina, P., Sanchez-Muniz, F.J., Rodenas, S. and Cuesta, C. (2001) Eicosanoid production, thrombogenic ratio, and serum and LDL peroxides in normo- and hypercholesterolaemic post-menopausal women consuming two oleic acid-rich diets with different content of minor components. *British Journal of Nutrition* 85, 41–47.

Perez-Jimenez, F., Castro, P., Lopez-Miranda, J., Paz-Rojas, E., Blanco, A., Lopez-Segura, F., Velasco, F., Marin, C., Fuentes, F. and Ordovas, J.M. (1999) Circulating levels of endothelial function are modulated by dietary monounsaturated fat. *Atherosclerosis* 145, 351–358.

Rasmussen, O., Thomsen, C., Ingerslev, J. and Hermansen, K. (1994) Decrease in von Willebrand factor levels after a high-monounsaturated-fat diet in non-insulin-dependent diabetic subjects. *Metabolism* 43, 1406–1409.

Roque, M., Reis, E.D., Fuster, V., Padurean, A., Fallon, J.T., Taubman, M.B., Chesebro, J.H. and Badimon, J.J. (2000) Inhibition of tissue factor reduces thrombus formation and intimal hyperplasia after porcine coronary angioplasty. *Journal of the American College of Cardiology* 36, 2303–2310.

Ross, R. (1999) Atherosclerosis – an inflammatory disease. *New England Journal of Medicine* 340, 115–126.

Ruf, J.C. (1999) Wine and polyphenols related to platelet aggregation and atherothrombosis. *Drugs Experimental Clinical Research* 25, 125–131.

Sambola, A., Fuster, V. and Badimon, J.J. (2003a) Role of coronary risk factors in blood thrombogenicity and acute coronary syndromes. *Revista Española de Cardiología*, 56, 1001–1009.

Sambola, A., Osende, J., Hathcock, J., Degen, M., Nemerson, Y., Fuster, V., Crandall, J. and Badimon, J.J. (2003b) Role of risk factors in

the modulation of tissue factor activity and blood thrombogenicity. *Circulation* 107, 973–977.

Sanders, T.A. (1996) Effects of unsaturated fatty acids on blood clotting and fibrinolysis. *Current Opinion in Lipidology* 7, 20–23.

Sanders, T.A., de Grassi, T., Miller, G.J. and Humphries, S.E. (1999) Dietary oleic and palmitic acids and postprandial factor VII in middle-aged men heterozygous and homozygous for factor VII R353Q polymorphism. *American Journal of Clinical Nutrition* 69, 220–225.

Schmidt, E.B., Varming, K., Ernst, E., Madsen, P. and Dyerberg, J. (1990) Dose-response studies on the effect of n-3 polyunsaturated fatty acids on lipids and haemostasis. *Thrombosis and Haemostasis* 63, 1–5.

Silva, K.D., Kelly, C.N., Jones, A.E., Smith, R.D., Wootton, S.A., Miller, G.J. and Williams, C.M. (2003) Chylomicron particle size and number, factor VII activation and dietary monounsaturated fatty acids. *Atherosclerosis* 166, 73–84.

Sirtori, C.R., Tremoli, E., Gatti, E., Montanari, G., Sirtori, M., Colli, S., Gianfranceschi, G., Maderna, P., Dentone, C.Z. and Testolin, G. (1986) Controlled evaluation of fat intake in the Mediterranean diet: comparative activities of olive oil and corn oil on plasma lipids and platelets in high-risk patients. *American Journal of Clinical Nutrition* 44, 635–642.

Smith, R.D., Kelly, C.N., Fielding, B.A., Hauton, D., Silva, K.D., Nydahl, M.C., Miller, G.J. and Williams, C.M. (2003) Long-term monounsaturated fatty acid diets reduce platelet aggregation in healthy young subjects. *British Journal of Nutrition* 90, 597–606.

Soejima, H., Ogawa, H., Yasue, H., Kaikita, K., Nishiyama, K., Misumi, K., Takazoe, K., Miyao, Y., Yoshimura, M., Kugiyama, K., Nakamura, S., Tsuji, I. and Kumeda, K. (1999) Heightened tissue factor associated with tissue factor pathway inhibitor and prognosis in patients with unstable angina. *Circulation* 99, 2908–2913.

Temme, E.H., Mensink, R.P. and Hornstra, G. (1999) Effects of diets enriched in lauric, palmitic or oleic acids on blood coagulation and fibrinolysis. *Thrombosis and Haemostasis* 81, 259–263.

Thogersen, A.M., Jansson, J.H., Boman, K., Nilsson, T.K., Weinehall, L., Huhtasaari, F. and Hallmans, G. (1998) High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. *Circulation* 98, 2241–2247.

Thompson, S.G., Kienast, J., Pyke, S.D., Haverkate, F. and van de Loo, J.C. (1995) Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. *New England Journal of Medicine* 332, 635–641.

Togna, G.I., Togna, A.R., Franconi, M., Marra, C. and Guiso, M. (2003) Olive oil isochromans inhibit human platelet reactivity. *Journal of Nutrition* 133, 2532–2536.

Toschi, V., Gallo, R., Lettino, M., Fallon, J.T., Gertz, S.D., Fernandez-Ortiz, A., Chesebro, J.H., Badimon, L., Nemerson, Y., Fuster, V. and Badimon, J.J. (1997) Tissue factor modulates the thrombogenicity of human atherosclerotic plaques. *Circulation* 95, 594–599.

Tremoli, E., Eligini, S., Colli, S., Maderna, P., Marangoni, F., Angeli, M.T., Sirtori, C.R. and Galli, C. (1994) Effects of omega 3 fatty acid ethyl esters on monocyte tissue factor expression. *World Reviews in Nutrition and Diet* 76, 55–59.

Turpeinen, A.M. and Mutanen, M. (1999) Similar effects of diets high in oleic or linoleic acids on coagulation and fibrinolytic factors in healthy humans. *Nutrition Metabolism and Cardiovascular Disease* 9, 65–72.

Vognild, E., Elvevoll, E.O., Brox, J., Olsen, R.L., Barstad, H., Aursand, M. and Osterud, B. (1998) Effects of dietary marine oils and olive oil on fatty acid composition, platelet membrane fluidity, platelet responses, and serum lipids in healthy humans. *Lipids* 33, 427–436.

Wagner, D.D. and Burger, P.C. (2003) Platelets in inflammation and thrombosis. *Arteriosclerosis, Thrombosis and Vascular Biology* 23, 2131–2137.

11 Monounsaturated Fat in the Pathogenesis and Treatment of Diabetes

JOHN WRIGHT

School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey, UK

1. Introduction

1.1. Prevalence and pathogenesis of diabetes

The prevalence of diabetes mellitus throughout the world is increasing alarmingly. An estimated 30 million people worldwide had diabetes in 1985. The most recent WHO estimate is that the global prevalence is now around 177 million, and that, if the current rate of increase continues, this will more than double by 2030 (World Health Organisation, 2005). The increase is largely occurring in those parts of the developing world that are rapidly adopting 'western' lifestyles, as well as in the developed world where the increase in diabetes parallels the increase in overweight and obesity. The vast majority of the increase is in type 2 diabetes which is approximately ten times more common than type 1, although there is also an unexplained increase in the incidence of type 1 diabetes in Europe and North America.

In terms of their pathogenesis, types 1 and 2 diabetes are very different. In type 1 diabetes there is partial or (more usually) complete loss of insulin secretion due to B-cell destruction resulting, for the most part, from an auto-immune process. Type 2 diabetes results from a combination of an insulin secretory defect and peripheral insulin resistance. There is a strong genetic component in the both types 1 and 2 diabetes. Inheritance of an insulin secretory defect in the B-cell may be the more important genetic factor in type 2 diabetes, but it is believed that lifestyle factors – most importantly physical inactivity and obesity – which lead to the development of insulin resistance are largely responsible for the dramatic increase in the prevalence of type 2 diabetes. Around 70–80% of people with type 2 diabetes are overweight. The relationship between weight and the risk of diabetes is continuous: for each 1 kg increase in weight in the population, the risk of diabetes increases by 4.5% (Ford *et al.*, 2002). The risk of diabetes is particularly associated with abdominal or central obesity (Vague, 1947; Björntorp, 1992; Després, 2001).

 Olive Oil and Health (eds J.L. Quiles, M.C. Ramírez-Tortosa and P. Yaqoob)

In the majority of patients with type 2 diabetes, the condition occurs as part of the metabolic syndrome. The features of the syndrome, first described by Reaven (1988), and subsequently termed Syndrome X, the Insulin Resistance Syndrome and, more recently, the metabolic (or plurimetabolic) syndrome, are outlined in Table 11.1.

The prevalence of the metabolic syndrome is unknown but circumstantial evidence suggests that perhaps 20–30% of Caucasians and probably more than 50% of some Asian populations may be genetically at risk and thus programmed to develop the clinical syndrome if weight is gained. In people with normal glucose tolerance, the prevalence (according WHO criteria) is between 10 and 15%, in people with impaired glucose tolerance around 50%, and in people with diabetes at least 80% (Isomaa *et al.*, 2001). By the time diabetes develops, other features of the metabolic syndrome may have been present for many years. There is now good evidence that progression to diabetes can be modified by lifestyle changes, particularly diet and exercise (Tuomilehto *et al.*, 2001; Knowler *et al.*, 2002).

1.2. Complications of diabetes

The major objective in the treatment of diabetes is to reduce the long-term complications of the condition, notably microvascular disease (retinopathy and nephropathy), macrovascular disease (coronary heart disease (CHD), peripheral vascular disease and cerebrovascular disease) and neuropathy. Micro- and macrovascular complications differ both in their natural history and pathogenesis. The lesions of diabetic retinopathy and nephropathy are peculiar to diabetes, and are not seen in the absence of diabetes or impaired glucose tolerance. On the other hand, the atherosclerotic macrovascular disease in diabetes is identical to

Table 11.1. Features of the metabolic syndrome.

Impaired insulin-stimulated glucose uptake (insulin resistance)
Hyperinsulinaemia*
Central (truncal) obesity*
Glucose intolerance/type 2 diabetes*
Dyslipidaemia*
• hypertriglyceridaemia
• decreased high-density lipoprotein (HDL)
• increased small dense low density lipoprotein (LDL) particles
• enhanced postprandial hypertriglyceridaemia
Hypertension*
Hypercoagulable state (increased PAI-1 and factor VII)*
Hyperuricaemia*
Microalbuminuria*
Increased markers of inflammation (C-reactive protein (CRP))*
Polycystic ovary syndrome

* Risk factor for cardiovascular disease.

that seen in the non-diabetic population, but in diabetes it develops at an earlier age and is more extensive.

While neuropathy and microvascular disease are major causes of morbidity, macrovascular disease (particularly CHD) is the greatest concern and accounts for around 50% of deaths in people with diabetes. The risk of developing microvascular complications is related to the duration of diabetes and to blood glucose levels. Good glycaemic control has been convincingly shown to reduce the progression of microvascular disease in both type 1 and type 2 diabetes (DCCT, 1993; UKPDS, 1998) but it has far less impact on the risk of macrovascular disease.

It is clear that the increased risk of cardiovascular disease in diabetes is, in large part, related to factors other than glycaemic control. As shown in Table 11.1, many of the features of the metabolic syndrome are risk factors for cardiovascular disease. CHD mortality is increased 3-fold in men with diabetes or metabolic syndrome and up to 6-fold in women with diabetes and metabolic syndrome (Stamler *et al.*, 1993; Isoma *et al.*, 2001), and results of the Quebec Cardiovascular Study have shown that the cluster of metabolic disturbances observed among subjects with the visceral obesity characteristic of the metabolic syndrome (hyperinsulinaemia, hyperapolipoprotein B and small, dense low-density lipoprotein (LDL) particles) was associated with a 20-fold increase in the risk of coronary heart disease in a sample of middle-aged men followed over 5 years (Després, 2001). Pharmacological treatment, in particular of hypertension and dyslipidaemia, has been shown to have a major impact on cardiovascular risk. Dietary management is similarly as important in reducing cardiovascular risk as in improving glycaemic control. In both of these respects, high-MUFA diets have been shown to have beneficial effects.

1.3. Dietary management of diabetes

As shown in Table 11.2, there has been a steady evolution in dietary advice over the past 80 years. The perception that high-carbohydrate diets might not be as

Table 11.2. Dietary advice on diabetes managements through time.

	Distribution of calories (% dietary energy)		
Year	Protein	CHO	Fat
Pre 1921	Starvation diets		
1921	10	20	70
1950	20	40	40
1971	20	45	35
1986	12–20	Up to 60	Less than 30
1994	10–20	Based on individual assessment and goals*	
1998–2003	10–20	45–60 CHO and MUFA together 60–70%	10–20 *

* Less than 10% as saturated fat. References for recent (1998–2003) recommendations: Ha and Lean, 1998; Diabetes and Nutrition Study Group of the EASD, 2000; Franz *et al.*, 2002.

adverse as initially thought (provided that refined carbohydrate was limited), along with the increasing perception that fat in general (and saturated fat in particular) increased cardiovascular risk, led to the encouragement of a high-carbohydrate (55% to 60% of energy), low fat (total fat less than 30%, saturated fat less than 10% of energy) diet, which was the mainstay of nutritional treatment for diabetes for many years (American Diabetes Association, 1998).

A number of factors have led to a reassessment of this policy. First, it has been known for many years that high-carbohydrate diets result in an increase in plasma triglyceride in both normal subjects and particularly in type 2 diabetes (Coulston *et al.*, 1987, 1989). This is due both to increased hepatic production of large, buoyant triglyceride-rich VLDL particles ($VLDL_1$), and reduced clearance of triglyceride from plasma; the underlying mechanisms have been extensively reviewed (Blades and Garg, 1995; Frayn and Kingman, 1995; Parks and Hellerstein, 2000). VLDL and chylomicrons share a common metabolic pathway; both are hydrolysed by lipoprotein lipase (LPL), predominantly in adipose tissue. In the presence of an excess of VLDL, there is a reduction in the hydrolysis and removal of chylomicron triglyceride as a result of competitive inhibition, leading to prolonged and enhanced postprandial hypertriglyceridaemia. Secondly, although it was hotly debated for many years, it is now accepted that hypertriglyceridaemia is a significant cardiovascular risk factor, independent of HDL-cholesterol, particularly in women (Holkanson and Austin, 1996). In terms of mechanisms, it has become clear that hypertriglyceridaemia is the factor which drives the development of the other features of the 'atherogenic lipoprotein phenotype' – the characteristic dyslipidaemia of the metabolic syndrome: in the presence of moderately elevated triglyceride levels (above 1.5 mmol/l) neutral lipid exchange between triglyceride-rich lipoproteins (of both intestinal and hepatic origin) and LDL mediated by the enzyme cholesteryl ester transfer protein (CETP) is enhanced, resulting in the generation of triglyceride-rich LDL particles which subsequently undergo delipidation by hepatic lipase with the production of increasingly small, dense LDL particles (Caslake *et al.*, 1992; Griffin *et al.*, 1994). These possess a number of characteristics that render them highly atherogenic: they penetrate more easily into the subendothelial space, where they are trapped by the glycoprotein matrix; they are more prone than larger particles to lipid oxidation; and they are taken up via the macrophage scavenger receptor, leading to the development of foam cells and, ultimately, fatty streaks.

One of the perceived benefits of high-carbohydrate diets was a lowering of plasma total cholesterol concentration. However, this is, in part at least, due to fall in HDL-C (Levy *et al.*, 1966); meta-analysis by Mensink and Katan (1992) showed that for every 1% of energy from carbohydrate as a replacement for saturated fat, there was a 0.012 mmol/l fall in HDL-C.

There are two alternative approaches to traditional high-carbohydrate diets: replacement of carbohydrate with fat – either polyunsaturated (PUFA) or monounsaturated; or replacement of high glycaemic index (GI) carbohydrate with low GI carbohydrate. The latter approach has gained favour recently, and has been shown to have beneficial effects on both glycaemic control and plasma lipids (Frost *et al.*, 1999) but will not be discussed further. As far as substitution of car-

bohydrate with PUFA or MUFA is concerned, they have been show n to have similar effects on lipoprotein levels (Mensink and Katan, 1989; Gardner and Kraemer, 1995) but in view of the risk of lipid peroxidation associated with high-PUFA (predominantly n-6 PUFA) diets, they are not recommended. Current European, American and British guidelines for the dietary management of diabetes are that carbohydrate and MUFA should together provide 60–70% of dietary energy with MUFA providing up to 20% (Diabetes and Nutrition Study Group of the European Association for the Study of Diabetes, 2000; Franz *et al.*, 2002; Nutrition Subcommittee of the Diabetes Care Advisory Committee of Diabetes UK, 2003).

These recommendations derive from the evidence for the potential beneficial effects of MUFA on numerous aspects of diabetes and the metabolic syndrome which have accumulated in recent years. These include:

- Effects on carbohydrate metabolism: improving insulin sensitivity, reducing dietary glycaemic load, improving glycaemic control, thereby reducing the incidence of diabetes and reducing the risk of microvascular (and possibly macrovascular) complications.
- Effects on lipid metabolism: improving plasma lipid and lipoprotein levels (particularly triglyceride), thereby reducing the risk of macrovascular complications.
- Effects on hypercoagulable state.
- Effects on blood pressure.
- Effects on inflammatory markers.

Evidence for the last three of these is limited. There are some encouraging data from a few studies, the majority in normal subjects (decreased PAI-1: Lopez-Segura *et al.*, 1996; reduced systolic and diastolic blood pressure, and reduced need for antihypertensive medication: Espino-Montoro *et al.*, 1996; Psaltopoulou *et al.*, 2004; Ferrara *et al.*, 2000; reduced CRP and interleukin 6: Esposito *et al.*, 2004; Baer, 2004) but there have been no specific studies in diabetes. Nevertheless, these are important components of the metabolic syndrome, and further studies are needed. These will not be discussed further in this chapter.

In addition, there is the potential for influencing the risk of the development of metabolic syndrome and the progression to diabetes in the population at large by modification of the proportion of different fatty acids, in particular MUFA, in the background diet.

2. The Effect of Dietary MUFA on Insulin Sensitivity and Carbohydrate Metabolism and the Risk of Diabetes

2.1. Observational studies

The effects of any dietary component on the risk of diabetes may be mediated indirectly via an effect on weight or via a direct effect on insulin sensitivity and carbohydrate metabolism or both. There is no evidence that any dietary factor has an impact on the insulin secretory defect, which is an important (albeit poorly understood) feature of type 2 diabetes (Gerich and Van Haeften, 1998).

Along with physical inactivity, overweight is the major modifiable risk factor for type 2 diabetes. Total energy consumption is doubtless the most important dietary determinant of weight gain but there is also evidence that individual nutrients may have specific effects on insulin secretion, insulin sensitivity, carbohydrate metabolism and, ultimately, the risk of type 2 diabetes (for review, see Parillo and Riccardi, 2004).

As far as dietary fatty acids are concerned, the most consistent effect is for saturated fat. In cross-sectional studies, saturated fatty acid intake has been shown to correlate with both fasting plasma glucose in men (Feskens and Kromhout, 1990), and both fasting and postprandial insulin levels in men[1] (Parker *et al.*, 1993; Feskens *et al.*, 1994; Storlien *et al.*, 1996; Vessby, 2000) and with risk of diabetes (van Dam *et al.*, 2002). Most prospective studies have shown a similar relationship between saturated fatty acid intake and fasting insulin concentration (Marshall *et al.*, 1997), IGT and diabetes (Feskens *et al.*, 1995), the exception being an exclusively female population in which there was no association (Salmerón *et al.*, 2001). Conversely, a high polyunsaturated fat intake has been shown to be associated with lower insulin levels (Feskens *et al.*, 1994), and, in prospective studies, with a reduction in the risk of diabetes (Salmerón *et al.*, 2001; Harding *et al.*, 2004).

Some studies have shown an inverse relationship between PUFA intake and insulin levels (Feskens *et al.*, 1994) or risk of diabetes (Harding *et al.*, 2004), but despite the potential beneficial effect of dietary MUFA, none was seen in these studies. Indeed, in a 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study (Feskens *et al.*, 1995), there was a positive relationship between MUFA intake and progression to carbohydrate intolerance. However, this relationship was no longer significant when adjusted for confounders, and the authors suggest that this may reflect the fact that MUFA intake in Northern Europe is largely from sources such as meat and milk products rather than olive oil, and therefore closely related with SFA intake.

Epidemiologically (in Europe at least), there appears to be no relationship between background dietary MUFA intake and prevalence of diabetes. According to WHO figures, for example, the prevalence of diabetes in Spain and Greece (where the dietary intake of MUFA is around 17% dietary energy) is around 7%, compared with around 3% in the UK and Germany where dietary MUFA intakes are around 11% and 9% respectively. This is hardly unexpected: diabetes is a complex disease of multifactorial origin, and it would be surprising if there were a simple relationship between a single nutritional factor and prevalence. The reason for the higher rate of diabetes in Southern Europe is unclear, particularly since it does not appear to be simply related to higher rates of overweight and obesity.

[1]A number of methods are used for the measurement of insulin sensitivity. In large population studies, methods such as the euglycaemic clamp or the intravenous glucose tolerance test are clearly impractical. Hyperinsulinaemia occurs as a consequence of insulin resistance, and elevated fasting and postprandial plasma insulin levels are acceptable markers of insulin sensitivity/resistance.

2.2. Prospective and intervention studies

In the face of a high total fat intake, a relatively high MUFA intake has no effect (and may have an adverse effect) on the conversion from impaired glucose tolerance (IGT) to type 2 diabetes. In a prospective study of 123 subjects with IGT, Marshall *et al.* (1994) found that total fat intake at baseline predicted both reversion to normal glucose tolerance and progression to type 2 diabetes over a 3-year period. In those subjects who reverted to normal glucose tolerance, dietary fat intake as a proportion of total dietary energy was 38.9% (of which 14.9% MUFA), in those who remained glucose intolerant 40.6% (of which 16.3% MUFA), and in those who converted to diabetes 43.9% (of which 17.1% MUFA). There was no significant difference between the three groups in saturated fat intake (14.0%, 14.6% and 16.1% of dietary energy respectively). The differences in carbohydrate intake in the three groups (45.1%, 43.5% and 40.5% respectively) were not significant, and therefore not thought to be an explanation for the differences in progression to diabetes.

In contrast, the KANWU study (Vessby *et al.*, 2001) showed a beneficial effect of MUFA compared with saturated fat on insulin sensitivity, but confirmed that this effect was lost when total fat intake was very high. One hundred and sixty-two healthy subjects (86 men and 76 women aged 30–65 years) were randomly allocated to a 3-month period on one of two high-fat diets calculated to contain 37% dietary energy as fat, one high-saturated fat (F37, S17, M14, P6), and the other high-MUFA (F37, S8, M23, P6).[2] (Actual intakes of SFA were slightly higher and those of MUFA slightly lower than target values on the two diets.) At the end of the dietary intervention, there was an overall significant reduction in insulin sensitivity relative to the background diet on the high-SFA diet compared with the high-MUFA diet (−10% and +2% respectively). However, this was seen only in those subjects in whom actual fat intake during the intervention was less than 37% total energy (mean 33.9%); in these subjects there was 12.5% reduction and an 8.8% increase in insulin sensitivity on the high-SFA and high-MUFA diets respectively. When actual total fat intake was higher (mean 40.2%), there was no significant difference in the change in insulin sensitivity on the two diets (−7.8% and −3.3% on the high-SFA and high-MUFA diets respectively). In addition, there was an increase in LDL-cholesterol on the high-SFA diet and a decrease on the high-MUFA diet, and an unexplained 12% increase in lipoprotein (a) on the high-MUFA diet. This study is important partly because of the rigorous methodology used to measure insulin sensitivity (the frequently-sampled intravenous glucose tolerance, Steil *et al.*, 1993), and partly because it clearly demonstrates the benefits in terms of insulin sensitivity of what would normally be considered high intakes of both total and monounsaturated fat (mean actual intake on the high-MUFA diet, 37.1% and 21.2% respectively).

[2]A similar format is used throughout to describe details of diets in quoted studies. For example, a diet in which 40% of dietary energy is given as fat, of which 20% of dietary energy as MUFA, 10% as saturated fat and 10% as polyunsaturated fat, and 45% dietary energy as carbohydrate is represented as: F40, M20, S10, P10, C45. In all cases, the percentages are rounded up or down to whole numbers.

In a short-term (4-week) cross-over study in healthy young people (n = 59), Pérez-Jiménez *et al.* (2001) confirmed that substitution of MUFA (F38, M22, S10, P6, C47) for saturated fat (F38, M12, S20, P6, C47) results in an improvement in insulin sensitivity as measured by a modified insulin suppression test. However, the fact that a similar improvement in insulin sensitivity was also seen after a high-carbohydrate diet (F28, M12, S10, P6, C57) indicates that the major effect on insulin sensitivity is due to the reduction in saturated fat. Similarly, Thomsen *et al.* (1999) showed in a group of 16 first-degree relatives of people with type 2 diabetes, that substitution of either a high-MUFA diet (F42, M24, S9, P6, C41) or a high-carbohydrate diet (F28, M8, S9, P7, C53) for a relatively high-saturated fat diet (F36, M10, S14, P5, C45) for 4 weeks resulted in similar levels of insulin sensitivity as assessed by the frequently-sampled intravenous glucose tolerance test (Bergman, 1989). Unfortunately, this study is weakened by the fact that no comparison was made with the baseline diet.

3. The Effect of Dietary MUFA on Body Weight and Fat Distribution

Along with physical inactivity, overweight is the major modifiable risk factor for type 2 diabetes. Around 70–80% of people with type 2 diabetes are overweight. The relationship between weight and the risk of diabetes is continuous: in a USA population, for example, it was found that for each 1 kg increase in weight over 10 years, the risk of diabetes increases by 4.5% (Ford *et al.*, 1997). The risk of diabetes is particularly associated with abdominal or central obesity (Vague, 1947; Björntorp, 1992; Després, 2001).

Control of weight is central to both the prevention and management of diabetes. One of the concerns about high-MUFA diets is that they may promote weight gain, and this has been, in part at least, responsible for the resistance to these diets in the diabetic community. There are obvious reasons why, as with any high-fat diet, this may be the case if energy intake is not restricted: fat is the least satiating macronutrient, more energy dense than carbohydrate, and the energy costs of converting dietary carbohydrate into adipose tissue are far greater than those of converting dietary fat. In general, however, there is little evidence that dietary fat is a specific determinant of obesity (Seidell, 1998; Willett and Leibel, 2002). Nevertheless, on *ad libitum* diets when energy intake is not controlled, subjects on higher-fat diets tend to overeat and gain more weight (for review see Shah and Garg, 1996). Similarly, in subjects on *ad libitum* diets, advice simply to reduce dietary fat without intentional restriction of energy intake results in weight loss (Astrup *et al.*, 2000). However, there is evidence that, in general, similar weight loss can be achieved with either low-fat or low-carbohydrate energy-restricted diets (Golay *et al.*, 1996) although when high-MUFA diets are compared with other, isoenergetic diets designed either to increase, maintain or reduce weight, there is some evidence that this may not be the case. Gerhard *et al.* (2004) gave either a low-fat, high-carbohydrate diet or a high-MUFA diet to 11 patients with type 2 diabetes for a period of 6 weeks. Patients were offered up to 125% of estimated energy requirement on both diets. There was a significant decrease in body weight (1.53 kg) only with the low-fat diet. There was a similar slight decrease in plasma total, LDL- and HDL-cholesterol concentrations on both diets.

There are relatively few direct comparisons of isocaloric high-carbohydrate and high-MUFA diets. Those that there are indicate that it is energy intake rather than macronutrient composition which is most important. A number of studies have compared high-carbohydrate with high-MUFA weight-reducing diets in both non-diabetic and diabetic subjects. In studies by Zambon *et al.* (1999) (in non-diabetic obese subjects) and Heilbronn *et al.* (1999) (in obese subjects with type 2 diabetes) there was no significant difference in weight loss in subjects treated with energy-matched hypocaloric high-carbohydrate or high-MUFA diets. In an interesting study, Low *et al.* (1996) compared the effects of a very high-MUFA (F70, M49, P13, C10) and a very high-carbohydrate (F10, M1, P8, C70), calorie restricted diet for six weeks in a group of obese patients with type 2 diabetes. At the end of the diet period, patients were kept on the formula diets for a further 4 weeks during which energy intake was titrated upwards to maintain weight (refeeding period). Weight loss was similar on the two diets (mean 7.8 kg) but fasting glucose and plasma triglycerides were lower in the high-MUFA group at the end of the 6-week diet period. Other lipid parameters were similar in the two groups. Twenty-four-hour glucose, insulin and C-peptide profiles were measured at the end of the diet and refeeding periods, and an oral glucose tolerance test was performed. Twenty-four-hour glucose levels were lower in the high-MUFA group at the end of the diet period and were similarly low after the refeeding period whereas in the high-carbohydrate group, the 24-hour glucose profile had risen back to pre-diet levels. The glucose response to the OGTT was similar in both groups; the insulin response appeared slightly higher in the high-MUFA group but this difference was not significant, but the C-peptide response was significantly higher. These results indicate that providing energy as MUFA allows a reduction in glycaemic load which, after a relatively short period, may result in a sustained improvement in insulin secretion.

In view of the importance of central adiposity as a risk factor for the development of insulin resistance and diabetes, the possibility that high-MUFA diets may influence weight distribution is of particular interest. Walker *et al.* (1996, 1999) compared the effect of high-carbohydrate, low-fat and high-MUFA diets on body composition in type 2 diabetes in two studies, one weight-stable (Walker *et al.*, 1996) and the other weight-reducing (Walker *et al.*, 1999). Although changes were small, there was evidence of preferential loss of upper body fat on the high-MUFA diet in both studies. However, Clifton *et al.* (2004) failed to confirm these findings in a study comparing similar (low-fat, high-carbohydrate *vs* high-MUFA) diets in a group of overweight but non-diabetic women, although they did find a relative preservation of lean body mass in postmenopausal women on the high-MUFA diet. All of these studies were of relatively short duration (12 weeks), and longer-term studies are clearly needed.

4. The Effect of Dietary MUFA on Lipid Abnormalities in Diabetes

4.1. Dietary intervention studies

In type 1 diabetes there is no characteristic dyslipidaemia but in type 2 diabetes and in the metabolic syndrome there is a characteristic dyslipidaemia (the

atherogenic lipoprotein phenotype, ALP), which comprises hypertriglyceridaemia, low HDL-cholesterol, a high proportion of small dense LDL particles and enhanced postprandial hypertriglyceridaemia. In view of the importance of hypertriglyceridaemia, the possibility of a reduction by dietary means is clearly important and there is increasing evidence that high-MUFA diets can help to achieve this.

Early studies in healthy, non-diabetic subjects showed that a high MUFA diet (F41, M24, C46) given for 36 days resulted in lower triglyceride and higher HDL levels than a high-complex carbohydrate diet (F22, M9, C63) (Mensinck and Katan, 1987). A number of subsequent studies have shown similar effects in type 2 diabetes. Nine studies comparing high-carbohydrate with high-MUFA diets in type 2 diabetes (Garg *et al.*, 1988, 1992b, 1994; Rivellese *et al.*, 1990; Parillo *et al.*, 1992, 1996; Rasmussen *et al.*, 1993; Campbell *et al.*, 1994; Lerman-Garber *et al.*, 1994) were subjected to a meta-analysis (Garg, 1998); the findings of these studies will be summarized here. All studies included in the meta-analysis were randomized, cross-over trials using iso-energetic, weight-maintaining diets. There was considerable variation in the proportion of fat and carbohydrate content of the diets as shown in Table 11.3. The majority (eight) of the studies used metabolic diets; two (Campbell *et al.*, 1994; Lerman-Garber *et al.*, 1994) used prescribed diets with specific recommendations for food items. A total of 133 patients were included in the nine studies.

Overall, there was no significant reduction in either total or LDL cholesterol, and only a small (0.05 mmol/l, 4%) increase in HDL-cholesterol. However, there was a marked reduction in both plasma triglyceride and VLDL (19% and 22.5% respectively). Very neatly, for each 1% substitution of MUFA for carbohydrate, there was a 0.1 mmol/l decrease in plasma triglyceride. The studies provided little information about specific apolipoproteins, although one study showed a significant (9%) increase in apo A-1 on a high-MUFA diet (Garg *et al.*, 1988).

A further three studies were excluded from Garg's meta-analysis because the order of the diets was not randomized (Bonanome *et al.*, 1991; Garg *et al.*, 1992a; Nielsen *et al.*, 1995). Otherwise, the fat and carbohydrate contents of the diets in these studies were similar to the included studies. Only the study by Garg *et al.* (1992a,b) showed any significant difference in lipid and lipoprotein levels with lower triglyceride and VLDL-cholesterol levels on the high-MUFA diet.

As pointed out by Vessby (1995), some caution is required in interpreting the results of metabolic ward studies. The high-carbohydrate diets used in most of these contained a relatively low proportion of dietary fibre and a high proportion of starch-rich, refined foods which tend to raise triglyceride levels in diabetes; this may have resulted in some exaggeration of the beneficial effects of the high-MUFA diets in these studies.

Since the publication of the above meta-analysis, a number of further studies of varied design have been published, and these have recently been reviewed (Ros, 2003). Only three of the six studies reviewed were in diabetic subjects. Luscombe *et al.* (1999) compared 4-week treatments with either a high-GI diet (F21, M7, C53), a low-GI diet (C51, F23, M7), or a high-MUFA, high-GI diet (F35, M17, C42) in a cross-over study in a group of 21 subjects with type 2

Table 11.3. Intervention studies comparing high-MUFA diets (HMD) and high-carbohydrate diets (HCD) in patients with type 2 diabetes.

				HCD					HMD					Results				
		Diets (see	Time on each diet	% energy as					% energy as									
Study	*n*	legend)	(wk)	Fat	M	P	S	C	Fat	M	P	S	C	TG*	VL-C*	HDL*	Gluc†	Wt†
Garg *et al.* (1988)	10	(a)	4	25	9	6	9	60	50	33	7	10	33	−25	−34	+11	↓	NSD
Rivellese *et al.* (1990)	8	(a)	2	20	NR	NR	NR	60	40	NR	NR	NR	40	−20	NR	NR	NR	NSD
Garg *et al.* (1992a,b)	8	(a)	3	25	12	5	8	60	50	32	7	11	35	−22	−22	+16	NSD	NSD
Parillo *et al.* (1992)	10	(a)	2	20	13	2	5	60	40	29	4	7	40	−15	NR	NSD	↓	NSD
Rasmussen *et al.* (1993)	15	(a)	3	32	11	7	11	49	50	30	7	10	36	NSD	NR	NSD	↓	NSD
Campbell *et al.* (1994)	10	(a)	2	24	9	7	8	52	38	21	8	8	40	−21	NR	NSD	↓	NSD
Lerman-Garber *et al.* (1994)	12	(a)	4	20	7	7	7	60	40	24	5	11	40	−22	NR	NSD	↓	NSD
Garg *et al.* (1994)	42	(a)	6	30	10	10	10	55	45	25	10	10	40	−20	−19	NSD	↓	NSD
Parillo *et al.* (1996)	9	(a)	2	20	13	2	5	60	40	29	4	7	40	−18	NR	NSD	±	NSD
Bonamone *et al.* (1991)	19	(a)	8	25	10	5	10	60	40	25	5	10	45	NSD	NSD	NSD	NSD	NSD
Garg *et al.* (1992a,b)	10	(aa)	4	20	11	6	3	65	45	31	10	5	38	−40	−42	NSD	↓	NSD
Neilsen *et al.* (1995)	10	(a)	3	30	9	8	10	48	50	30	7	10	34	NSD	NSD	NSD	NSD	NSD
Walker *et al.* (1996)	16	(a)	12	31	12	6	13	44	35	20	5	10	40	NR	NR	NR	NR	NSD
Heilbronn *et al.* (1999)	35	(b)	12	10	3	2	4	32	15	15	9	7	50	NSD	NSD	NSD	NSD	NSD
Walker *et al.* (1999)	21	(a)	12	22	9	4	9	52	33	18	5	10	43	NSD	NSD	NSD	NSD	NSD
Gumbiner *et al.* (1998)	9	(b)	6	10	1	8	1	70	70	49	13	8	10	−36	NR	NSD	↓	NSD
Rodríguez-Villar *et al.* (2000)	12	(a)	6	29	12	NR	6	54	40	25	NR	8	43	NSD	NSD	NSD	NSD	NSD
Clifton *et al.* (2004)	70	(b)	12	12	4	2	4	66	35	20	7	6	44	NSD	NR	↑	NSD	NSD
Gerhard *et al.* (2004)	11	(c)	6	21	8	6	4	65	40	25	6	6	45	NSD	NSD	↓	NSD	↑

Study diets: (a): isocaloric, weight-maintaining; (aa): as (a) but 'simple' carbohydrate (31% as glucose); (b): isocaloric, weight-reducing; (c): *ad libitum*, non-isocaloric. Fat: total fat; M: MUFA; P: PUFA; S: SFA; C: carbohydrate. * % reduction in HMD group compared with HCD group at end of study diets; comparison with baseline not shown. † Change in weight HMD group compared with HCD group at end of study diets; comparison with baseline not shown. TG: plasma triglyceride. VL-C: VLDL-cholesterol. HDL: HDL cholesterol. Gluc: Glucose – various parameters measured (fasting, postprandial, 24-hour profile). NSD: not significantly different. NR: not reported.

diabetes. The one significant finding was a lower HDL-cholesterol on the high-GI diet, indicating the similar effects of either a low-GI diet and a high-MUFA diet on HDL-cholesterol levels. In a study primarily designed to look at postprandial parameters (see below), Rodríguez-Villar *et al.* (2000), using a cross-over study design, compared the effect of 6-week treatment periods of a traditional high-carbohydrate diet (F30, M12, C55) with a low carbohydrate, high-fat, high-MUFA diet (F40, M25, C45) in 12 patients with good diabetic control (mean HbA_{1C} 6.4%). There were no significant differences in fasting glucose, insulin or lipid levels on the two diets (although there was a non-significant 32% reduction in VLDL-cholesterol). In a further study primarily designed to look at susceptibility of LDL to oxidative modification, Rodríguez-Villar *et al.* (2004) found no significant difference in the effects of low and high-fat diets (28% *vs* 40% of energy) on fasting lipid levels.

Lowering triglyceride levels should, in principle, result in a reduction in the proportion of small dense LDL particles in the circulation, and such effects have been shown in response to lipid-lowering drugs (particularly fibrates) in diabetes (Feher *et al.*, 1999). To date, no such effects have been demonstrated in response to high-MUFA diets.

The situation in type 1 diabetes is unclear. Georgopoulos *et al.* (2000) suggested that a high-MUFA diet might have disadvantages since, in addition to an apparently adverse effect on postprandial lipid metabolism (see below), they also found that VLDL particles were larger and therefore potentially more atherogenic following a high-MUFA (25% energy) compared with a high carbohydrate (61% energy) diet. These results are difficult to interpret in the absence of appropriate turnover studies. More reassuringly, Strychar *et al.* (2003) demonstrated significant improvements in the lipid profile of a group of subjects with type 1 diabetes following two months on a high-MUFA (C43–46, F37–40, M17–20) compared with a high-carbohydrate (C54–57, F 27–30, M10–13), with marked falls in plasma triglyceride (−18%), VLDL-triglyceride (−26%) and VLDL-cholesterol (−48%). Further studies are needed not only to clarify these findings but also to compare the palatability and acceptability of these diets in younger people with diabetes.

4.2. Postprandial lipid metabolism

4.2.1. Type 2 diabetes

Enhanced postprandial lipaemia is one of the key features of the dyslipidaemia of the metabolic syndrome and type 2 diabetes (Lewis *et al.*, 1991; Chen *et al.*, 1993). Similar changes are seen in a high proportion of patients with coronary heart disease, giving rise to the hypothesis that 'atherogenesis is a postprandial phenomenon' (Zilversmit, 1979). The mechanism underlying this is believed to be the remodelling which occurs in the presence of hypertriglyceridaemia of both HDL and LDL towards smaller, denser HDL_3 and LDL_3 particles which are, respectively, less protective and more atherogenic (Patsch *et al.*, 1987; Nikkila *et al.*, 1994).

Rasmussen *et al.* (1996) compared the effect of adding varying amounts of either butter (50 g and 100 g) or olive oil (40 g and 80 g) to a meal of mashed potato (300 g) in a group of patients with type 2 diabetes. The postprandial serum insulin response was increased, and the blood glucose response decreased by the addition of the fats, but only the changes seen with butter were statistically significant. The possible mechanisms include a delay in gastric emptying and/or an incretin effect since fat is the most potent dietary stimulus of GIP (glucose-dependent insulinotrophic polypeptide). The lack of an effect of MUFA in this study may be due to the lower amounts given compared with butter. However, there appeared to be a difference between the higher doses of butter and olive oil in terms of the postprandial clearance of triglyceride, with a fall in plasma levels of triglyceride from 120 minutes postprandially with olive oil compared with sustained hypertriglyceridaemia following butter.

Several cross-over studies have compared the effect of high-MUFA diets with high-carbohydrate diets on the postprandial metabolic profile in patients with type 2 diabetes (Campbell *et al.*, 1994; Chen *et al.*, 1995) or the response to a single test meal (Rodríguez-Villar *et al.*, 2000). Campbell *et al.* (1994) found lower glucose (plasma and urinary) levels following the high-MUFA diet, and lower fasting but not 6-hour triglyceride levels. Chen *et al.* (1995) found very similar glucose and insulin profiles, both lower in the first 10 or so hours of the day following the high-MUFA diet. Subjects also had consistently lower triglyceride levels throughout the 24-hour period following the high-MUFA diet. In addition, this study provided insights into the mechanisms involved: as expected from what is known about the effects of high-carbohydrate diets, VLDL production rate and pool size were increased following high carbohydrate intake. The more original finding of this study was that the fractional clearance rate of VLDL was increased on the high-fat, high-MUFA diet.

Rodríguez-Villar *et al.* (2000) provided some (albeit weak) evidence for an effect of MUFA on triglyceride clearance. Although they found no overall difference in postprandial lipid, lipoprotein, glucose or insulin levels following a test meal challenge, VLDL-cholesterol and total triglyceride levels were significantly lower in the late postprandial phase following the period on the high-MUFA diet.

The above studies raise the question of whether the observed effects are attributable to MUFA *per se* or whether they are due to a reduction in dietary carbohydrate, in which case MUFA and PUFA might be expected to have similar effects. This question has been addressed to some extent by two studies which have compared the effects of MUFA with those of PUFA. In a randomized cross-over study, Higashi *et al.* (2001) compared the effects of two low (25%) fat diets, one oleic acid-enriched (S5, M15, P5), and the other linoleic acid-enriched (S5, M5, P15), each given for three weeks to a group of eight patients with type 2 diabetes. There was a reduction in postprandial remnant-like particles (RLP) following the linoleic acid-enriched diet. However, RLP were measured using an apoB-100 antibody which may or may not have detected apoB-48-containing (intestinally-derived) particles. Furthermore, adjusted for differences in fasting levels, it is unlikely that the differences in RLP excursion would have been significantly different. In any event, the diets in this study remained high in carbohydrate (58% energy) which may well have dominated the metabolic response to

the meal challenge. Of more interest is the study by Madigan *et al.* (2000) who assessed the effects of a high-MUFA or high-PUFA diet (incorporating either 30 ml of olive oil or sunflower oil per day respectively) given for 2 weeks to a group of 11 patients with type 2 diabetes in a crossover design. At the end of each diet period subjects were given a test meal. Postprandial levels of both chylomicron and VLDL B48 and B100 were significantly lower following the high-MUFA diet, indicating better clearance of triglyceride-rich lipoproteins of both intestinal and hepatic origin.

4.2.2. Type 1 diabetes

There is a paucity of data on the effect of high-MUFA diets in type 1 diabetes, but some suggestion that the situation may be different. Georgopoulos *et al.* (1998) compared the responses to a test meal in a group of patients with type 1 diabetes (average HbA_{1C} 9.9%) following 4 weeks on either a high-fat, high-MUFA diet (F40, M25, C45) or a high-carbohydrate diet (F24, M9, C61) in a randomized cross-over design. There were marked differences with the findings in type 2 diabetes, with higher triglyceride levels, higher levels of both triglyceride and retinyl ester in chylomicrons and chylomicron remnants, and higher levels of small dense LDL particles on the high-fat, high-MUFA diet. These findings are similar in many respects to those in normal subjects on a high MUFA diet in whom triglyceride and apoB-48 levels which are higher in the early postprandial phase (presumably due to more efficient packaging into chylomicrons) fall rapidly in the late postprandial phase due to more efficient clearance from plasma (Roche *et al.*, 1998; Zampelas *et al.*, 1998).

5. Effects of MUFA on Composition and Function of Lipoproteins

In addition to quantitative changes, there is also evidence of qualitative changes in lipoproteins in diabetes. These include changes in the size profile of both LDL and HDL with a predominance of smaller, more dense particles, and both glycation and oxidation of lipoproteins.

5.1. Glycation and oxidation

Reduced clearance of lipoproteins in diabetes, and thus their increased residence time in plasma, increases their exposure to glucose and their susceptibility to nonenzymic glycation. By reducing triglyceride levels and thereby increasing the efficiency of lipoprotein clearance from plasma, MUFA has the potential to reduce the susceptibility of LDL particles both to glycation and subsequent oxidation, but direct evidence for this is lacking. Glycation *per se* may increase the atherogenic potential of LDL, possibly by enhancing uptake by macrophage scavenger receptors. Both glycation (Dimitriadis *et al.*, 1995, 1996) and small particle size combine to increase the susceptibility of LDL to oxidative modification, thereby further increasing its atherogenic potential (for review, see Reaven

and Witzum, 1996); small LDL particles are more prone to oxidation probably due to loss of protective antioxidants during intravascular delipidation and remodelling. However, the main determinant of susceptibility to lipid peroxidation is the fatty acid composition of the particle, those with a high PUFA content being most susceptible. There is good evidence (although not specifically in diabetes) that dietary substitution of MUFA for PUFA reduces the *in vitro* susceptibility of LDL to oxidative modification. Dimitriadis *et al.* (1995, 1996) showed that both LDL and HDL from diabetic subjects have a higher linoleic acid content and are more susceptible to oxidation compared with controls. After 4 weeks on a high MUFA diet (M12, P9, S16), there was a significant reduction in linoleic acid content and increase in oleic content in lipoprotein cholesteryl esters, and a concomitant reduction in susceptibility to oxidation. The effect of increasing dietary MUFA appears to be due to the reduction in dietary PUFA (and therefore, LDL PUFA content) since similar effects are seen on high-carbohydrate, low PUFA diets both in diabetic and non-diabetic subjects (Hargrove *et al.*, 2001; Rodriguez-Villar *et al.*, 2004).

The ability of the LDL of diabetic subjects to down-regulate *de novo* cellular cholesterol synthesis also appears to be diminished (Owens *et al.*, 1990). This may also be related to a high LDL linoleic acid content, and is improved in diabetics fed a high oleic acid diet (Griffin *et al.*, 1996).

6. The Effect of Dietary MUFA on Insulin Sensitivity and Carbohydrate Metabolism in Diabetes

In the majority of the intervention studies in Table 11.2, the parameters of carbohydrate metabolism assessed are limited to measurements of fasting, postprandial or 24-hour profiles of plasma glucose and insulin, plus measurements of glycated haemoglobin or fructosamine. As shown, several studies show some improvement in these measures although, in general, the differences are relatively small but clearly consistent; in no study was carbohydrate tolerance worse on a high-MUFA diet. However, direct measurements of insulin sensitivity (using a euglycaemic, hyperinsulinaemic clamp) were performed in only two studies with conflicting results. In the study by Parillo *et al.* (1992) insulin sensitivity (whole body glucose disposal) was higher after just 2 weeks on a high-MUFA diet in a group of 10 middle-aged patients with type 2 diabetes than after the same period on a high-carbohydrate diet. In contrast, no differences in insulin sensitivity were found by Gerhard *et al.* (2004) in a similar group of patients (n = 11) after 6 weeks on high-MUFA and high-carbohydrate diets. However, although the macronutrient composition of the diets was similar in the two studies, their designs were significantly different. In the study by Parillo *et al.* (1992). The two diets were isocaloric and weight-maintaining, whereas in the study by Gerhard *et al.* (2004) the subjects were offered up to 125% of their estimated energy requirement. The resulting energy intake was higher on the high-MUFA diet (2901 ± 469 *vs* 2689 ± 377 kcal per day), and there was a significant weight loss of 1.53 kg on the high-carbohydrate diet which may have resulted in a concomitant increase in insulin sensitivity in this group.

There are few studies of the effects of MUFA on glycaemic control in type 1 diabetes. In a group of adolescents with diabetes, Donoghue *et al.* (2000) there was a small fall in HbA_{1C} on a high-MUFA diet which was correlated with the increase in oleic acid in red cell phospholipid. In addition, there was a similar significant fall in total and LDL cholesterol. These results are encouraging in view of the doubt cast upon the benefit of high-MUFA diets in type 1 diabetes by Georgopoulos *et al.* (1998) (see above).

7. Effects of MUFA on B-cell Function and the Enteroinsular Axis

7.1. Direct protective effects on B-cell function

Type 2 diabetes is due to a combination of insulin resistance and defective insulin secretion. Whatever the nature of the B-cell, there is a gradual deterioration of B-cell function with time in almost all patients. Ultimately, insulin secretion fails to compensate for insulin resistance resulting in increasing glycaemia and need for treatment with insulin (De Fronzo, 1992). This has long been attributed 'glucose toxicity', the mechanisms of which have been poorly understood. Recent studies in cultured rodent and human islets have shown that high concentrations of both glucose and saturated fatty acids (palmitic acid) induce B-cell apoptosis, decrease B-cell proliferation and impair B-cell function, and that co-administration of MUFA (either oleic or palmitoleic acid) can protect the B-cell against both glucotoxic and lipotoxic effects (Maedler *et al.*, 2001, 2003).

7.2. Effects on the enteroinsular axis

There is evidence that MUFA may influence the secretion of the two major hormone of the enteroinsular axis – glucose-dependent insulinotrophic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). The secretion of both hormones is influenced by a variety of factors, most importantly fat ingestion. In the case of GLP-1, there is some evidence from *in vitro* studies that secretion of the hormone is stimulated by MUFA but not by saturated fatty acids (Rocca and Brubaker, 1995).

An effect of MUFA on GIP secretion was seen in a study comparing the postprandial lipid, lipoprotein and hormone responses to a test meal in subjects from Northern Europe (Dublin, Ireland and Guildford, UK) and from Southern Europe (Athens and Crete, Greece) (Jackson *et al.*, 2000). The subjects were well matched but differed significantly in terms of their habitual diet: total fat intake was higher in the Southern Europeans (43 *vs* 37% total energy; $p < 0.05$), as was MUFA intake (18.3 *vs* 11%; $p < 0.01$). Saturated fat intake was similar in the two groups. Fasting cholesterol and triglycerides were similar in the two groups but HDL-cholesterol was lower in the Southern Europeans (0.80 *vs* 0.96 mmol/l; $p < 0.05$). Interestingly (and unexpectedly) both postprandial insulin and GIP levels were markedly greater in the Southern Europe group ($p < 0.0001$ and $p < 0.0002$ respectively). Postprandial triglyceride clearance was more efficient in the Southern Europe group, with a late second triglyceride and apo B-48 peak

in the Northern Europeans probably indicating the accumulation of triglyceride-rich remnant particles in the late postprandial phase.

Effects of MUFA on GLP-1 have also been reported but results are conflicting. Brynes *et al.* (2000) compared the effects of a high-MUFA diet (20.3% of energy *vs* 9.1% in baseline diet) and a high-PUFA diet (13.4% of energy *vs* 5.2% in baseline diet) on GLP-1 secretion and insulin sensitivity in a group of patients with type 2 diabetes. Total fat intake was slightly higher on both the intervention diets although total carbohydrate intake was not significantly different. Each diet was given for 24 days in a cross-over design. There were no significant changes from baseline in either insulin sensitivity (measured by a short intravenous insulin tolerance test) or GLP-1 secretion on either diet.

Rocca *et al.* (2001) fed either a saturated fat or MUFA diet (fat 5% total energy intake either as coconut oil or olive oil) to rats for two weeks at the end of which oral glucose tolerance tests were performed. Insulin responses were similar in the two groups but the glycaemic response was significantly lower ($p < 0.05$) in the MUFA-fed animals. There was also an early (10 minute) GLP-1 peak in the MUFA-fed animals which was not seen in the saturated fat group. In a parallel *in vitro* study using cells derived from a gut tumour cell line, the same group showed that the GLP-1 response to GIP (a recognized GLP-1 secretogogue) was greater in cells preincubated with oleic acid than with palmitic acid.

Finally, there is some evidence that MUFA may enhance GLP-1 secretion in man. Thomsen *et al.* (2003) fed three test meals containing carbohydrate alone or with additional saturated fat (butter) or MUFA (olive oil) to a group of subjects with type 2 diabetes. Differences in postprandial glucose and insulin responses to carbohydrate only *vs* carbohydrate plus fat are difficult to interpret due to clear differences in the time course of the responses presumably due to differences in gastric emptying. However, the glucose response was significantly lower in the meal with MUFA than the meal with butter despite similar insulin responses. The GLP-1 response to CHO alone was markedly less than to CHO plus fat (unsurprising given the known stimulatory effect of fat on GLP-1 secretion). However, although not entirely clear-cut, there was a greater postprandial GLP-1 response with olive oil than with butter.

8. Effects of MUFA on Endothelial Function in Diabetes

Endothelial dysfunction is an important element of the cardiovascular risk profile in type 2 diabetes (McVeigh *et al.*, 1992; Enderle *et al.*, 1998), and is associated with a subclinical inflammatory state (Yudkin *et al.*, 1999). The precise cause of endothelial dysfunction is uncertain but it is associated with a number of interrelated features of type 2 diabetes and metabolic syndrome including insulin resistance, oxidized LDL, and postprandial hypertriglyceridaemia, all of which, as discussed above, may be ameliorated by a high MUFA diet. The direct effects of high-MUFA diets on endothelial dysfunction have been examined in a small number of studies in non-diabetic subjects with equivocal results (for review, see Ros, 2003), and in two dietary intervention studies in subjects with metabolic syndrome or type 2 diabetes. Ryan *et al.* (2000) substituted oleic acid-

rich cooking oils and spreads for habitual high-PUFA (linoleic acid) products for two months in a group of subjects with type 2 diabetes and demonstrated an improvement in endothelium-dependent flow-mediated dilatation (FMD) which correlated with the increase in adipocyte membrane oleic:linoleic acid ratio. They also found a similar correlation with *ex vivo* insulin-mediated glucose transport in adipocytes.

In a large dietary intervention study, Esposito *et al.* (2004) randomized 180 people with metabolic syndrome (as defined by ATPIII criteria) to either a control 'prudent' diet or a Mediterranean diet for 2 years. In addition to an increase in dietary MUFA from 9% to 12.4% of dietary energy on the Mediterranean diet, there were also significant changes in a number of other food items including SFA and cholesterol (decreased), and complex carbohydrate, fibre, n-3 PUFA, grains, fruit and vegetable (all increased). Endothelial function (L-arginine test) was one many parameters which improved on the Mediterranean diet (there were also improvements in weight, waist circumference, HOMA score, plasma triglyceride, systolic blood pressure and inflammatory markers). Although the results of this study are impressive, the fact that there are so many other variables makes it impossible to attribute the effects seen to the increase in MUFA consumption.

9. Summary and Conclusions

There is convincing evidence that high MUFA diets may be of benefit in both the prevention and management of diabetes. The majority of these beneficial effects, particularly those on carbohydrate and lipid metabolism, are due to the substitution of MUFA for either saturated fat or carbohydrate in the diet. The design of diets with a high MUFA content using currently-available foods is not straightforward, and care must be taken to avoid simply adding extra calories. In Southern Europe where olive oil is the major source of dietary MUFA, changes can be more easily implemented, but in Northern Europe this is less easy. In the United Kingdom, for example, where over 25% of dietary oleic acid is obtained from meat and meat products, a more fundamental change to dietary habits – and one which would involve a major commitment from the food industry – may be required. If this could be achieved, it would complement the current enthusiasm for low glycaemic index diets in diabetes while considerably increasing dietary choice.

10. References

American Diabetes Association (1998) Nutrition recommendations and principles for people with diabetes mellitus. *Diabetes Care* 21 (Supp(1), 532–535.

Astrup, A., Grunwald, G.K., Melanson, E.L., Saris, W.H.M. and Hill, J.O. (2000) The role of low-fat diets in body weight control: a meta-analysis of *ad libitum* dietary intervention studies. *International Journal of Obesity* 24, 1545–1552.

Baer, D.J., Judd, J.T., Clevidence, B.A. and Tracy, R.P. (2004) Dietary fatty acids affect plasma

markers of inflammation in healthy men fed controlled diets: a randomized crossover study. *American Journal of Clinical Nutrition* 79, 969–973.

Bergman, R.N. (1989) Lilly Lecture, 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. *Diabetes* 38, 1512–1527.

Björntorp, P. (1992) Abdominal fat distribution and disease: an overview of epidemiological data. *Annals of Medicine* 24, 15–18.

Blades, B. and Garg, A. (1995) Mechanism of increase in plasma triacylglycerol concentrations in patients with non-insulin-dependent diabetes mellitus. *American Journal of Clinical Nutrition* 62, 996–1002.

Bonamone, A., Visonà, A., Lusiani, L., Beltramello, G., Confortin, L., Biffanti, S., Sorgato, F., Costa, F. and Pagnan, A. (1991) Carbohydrate and lipid metabolism in patients with non-insulin-dependent diabetes mellitus: effects of a low-fat, high-carbohydrate diet vs a diet high in monounsaturated fatty acids. *American Journal of Clinical Nutrition* 54, 586–590.

Brynes, A.E., Edwards, C.M., Jadhav, A., Ghatei, M.A., Bloom, S.R. and Frost, G.S. (2000) Diet-induced change in fatty acid composition of plasma triacylglycerol is not associated with change in glucagon-like peptide 1 or insulin sensitivity in people with type 2 diabetes. *American Journal of Clinical Nutrition* 72, 1111–1118.

Campbell, L.V., Marmot, P.E., Dyer, J.A., Borkman, M. and Storlien, P.E. (1994) The high-monounsaturated fat diet as a practical alternative for NIDDM. *Diabetes Care* 17, 177–182.

Caslake, M.J., Packard, C.J., Series, J.J., Yip, B., Dagen, M.M. and Shepherd, J. (1992) Plasma triglyceride and low density lipoprotein metabolism. *European Journal of Clinical Investigation* 22, 96–104

Chen, Y.-D.I., Hollenbeck, C.B., Coulston, A.M., Reaven, G.M. and Zhou, M.Y. (1995) Why do low-fat high-carbohydrate diets accentuate postprandial lipemia in patients with NIDDM? *Diabetes Care* 18, 10–16.

Chen, Y.-D.I., Swami, S., Skowronski, R., Coulston, A. and Reaven, G.M. (1993) Differences in postprandial lipemia between patients with normal glucose tolerance and non-insulin-dependent diabetes. *Journal of Clinical Endocrinology and Metabolism* 76, 172–177.

Clifton, P.M., Noakes, M. and Keogh, J.B. (2004) Very low fat (12%) and high monounsaturated fat (35%) diets do not differentially affect fat loss in overweight, nondiabetic women. *Journal of Nutrition* 134, 1741–1745.

Coulston, A.M., Hollenbeck, C.B., Swislocki, A.L.M., Chen, Y.-D.I. and Reaven, G.M. (1987) Deleterious metabolic effects of high-carbohydrate sucrose-containing diets in patients with non-insulin-dependent diabetes mellitus. *American Journal of Medicine* 82, 213–220.

Coulston, A.M., Hollenbeck, C.B., Swislocki, A.L.M. and Reaven, G.M. (1989) Persistence of hypertriglyceridaemic effect of low-fat high-carbohydrate diets in NIDDM patients. *Diabetes Care* 12, 94–101.

De Fronzo, R.A. (1992) Pathogenesis of NIDDM: a balanced overview *Diabetologia* 35, 389–397.

Després, J.P. (2001) Health consequences of visceral obesity. *Annals of Medicine* 33, 534–541.

Diabetes and Nutrition Study Group of the European Association for the Study of Diabetes (2000) Recommendations for the nutritional management of patients with diabetes mellitus. *European Journal of Clinical Nutrition* 54, 353–355.

DCCT (1993) (Diabetes Control and Complications Trial Research Group.) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *New England Journal of Medicine* 329, 977–986.

Dimitriadis, E., Griffin, M., Owens, D., Johnson, A. and Tomkins, G.H. (1995) Oxidation of low-density lipoprotein in NIDDM: its relationship to fatty acid composition. *Diabetologia* 38, 1300–1306.

Dimitriadis, E., Griffin, M., Collins, P., Johnson, A., Owens, D. and Tomkins, G.H. (1996) Lipoprotein composition in NIDDM: effects of dietary oleic acid on the composition, oxidisability and function of low and high density lipoproteins. *Diabetologia* 39, 667–676.

Donaghue, K.C., Pena, M.M., Chan, A.K., Blades, B.L., Storlien, L.H. and Silink, M. (2000) Beneficial effects of increasing monounsaturated fat intake in adolescents with type 1 diabetes. *Diabetes Research and Clinical Practice* 48, 193–199.

Enderle, M.D., Benda, N., Schmuelling, R.M., Haering, H.U. and Pfohl, M. (1998) Preserved endothelial function in IDDM patients, but not in NIDDM patients, compared with healthy subjects. *Diabetes Care* 21, 271–277.

Espino-Montoro, A., López-Miranda, J., Castro, P., Rodríguez, M., López-Segura, F., Blanco, A., Jiménez-Perepérez, J.A., Ordovás, J.M. and Pérez-Jiménez, F. (1996) Monounsaturated fatty acid enriched diets lower plasma insulin levels and blood pressure in healthy young men. *Nutrition, Metabolism and Cardiovascular Disease* 6, 147–154.

Esposito, K., Marfella, R., Ciotola, M., Di Palo, C., Giugliano, F., Giugliano, G., D'Armiento, M., D'Andrea, F. and Giugliano, D. (2004) Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomised trial. *Journal of the American Medical Association* 292, 1490–1492.

Feher, M.D., Caslake, M., Foxton, J., Cox, A. and Packard, C.J. (1999) Atherogenic lipoprotein phenotype in type 2 diabetes: reversal with micronised fenofibrate. *Diabetes/Metabolism Research Reviews* 15, 395–399.

Ferrara, L.A., Raimondi, A.S., d'Episcopo, L., Guida, L., Dello Russo, A. and Marotta, T. (2000) Olive oil and reduced need for antihypertensive medications. *Archives of Internal Medicine* 160, 837–842.

Feskens, E.J. and Kromhout, D. (1990) Habitual dietary intake and glucose tolerance in euglycaemic men: the Zutphen Study. *International Journal of Epidemiology* 19, 953–959.

Feskens, E.J., Loeber, J.G. and Kromhout, D. (1994) Diet and physical activity as determinants of hyperinsulinaemia in the Zutphen Elderly Study. *American Journal of Epidemiology* 140, 350–360.

Feskens, E.J., Stengård, J., Virtanen, S.M., Pekkanen, J., Räsänen, L., Nissinen, A., Tuomilehto, J. and Kromhout, D. (1995) Dietary factors determining diabetes and impaired glucose tolerance. A 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study. *Diabetes Care* 18, 1104–1112.

Ford, E.S., Williamson, D.F. and Liu, S. (1997) Weight change and diabetes incidence: findings from a national cohort of US adults. *American Journal of Epidemiology* 146, 214–222.

Ford, E.S., Giles, W.H. and Dietz, W.H. (2002) Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *Journal of the American Medical Association* 287, 356–359.

Franz, M.J., Bantle, J.P., Beebe, C.A., Brunzell, J.D., Chiasson, J.-L., Garg, A., Holzmeister, L.A., Hoogwerf, B., Mayer-Davis, E., Mooradian, A.D., Purnell, J.Q. and Wheeler, M. (2002) Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. *Diabetes Care* 25, 148–198.

Frayn, K.N. and Kingman, S.M. (1995) Dietary sugars and lipid metabolism in humans. *American Journal of Clinical Nutrition* 62 (suppl), 250S–263S.

Frost, G., Leeds, A.A., Doré, C.J., Madeiros, S., Brading, S. and Dornhorst, A. (1999) Glycaemic index as a determinant of serum HDL-cholesterol concentration. *Lancet* 353, 1045–1048.

Gardner, C.D. and Kraemer, H.C. (1995) Monounsaturated versus polyunsaturated dietary fat and serum lipids: a meta-analysis. *Arteriosclerosis, Thrombosis and Vascular Biology* 15, 1917–1927.

Garg, A. (1998) High-monounsaturated fat diets for patients with diabetes mellitus: a meta-analysis. *American Journal of Clinical Nutrition* 67 (suppl), 577S–582S.

Garg, A., Bantle, J.P., Henry, R.R., Coulston, A.M., Griver, K.A., Raatz, S.K., Brinkley, L., Ida Chen, Y.-D., Grundy, S.M., Huet, B.A. and Reaven, G.M. (1994) Effects of varying carbohydrate content of diet in patients with non-insulin-dependent diabetes mellitus. *Journal of The American Medical Association* 271, 1421–1428.

Garg, A., Bonanome, A., Grundy, S.M., Zhang, Z.-J. and Unger, R.H. (1988) Comparison of a high carbohydrate diet with a high monounsaturated fatty acid diet in patients with non-insulin-dependent diabetes mellitus. *New England Journal of Medicine* 319, 829–834.

Garg, A., Grundy, S.M. and Koffler, M. (1992a) Effect of high carbohydrate intake on hyperglycemia, islet function, and plasma lipoproteins in NIDDM. *Diabetes Care* 15, 1572–1580.

Garg, A., Grundy, S.M. and Unger, R.H. (1992b) Comparison of effects of high and low carbohydrate diets on plasma lipoproteins and insulin sensitivity in patients with mild NIDDM. *Diabetes* 41, 1278–1285.

Georgopoulos, A., Bantle, J.P., Noutsou, M. and Hoover, H.A. (2000) A high carbohydrate versus a high monounsaturated fatty acid diet lowers the atherogenic potential of big VLDL particles in patients with type 1 diabetes. *Journal of Nutrition* 130, 2503–2507.

Georgopoulos, A., Bantle, J.P., Noutsou, M., Swaim, W.R. and Parker, S.J. (1998) Differences in the metabolism of postprandial lipoproteins after a high-monounsaturated-fat versus a high-carbohydrate diet in patients with type 1 diabetes mellitus. *Arteriosclerosis, Thrombosis and Vascular Biology* 18, 773–782.

Gerhard, G.T., Ahmann, A., Meeuws, K., McMurry, M.P., Barton Duell, P.B. and Connor, W.E. (2004) Effects of a low-fat diet compared with those of a high-monounsaturated fat diet on body weight, plasma lipids and lipoproteins, and glycaemic control in type 2 diabetes. *American Journal of Clinical Nutrition* 80, 668–673.

Gerich, J. and Van Haeften, T. (1998) Insulin resistance versus impaired insulin secretion as the genetic basis for type 2 diabetes. *Current Opinion in Endocrinology and Diabetes* 5, 144–148.

Golay, A., Allaz, A.-F., Morel, Y., de Tonnac, N., Tankova, S. and Reaven, G.R. (1996) Similar weight loss with low- or high-carbohydrate diets. *American Journal of Clinical Nutrition* 63, 174–178.

Griffin, B.A., Freeman, D.J., Tait, G.W., Thomson, J., Caslake, M.J., Packard, C.J. and Shepherd, J. (1994) Role of plasma triglyceride in the regulation of plasma low density lipoprotein (LDL) subfractions: relative contribution of small dense LDL to coronary heart disease risk. *Atherosclerosis* 106, 241–253.

Griffin, M.E., Dimitriadis, E., Lenehan, K., Owens, D., Collins, P., Johnson, A. and Tomkin, G.H. (1996) Non-insulin-dependent diabetes mellitus: dietary monounsaturated fatty acids and low-density lipoprotein composition and function. *Quarterly Journal of Medicine* 89, 211–216.

Ha, T.K.K. and Lean, M.E.J. (1998) Recommendations for the nutritional management of patients with diabetes mellitus. *European Journal of Clinical Nutrition* 52, 467–481.

Harding, A.-H., Day, N.E., Khaw, K.-T., Bingham, S., Luben, R., Welsh, A. and Wareham, N.J. (2004) Dietary fat and the risk of clinical type 2 diabetes. *American Journal of Epidemiology* 159, 73–82.

Hargrove, R.L., Etherton, T., Pearson, T.A., Harrison, E.H. and Kris-Etherton, P.M. (2001) Low fat and high monounsaturated fat diets decrease human low density lipoprotein oxidative susceptibility in vitro. *Journal of Nutrition* 131, 1758–1763.

Heilbronn, L.K., Noakes, M. and Clifton, P.M. (1999) Effect of energy restriction, weight loss and diet composition on plasma lipids and glucose in patients with type 2 diabetes. *Diabetes Care* 22, 889–895.

Higashi, K., Shige, H., Ito, T., Nakajima, K., Ishikawa, T., Nakamura, H. and Ohsuzu, F. (2001) Effect of a low-fat diet enriched with oleic acid on postprandial lipemia in patients with type 2 diabetes mellitus. *Lipids* 36, 1–6.

Holkanson, J.E. and Austin, M.A. (1996) Plasma triglyceride level is a risk factor for cardiovascular disease independent of HDL-cholesterol: a meta-analysis of population-based prospective studies. *Journal of Cardiovascular Risk* 3, 213–219.

Isomaa, B., Lahti, K., Almgren, P., Nissé, M., Tuomi, T., Taskinen, M.-R., Forsén, B. and Groop, L. (2001) Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care* 24, 683–689.

Jackson, K.G., Zampelas, A., Knapper, J.M.E.,

Roche, H.M., Gibney, M.J., Kafatos, A., Gould, B.J., Wright, J.W. and Williams, C.M. (2000) Differences in glucose-dependent insulinotrophic polypeptide hormone and hepatic lipase in subjects of southern and northern Europe: implications for postprandial lipaemia. *American Journal of Clinical Nutrition* 71, 13–20.

Knowler, W.C., Barrett-Connor, E., Fowler, S.E., Hamman, R.F., Lachin, J.M., Walker, E.A., Nathan, D.M., Diabetes Prevention Program Research Group (2002) Reduction in the incidence of Type 2 diabetes with lifestyle intervention or metformin. *New England Journal of Medicine* 346, 393–403.

Lerman-Garber, I., Cardoso-Saldaña, G., Ichazo-Cerro, S., Posadas-Romero, C. and Zamora-Gonsález, J. (1994) Effect of a high-monounsaturated fat diet enriched with avocado in NIDDM patients. *Diabetes Care* 17, 311–315.

Levy, R.I., Lees, R.S. and Fredrickson, D.S. (1966) The nature of pre-beta (very low density) lipoproteins. *Journal of Clinical Investigation* 45, 63–77.

Lewis, G.F., O'Meara, N.M., Soltys, P.A., Blackman, J.D., Iverius, P.H., Pugh, W.L., Getz, G.S. and Polonsky, K.S. (1991) Fasting hypertriglyceridaemia in non-insulin-dependent diabetes mellitus is an important predictor of postprandial lipid and lipoprotein abnormalities. *Journal of Clinical Endocrinology and Metabolism* 72, 934–944.

Lopez-Segura, F., Velasco, F., Lopez-Miranda, J., Castro, P., Lopez-Pedrera, R., Blanco, A., Jimenez-Pereperez, J., Torres, A., Trujillo, J., Ordovas, J.M. and Perez-Jimenez, F. (1996) Monounsaturated fatty acid-enriched diet decreases plasminogen activator inhibitor type 1. *Arteriosclerosis, Thrombosis and Vascular Biology* 16, 82–88.

Low, C., Grossman, E.B. and Gumbiner, B. (1996) Potentiation of effects of weight loss by monounsaturated fatty acids in obese NIDDM patients. *Diabetes* 45, 569–575.

Luscombe, N.D., Noakes, M. and Clifton, P.M. (1999) Diets high and low in glycemic index versus high monounsaturated fat diets: effects on glucose and lipid metabolism in NIDDM. *European Journal of Clinical Nutrition* 53, 473–478.

Madigan, C., Ryan, M., Owens, D., Collins, P. and Tomkin, G.H. (2000) Dietary unsaturated fatty acids in type 2 diabetes. Higher levels of postprandial lipoprotein on a linoleic acid-rich diet compared with an oleic acid-rich olive oil diet. *Diabetes Care* 23, 1472–1477.

Maedler, K., Spinas, G.A., Dyntar, D., Moritz, W., Kaiser, N. and Donach, M.Y. (2001) Distinct effects of saturated and monounsaturated fatty acids on β-cell turnover and function. *Diabetes* 50, 69–76.

Maedler, K., Oberholzer, J., Bucher, P., Spinas, G.A. and Donath, M.Y. (2003) Monounsaturated fatty acids prevent the deleterious effects of palmitate and high glucose on human pancreatic β-cell turnover and function. *Diabetes* 52, 726–733.

Marshall, J.A., Shetterly, S., Hoag, S. and Hamman, R.F. (1994) Dietary fat predicts conversion from impaired glucose tolerance to NIDDM. *Diabetes Care* 17, 50–56.

Marshall, J.A., Bessesen, D.H. and Hamman, R.F. (1997) High saturated fat and low starch diets are associated with hyperinsulinaemia in a non-diabetic population: the San Luis Valley Diabetes Study. *Diabetologia* 40, 430–438.

McVeigh, G.E., Brennan, G.M., Johnston, G.D., McDermott, B.J., McGrath, L.T., Henry, W.R., Andrews, J.W. and Hayes, J.R. (1992) Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 35, 771–776.

Mensink, R.P. and Katan, M.B. (1989) Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. *New England Journal of Medicine* 321, 436–441.

Mensink, R.P. and Katan, M.B. (1987) Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoprotein in healthy men and women. *Lancet* i, 122–124.

Mensink, R.P. and Katan, M.B. (1992) Effect of dietary fatty acids on serum lipids and lipoproteins – a meta-analysis of 27 trials. *Arteriosclerosis, Thrombosis and Vascular Biology* 12, 911–919.

Nielsen, S., Hermansen, K., Rasmussen, O.W.,

Thomsen, C. and Mogensen, C.E. (1995) Urinary albumin excretion rate and 24-h ambulatory blood pressure in NIDDM with microalbuminuria: effects of a monounsaturated-enriched diet *Diabetologia* 38, 1069–1075.

Nikkila, M., Solakivi, T., Lehtimaki, T., Koivula, T., Laippala, P. and Astrom, B. (1994) Postprandial plasma lipoprotein changes in relation to apolipoprotein E phenotype and low density lipoprotein size in men with and without coronary artery disease. *Atherosclerosis* 106, 149–157.

Nutrition Subcommittee of the Diabetes Care Advisory Committee of Diabetes UK (2003) The implementation of nutritional advice for people with diabetes. *Diabetic Medicine* 20, 786–807.

Owens, D., Maher, V., Collins, P., Johnson, A. and Tomkin, G.H. (1990) Cellular cholesterol regulation – a defect in the type 2 diabetic in poor metabolic control. *Diabetologia* 33, 93–99.

Parillo, M. and Riccardi, G. (2004) Diet composition and the risk of type 2 diabetes: epidemiological and clinical evidence. *British Journal of Nutrition* 92, 7–19.

Parillo, M., Rivellese, A.A., Ciardullo, A.V., Capaldo, B., Genivese, S. and Riccardi, G. (1992) A high monounsaturated fat/low carbohydrate diet improves peripheral insulin sensitivity in non-insulin-dependent diabetic patients. *Metabolism* 41, 1373–1378.

Parillo, M., Rivellese, A.A., Giacco, R., Riccardi, G. and Ciardullo, A.V. (1996) Does a high-carbohydrate diet have different effects in NIDDM patients treated with diet alone or hypoglycaemic drugs? *Diabetes Care* 19, 498–500.

Parker, D.R., Weiss, S.T., Troisi, R., Cassano, P.A., Vokonas, P.S. and Landsberg, L. (1993) Relationship of dietary saturated fatty acids and body habitus to serum insulin concentrations. *American Journal of Clinical Nutrition* 58, 129–136.

Parks, E.J. and Hellerstein, M.K. (2000) Carbohydrate-induced hypertriacylglycerolemia: historical perspective and review of biological mechanisms. *American Journal of Clinical Nutrition* 71, 412–433.

Patsch, J.R., Prasad, S., Gotto, A.M. and Patsch, W. (1987) High density lipoprotein$_2$: relationship of plasma levels of this lipoprotein species to its composition, to the magnitude of postprandial lipemia, and to the activities of lipoprotein lipase and hepatic lipase. *Journal of Clinical Investigation* 80, 341–347.

Pérez-Jiménez, F., López-Miranda, J., Pinillos, M.D., Gómez, P., Paz-Rojas, E., Marín, C., Velasco, M.J., Blanco-Molina, A., Jiménez Perepérez, J.A., Ordovás, J.M. (2001) A Mediterranean diet and a high-carbohydrate diet improve glucose metabolism in healthy young persons. *Diabetologia* 44, 2038–2043.

Psaltopoulou, T., Naska, A., Orfanos, P., Trichopoulos, D., Mountokalakis, D. and Trichopoulou, A. (2004) Olive oil, the Mediterranean diet, and arterial blood pressure: the Greek European Prospective Investigation into Cancer and Nutrition. *American Journal of Clinical Nutrition* 80, 1012–1018.

Rasmussen, O., Lauszus, F.F., Christiansen, C., Thomsen, C. and Hermansen, K. (1996) Differential effects of saturated and monounsaturated fat on blood glucose and insulin responses in subjects with non-insulin-dependent diabetes mellitus. *American Journal of Clinical Nutrition* 63, 249–253.

Rasmussen, O.W., Vesterlund, M., Thomsen, C., Winther, E., Hansen, K.W. and Hermansen, K. (1993) Effects on blood pressure, glucose and lipid levels of a high-monounsaturated fat diet compared with a high-carbohydrate diet in NIDDM subjects. *Diabetes Care* 16, 1565–1571.

Reaven, G.M. (1988) Role of insulin resistance in human disease. *Diabetes* 37, 1595–1607.

Reaven, P.D. and Witzum, J.L. (1996) Oxidized low density lipoprotein in atherogenesis; role of dietary modification. *Annual Review of Nutrition* 16, 51–71.

Rivellese, A.A., Giacco, R., Genovese, S., Patti, L., Marotta, G., Pacioni, D., Annuzzi, G. and Riccardi, G. (1990) Effects of changing amount of carbohydrate in diet on plasma lipoproteins and apolipoproteins in type II diabetic patients. *Diabetes Care* 13, 446–448.

Rocca, A.S. and Brubaker, P.L. (1995) Stereospecific effects of fatty acids on proglucagon-derived peptide secretion in fetal rat intestinal cultures. *Endocrinology* 136, 5593–5599.

Rocca, A.S. LaGreca, J., Kalitsky, J. and Brubaker, P.L. (2001) Monounsaturated fatty acid diets improve glycemic tolerance through increased secretion of glucagon-like peptide-1. *Endocrinology* 142, 1148–1155.

Roche, H.R., Zampelas, A., Knapper, J.M.E., Webb, D., Brooks, C., Jackson, K.G., Wright, J.W., Gould, B.J., Kafatos, A., Gibney, M.J. and Williams, C.M. (1998) Effect of long-term olive oil dietary intervention on postprandial triacylglycerol and factor VII metabolism. *American Journal of Clinical Nutrition* 68, 552–560.

Rodríguez-Villar, C., Manzanares, J.M., Casals, E., Pérez-Heras, A., Zambón, D., Gomis, R. and Ros, E. (2000) High-monounsaturated fat, olive oil-rich diet has effects similar to a high-carbohydrate diet on fasting and postprandial state and metabolic profiles of patients with type 2 diabetes. *Metabolism* 49, 1511–1517.

Rodríguez-Villar, C., Pérez-Heras, A., Mercadé, I., Casals, E. and Ros, E. (2004) Comparison of a high-carbohydrate and a high monounsaturated fat, olive oil-rich diet on the susceptibility of LDL to oxidative modification in subjects with type 2 diabetes mellitus. *Diabetic Medicine* 21, 142–149.

Ros, E. (2003) Dietary *cis*-monounsaturated fatty acids and metabolic control in type 2 diabetes. *American Journal of Clinical Nutrition* 78 (suppl.), 617S–625S.

Ryan, M., McInerney, D., Owens, D., Collins, P., Johnson, A. and Tomkin, G.H. (2000) Diabetes and the Mediterranean diet: a beneficial effect of oleic acid on insulin sensitivity, adipocyte glucose transport and endothelium-dependent vasoreactivity. *Quarterly Journal of Medicine* 93, 85–91.

Salmerón, J., Hu, F.B., Manson, J.E., Stampfer, M.J., Colditz, G.A., Rimm, E.B. and Willett, W.C. (2001) Dietary fat intake and risk of type 2 diabetes in women. *American Journal of Clinical Nutrition* 73, 1019–1026.

Seidell, J.C. (1998) Dietary fat and obesity: an epidemiologic perspective. *American Journal of Clinical Nutrition* 69 (suppl.), 546S–550S.

Shah, M. and Garg, A. (1996) High-fat and high-carbohydrate diets and energy balance. *Diabetes Care* 19, 1142–1152.

Stamler, J., Vaccaro, O., Neaton, J.D. and Wentworth, D. (1993) Diabetes, other risk factors and 12-year cardiovascular mortality for men screened in the MRFIT. *Diabetes Care* 16, 434–444.

Steil, G.M., Volund, A., Kahn, S.E. and Bergman, R.N. (1993) Reduced sample number for calculation of insulin sensitivity and glucose effectiveness from the minimal model. Suitability for use in population studies. *Diabetes* 42, 250–256.

Storlien, L.H., Baur, L.A., Kriketos, A.D., Pan, D.A., Cooney, G.J., Calvert, G.D. and Campbell, L.V. (1996) Dietary fats and insulin action. *Diabetologia* 39, 621–631.

Strychar, I., Ishac, A., Rivard, M., Lussier-Cacan, S., Beauregard, H., Arid-Jilwan, N., Radwan, F. and Yale, J.F. (2003) Impact of a high-monounsaturated-fat diet on lipid profile in subjects with type 1 diabetes. *Journal of the American Dietetic Association* 103, 467–474.

Thomsen, C., Christiansen, C., Pedersen, E., Vesterlund, M., Storm, H., Ingerslev, J. and Hermansen, K. (1999) Comparison of the effects of a monounsaturated fat diet and high carbohydrate diet on cardiovascular risk factors in first degree relatives to type-2 diabetic subjects. *European Journal of Clinical Nutrition* 52, 818–823.

Thomsen, J.C., Rasmussen, O., Christiansen, C., Pedersen, E., Vesterlund, M., Storm, H., Ingerslev, J. and Hermansen, K. (1999) Comparison of the effects of a monounsaturated fat diet and a high carbohydrate diet on cardiovascular risk factors in first degree relatives to type-2 diabetic subjects. *European Journal of Clinical Nutrition* 53, 818–823.

Thomsen, C., Storm, H., Hoslt, J.J. and Hermansen, K. (2003) Differential effects of saturated and monounsaturated fats on postprandial lipemia and glucagon-like peptide 1 responses in patients with type 2 diabetes. *American Journal of Clinical Nutrition* 77, 605–611.

Tuomilehto, J., Lindström, J. and Eriksson, J.G. (2001) Prevention of type 2 diabetes by changes in lifestyle among subjects with impaired glucose tolerance. *New England Journal of Medicine* 344, 1343–1350.

UKPDS (1998) (UK Prospective Diabetes Study Group) Intensive blood glucose control with sulphonylureas or insulin compared with conventional treatment and risk of compli-

cations in patients with type 2 diabetes. *Lancet* 352, 837–853.

Vague, J. (1947) La différentiation sexuelle. Facteur déterminant des formes de l'obésité. *Presse Médicale* 55, 339–341.

Van Dam, R.M., Stampfer, M.J., Willett, W.C., Hu, F.B. and Rimm, E.B. (2002) Dietary fat and meat intake in relation to risk of type 2 diabetes in men. *Diabetes Care* 25, 417–424.

Vessby, B. (2000) Dietary fat and insulin action in humans. *British Journal of Nutrition* 83, Suppl. 1 S91–S96.

Vessby, B. (1995) Nutrition, lipids and diabetes mellitus. *Current Opinion in Lipidology* 6, 3–7.

Vessby, B., Uusitupa, M., Hermansen, K., Riccardi, G., Rivellese, A.A., Tapsell, L.C., Berglund, L., Louheranta, A., Rasmussen, B.M., Calvert, G.D., Maffetone, A., Pedersen, E., Gustafsson, I.-B. and Storlien, L.H. (2001) Substituting dietary saturated fat for monounsaturated fat impairs insulin sensitivity in healthy men and women: the KANWU study. *Diabetologia* 44, 312–319.

Walker, K.Z., O'Dea, K., Johnson, L., Sinclair, A.J., Piers, L.S., Nicholson, G.C. and Muir, J.G. (1996) Body fat distribution and non-insulin-dependent diabetes: comparisons of a fiber-rich, high-carbohydrate, low-fat (23%) diet and a 35% fat diet high in monounsaturated fat. *American Journal of Clinical Nutrition* 63, 254–260.

Walker, K.Z., O'Dea, K. and Nicholson, G.C. (1999) Dietary composition affects regional fat distribution and levels of dehydroepiandrosterone sulphate (DHEAS) in post-menopausal women with type 2 diabetes. *European Journal of Clinical Nutrition* 53, 700–705.

Willett, W.C. and Leibel, R.L. (2002) Dietary fat is not a major determinant of body fat. *American Journal of Medicine* 113, 47S–59S.

World Health Organisation (2005) Diabetes Mellitus. Prevalence of diabetes. *http://www.who.int/diabetes/facts/world_figures/en/*

Yudkin, J.S., Stehouwer, C.D.A., Emeis, J.J. and Coppack, S.W. (1999) C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction; a potential role for cytokines originating from adipose tissue? *Arteriosclerosis, Thrombosis, and Vascular Biology* 19, 972–978.

Zambon, A., Sartore, G., Passera, D., Francini-Pesenti, F., Bassi, A., Basso, C., Zambon, S., Manzato, E. and Crepaldi, G. (1999) Effects of hypocaloric dietary treatment enriched in oleic acid on LDL and HDL subclass distribution in mildly obese women. *Journal of Internal Medicine* 246, 191–201.

Zampelas, A., Roche, H., Knapper, J.M., Jackson, K.G., Tornaritis, M., Hatzis, C., Gibney, M.J., Kafatos, A., Gould, B.J., Wright, J. and Williams, C.M. (1998) Differences in postprandial lipaemic response between Northern and Southern Europeans. *Atherosclerosis* 139, 83–93.

Zilversmit, D.B. (1979) Atherogenesis: a postprandial phenomenon. *Circulation* 60, 473–485.

12 Olive Oil and Immune Function

PARVEEN YAQOOB

The Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, The University of Reading, Reading, UK

1. Introduction

The immune system acts to protect the host from infectious agents which exist in the environment (bacteria, viruses, fungi, parasites) and from other noxious insults. The immune system has two functional divisions: the innate (or natural) immune system and the acquired (also termed specific or adaptive) immune system. Innate immunity consists of physical barriers, soluble factors and phagocytic cells, which include granulocytes (neutrophils, basophils, eosinophils), monocytes and macrophages and provides a first line of defence against invading pathogens. However, an immune response often requires the coordinated actions of both innate immunity and the more powerful and flexible acquired immunity. Acquired immunity involves the specific recognition of molecules (antigens) on an invading pathogen which distinguish it as being foreign to the host. Lymphocytes, which are subdivided into T and B lymphocytes, effect this form of immunity. While effective immune responses are highly desirable, some aspects of immunity have undesirable consequences. For example, bactericidal and inflammatory mediators secreted by macrophages are toxic not only to pathogens, but also to host tissues, resulting in unavoidable tissue damage. For this reason, immune responses, and macrophage responses in particular, need to be tightly controlled and the self-regulatory properties of the immune system highly effective. Unfortunately the immune system can become dysfunctional or dysregulated, resulting in inappropriate activation of some components. In some individuals the immune system recognizes a host ('self') antigen and then proceeds to direct its destructive activities against host tissues. These diseases are termed chronic inflammatory diseases and examples include rheumatoid arthritis, psoriasis, systemic lupus erythmatosus, multiple sclerosis, ulcerative colitis and Crohn's disease. In some other individuals the immune system becomes inappropriately sensitized to a normally benign antigen, termed an allergen, and so reacts vigorously when that antigen is encountered. These are the atopic

 Olive Oil and Health
(eds J.L. Quiles, M.C. Ramírez-Tortosa and P. Yaqoob)

diseases, which include allergies, asthma and atopic eczema. It is now recognized that atherosclerosis has an immunological component and clearly some cancers arise and develop as a result of diminished immunosurveillance. Thus, modulation of immune function by dietary components might be an effective means for altering the course of these diseases. Furthermore, diet may underlie the development of some of these diseases.

There are a number of reasons why fatty acids might be expected to have some impact on immune function. First, alteration of the fatty acid composition of the diet can modulate the fatty acid composition of cells of the immune system. Since cell membrane phospholipids are crucial to many intracellular signalling pathways, altering their fatty acid composition may alter the actions of intracellular second messengers in cells of the immune system. Second, eicosanoids are important immunoregulatory molecules derived from arachidonic acid, which is released from membrane phospholipids. Alteration of the fatty acid composition of these phospholipids may influence eicosanoid synthesis. Finally, fatty acids may alter cell function by direct interaction with intracellular targets, including transcription factors, which in turn could alter gene expression.

In order to evaluate the full impact of olive oil on immune function, it is important to consider the effects of both the lipid and non-lipid components. This chapter evaluates the evidence for the effects of olive oil on immune responses in both animals and humans, presents evidence suggesting that both oleic acid and the non-lipid components of olive oil can separately modulate selected immune responses, discusses the use of olive oil-containing emulsions in clinical settings and reviews evidence for protective effects of olive oil in chronic inflammatory disorders.

2. Effects of Olive Oil on *ex vivo* Lymphocyte Proliferation

The *in vitro* effects of fatty acids on lymphocyte proliferation have been studied since the early 1970s and have been reviewed in detail elsewhere (Gurr, 1983; Calder *et al.*, 2002). These studies have investigated the effects of a large range of fatty acids, including oleic acid, on immune responses, but the results are disparate and comparisons between studies are made difficult by the differences in the concentrations of fatty acids used, the cell type studied, the means by which they were presented to cells and the conditions of incubation. The *in vitro* studies therefore remain contradictory, some showing no effect of oleic acid and some showing a suppression of lymphocyte proliferation (Gurr, 1983; Calder *et al.*, 2002).

A number of animal studies have examined the effects of dietary fats on *ex vivo* lymphocyte proliferation. In one such series of dietary studies, the effects of feeding rats a range of high-fat (200 g/kg) diets, each with a characteristic fatty acid composition, or a low-fat (25 g/kg corn oil) diet on lymphocyte fatty acid composition and on a number of lymphocyte functions were compared (Yaqoob *et al.*, 1994a, 1994b, 1995a). In these studies, the animals were fed on hydrogenated coconut oil, olive oil, safflower oil, evening primrose oil or fish (menhaden) oil for a period of 10 weeks. The first of these studies reported a

significant suppressive effect of olive oil on the *ex vivo* proliferation of mesenteric lymph node lymphocytes in response to the T-cell mitogen, Concanavalin A (Con A) when compared with feeding a low-fat diet or diets rich in hydrogenated coconut oil or safflower oil (Yaqoob *et al.*, 1994a). The effect of the olive oil diet was similar in magnitude to that resulting from the feeding of fish oil or evening primrose oil (Yaqoob *et al.*, 1994a) and was also demonstrated in whole blood cultures stimulated with Con A (Yaqoob *et al.*, 1995a). All of the high fat diets were shown to modulate the fatty acid composition of lymphocytes, resulting in characteristic profiles for each dietary group (Yaqoob *et al.*, 1995b). In contrast to these studies, De Pablo *et al.* (1998a) demonstrated that olive oil and hydrogenated coconut oil enhanced mitogen-stimulated lymphocyte proliferation compared with sunflower (all fed at 15% by weight) in Balb/c mice. However, it may be important to note that in the study by De Pablo *et al.* (1998a), lymphocytes were cultured in 10% foetal calf serum prior to assessment, whereas Yaqoob *et al.* (1994a, 1995a) conducted experiments using autologous serum or whole blood cultures and showed that culturing cells for 48 h in foetal calf serum, but not autologous serum, reversed the changes in fatty acid composition brought about by dietary lipid manipulation (Yaqoob *et al.*, 1995b) and masked the effects of dietary lipid manipulation on cell function (Yaqoob *et al.*, 1994a). Thus the effects of dietary fats on immune function could potentially be altered by subsequent cell culture conditions. This issue is also relevant to the study of Berger *et al.* (1993), which compared the effects of feeding a low fat (30 g/kg fat), olive oil (100 g/kg), safflower oil (100 g/kg), linseed oil (100 g/kg) or fish plus safflower oil (90+10 g/kg) diet to dams for 5 months on the proliferation of Con A-stimulated murine spleen lymphocytes of their pups prior to weaning. They reported no effect of dietary manipulation (Berger *et al.*, 1993). This could be due to the fact that the high-fat diets in their study contained a lower amount of fat than those employed by Yaqoob *et al.*, (1994a, 1995a) and De Pablo *et al.* (1998a). It could also be explained by the fact that, Berger *et al.* (1993) fed murine dams on each of the diets and subsequently tested lymphocyte proliferation using cells from the pups before weaning, making it possible that the suckling pups had not been exposed to milk of sufficiently differing fatty acid composition to allow dietary lipid manipulation to occur through this transition. Furthermore, the lymphocytes were cultured in 10% foetal calf serum, which, as indicated above, might reverse the changes in fatty acid composition brought about by dietary lipid manipulation and mask any effects of dietary lipid manipulation on cell function.

The studies outlined above used relatively large amounts of a single oil and as such they represent very extreme diets, which are unlikely to be encountered by free-living human beings. Furthermore, the use of such oils inevitably results in variation in the levels of several fatty acids together and not only the one under investigation. A further study therefore investigated the effects of relatively small changes in the levels of commonly consumed fatty acids in a controlled manner in which one fatty acid was exchanged for another, without altering the levels of other fatty acids in the diet (Jeffery *et al.*, 1997). The nine diets used in this study contained 178 g fat/kg, and differed in their proportions of palmitic, oleic, linoleic and α-linolenic acids whilst maintaining a constant

n-6 PUFA: n-3 PUFA ratio of 7. The effect on lymphocyte proliferation of replacing one fatty acid with another appeared to be influenced by the level of other fatty acids in the diet such that lymphocyte proliferation was decreased with increasing dietary oleic acid levels up to an oleic acid level of 35.6 g/ 100g fatty acids, but increasing the oleic acid level above this level did not result in any further increase (Jeffery *et al.*, 1997). These results suggest that the discrepancies which exist between studies investigating the effects of olive oil on immune function in rodents are not likely to be due to differing quantities of fat fed, since all of the studies tested the effects of olive oil at 100 g/kg or above (Berger *et al.*, 1993; Yaqoob *et al.*, 1994a, 1995a; De Pablo *et al.*, 1998a).

One study to date has examined the effects of a monounsaturated fat (MUFA)-enriched diet on lymphocyte proliferation in humans (Yaqoob *et al.*, 1998). In this study, middle-aged men were randomly assigned to consume either a control diet (designed to reproduce the current UK diet fatty acid composition) or a diet containing foods enriched with highly-refined olive oil for 8 weeks. Foods provided for subjects included the main meal of the day (as a frozen recipe meal), cooking oils and spreads, biscuits and puddings. Subjects on the MUFA diet consumed significantly less saturated fat (% energy) compared with those on the control diet and significantly more MUFA; MUFA contributed 18.4% energy in this group compared with 11.3% in the control group (Yaqoob *et al.*, 1998). Consumption of the MUFA-rich diet did not affect the proliferative response of either whole blood cultures or peripheral blood mononuclear cells (PBMNC) to the T-cell mitogen, Concanavalin A (Yaqoob *et al.*, 1998). This observation contrasts with results obtained by the same group using laboratory animals (Yaqoob *et al.*, 1994a, 1995a). The lack of a clear effect of MUFA may be attributable to the higher level of monounsaturated fat used in the animal studies, where rats were fed for 10 weeks on diets containing 200 g/kg olive oil (MUFA therefore contributed approximately 30% of total energy intake), whereas in the human study, MUFA contributed approximately 18% of the total energy intake. While it is possible that a higher level of dietary MUFA may have resulted in suppression of proliferation, it is clear that at levels corresponding to Mediterranean intakes (Ferro-Luzzi and Branca, 1995) and levels which can readily be achieved through consumption of meals which use olive oil as the primary cooking fat, MUFA do not affect lymphocyte proliferation.

3. Effects of Olive Oil on *ex vivo* Natural Killer Cell Activity

One of the most important mechanisms by which the immune system deals with foreign cells is to damage or destroy them. Typical target cells include malignant cells, normal cells of the host that are infected with viruses or other microorganisms and normal cells from individuals unrelated to the responding host. Natural killer (NK) cells are a subset of lymphocytes found mainly in blood and the spleen. They are derived from the bone marrow, but are neither T-cells nor B-cells and they do not undergo thymic maturation. Killing by NK cells is part of natural rather than specific immunity, since it is not induced by a specific antigen and is not restricted by MHC molecules.

Feeding rats for 10 weeks on a diet containing 200 g/kg olive oil resulted in significant suppression of NK cell activity compared with feeding a low fat diet or diets containing 200 g/kg hydrogenated coconut oil or safflower oil (Yaqoob *et al.*, 1994b) (Table 12.1). The inhibition of NK activity was greater than that produced by feeding a diet rich in evening primrose oil, but not as great as that resulting from feeding a diet containing 200 g/kg fish oil (Yaqoob *et al.*, 1994b) (Table 12.1). In the study by De Pablo *et al.* (1998a), an olive oil-rich diet (15% by weight) significantly suppressed NK cell activity compared with hydrogenated coconut oil and, at some time points, sunflower oil, in Balb/c mice. This effect was observed despite the fact that cells were incubated in the presence of FCS, although in this case for a much shorter duration (4 h) than in the proliferation experiments. Thus, it is possible that there was insufficient time for the FCS to modify the dietary lipid-induced changes in cell composition and reverse the effects in these experiments. However, Berger *et al.* (1993) showed no effect of an olive oil-rich diet on NK cell activity in mice when compared with a low fat, safflower oil or linseed oil diet; once again, the lack of effect may be attributable to the amount of fat in the diet and/or the protocol used (dams fed for 5 months and pups subsequently used prior to weaning).

In a study comparing the effects of nine diets containing 178 g fat/kg, and differing in their proportions of palmitic, oleic, linoleic and α-linolenic acids, on NK activity, there was a significant negative linear relationship between the oleic acid content of the diet and NK cell activity, suggesting that dietary oleic acid suppresses NK cell activity (Jeffery *et al.*, 1997). Furthermore, there was a negative relationship between the oleic acid:linoleic acid ratio in the diet and NK cell activity and a weak negative relationship ($r-0.289$; $P = 0.092$) between NK cell activity and the level of oleic acid in spleen lymphocytes (Jeffery *et al.*, 1997).

In healthy, middle-aged men, consumption of a MUFA diet produced a small decrease in natural killer cell activity at 2 months, but not 1 month (Yaqoob *et*

Table 12.1. The effect of dietary lipid manipulation on NK activity in freshly prepared rat spleen lymphocytes.

Diet	% Cytolysis
LF	46.5 ± 2.0^{cef}
HCO	40.4 ± 2.4^{f}
OO	36.4 ± 2.8^{a}
SO	42.1 ± 1.5^{f}
EPO	40.7 ± 0.7^{af}
MO	29.4 ± 2.1^{abde}

Cytolysis of YAC-1 (target) cells by rat spleen lymphocytes (effector cells) was measured by release of $_{51}$Cr by pre-loaded YAC-1 cells at a ratio of 100:1 effector:target cells. Results are expressed as % cytolysis. Statistical significance for $P < 0.05$ at least is indicated as follows: a, *vs* LF; b, *vs* HCO; c, *vs* OO; d, *vs* SO; e, *vs* EPO; f, *vs* MO. Data are taken from Yaqoob *et al.* (1994b) and the Table modified from Yaqoob (1998), with permission.

al., 1998; Table 12.2). However, this was not statistically significant when compared either with the baseline or with the control group. Natural killer cell activity was unaffected by consumption of the control diet (Yaqoob *et al.*, 1998). As with the effects of olive oil on lymphocyte proliferation, this observation contrasts with some of the animal studies (Yaqoob *et al.*, 1994b; De Pablo *et al.*, 1998a) and may be attributable to the higher level of monounsaturated fat used in the animal studies. It is interesting to note, however, that the small (non-significant) changes in natural killer cell activity and proliferation observed after 2 months of consumption of the MUFA diet in the human study were accompanied by a small, but significant increase in the proportion of oleic acid in plasma phospholipids and in PBMNC (Yaqoob *et al.*, 1998).

4. Effects of Olive Oil on Expression of Adhesion Molecules

Recent advances in our understanding of basic mechanisms of inflammation, of cell–cell interactions and of leukocyte trafficking have highlighted the importance of adhesive interactions between leukocytes and cellular or extracellular components of tissues. There has been a significant expansion of knowledge regarding the role of cell surface adhesion molecules in these processes over the last 10 years and a number of adhesion molecules have been classified into families according to sequence homology and functions. It has been suggested that some adhesion molecules may have pathophysiological as well as physiological roles; some adhesion molecules have been implicated in the transendothelial migration of leukocytes into synovial tissue and fluid in rheumatoid arthritis and in leukocyte-endothelium interactions which lead to the formation of atherosclerotic plaques (Munro, 1993). There has consequently been a great deal of interest in recent years in the potential modulation of the expression and/or functions of adhesion molecules by fatty acids.

In a study by De Caterina *et al.* (1994), human saphenous vein endothelial cells (HSVEC) were preincubated for 24 h with 10 μM arachidonic acid (AA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) or oleic acid (OA)

Table 12.2. Effect of MUFA consumption on expression of ICAM-1 by PBMNC in healthy middle-aged men.

	% ICAM-1 positive cells		
	Baseline	1 month	2 months
Control	19.0 ± 1.3	19.1 ± 1.2	20.0 ± 1.5
MUFA	20.8 ± 1.4	16.4 ± 1.4	15.9 ± 1.1*§

Data are mean ± SEM for n = 18–23 subjects. Significant differences between groups (two-way repeated measures ANOVA, followed by appropriate post-hoc tests) are indicated as follows: *$P < 0.05$ *vs* control group; §$P < 0.01$ *vs* baseline. Data are taken from Yaqoob *et al.* (1998) and the Table modified from Yaqoob (1998), with permisssion.

prior to 6 h of stimulation with tumour necrosis factor-α (TNFα) and subsequent measurement of the surface expression of vascular call adhesion molecule-1 (VCAM-1). It was reported that DHA (but not EPA) and oleic acid significantly decreased the expression of VCAM-1 by HSVEC (De Caterina *et al.*, 1994). In a further study, the same group demonstrated that oleic acid inhibited the adhesion of a monocytic cell line to human umbilical vein endothelial cells *in vitro*, but that the effects were less potent than those of polyunsaturated fatty acids (Carluccio *et al.*, 1999). In a dietary study comparing the effects of a low fat (25 g/kg) and high fat diets containing 200 g/kg hydrogenated coconut oil, olive oil, safflower oil, evening primrose oil or fish oil, the level of expression of the adhesion molecules CD2, ICAM-1 and LFA-1 on rat spleen lymphocytes was decreased by both olive oil and fish oil (Sanderson *et al.*, 1995a). A further study demonstrated that the same diets decreased the adhesion of both freshly-prepared and mitogen-stimulated lymphocytes to macrophage monolayers and the adhesion of mitogen-stimulated lymphocytes to endothelial cells *ex vivo* (Sanderson and Calder, 1998).

A MUFA-rich diet resulted in a significant decrease in the expression of the leucocyte adhesion molecule, intercellular adhesion molecule-1 (ICAM-1), after 2 months compared both with baseline values and with those from the control group in a human study (Yaqoob *et al.*, 1998). The expression of ICAM-1 did not change during the consumption of the control diet (Yaqoob *et al.*, 1998) (Table 12.2). ICAM-1 is a member of the Ig superfamily of adhesion molecules and is involved in leukocyte–leukocyte adhesion (Chapman and Haskard, 1995) as well as adhesion of leukocytes to endothelial cells (Munro, 1993) and to fibrinogen, a plasma adhesive protein (Languino *et al.*, 1995). ICAM-1 is expressed on mononuclear cells that infiltrate inflamed synovium in patients suffering from rheumatoid arthritis and in some cases, such patients may also have high levels of serum soluble ICAM-1 (Cronstein, 1994). The formation of plaques in atherosclerosis shows many features which are common to the inflammation seen in rheumatoid arthritis, such as adhesive interactions between endothelial cells and leukocytes and extravascular leukocyte accumulation and ICAM-1 is thought to play a pivotal role in the recruitment of mononuclear cells to, and therefore the growth of, the atherosclerotic plaque (Poston *et al.*, 1992). Thus, these results suggest that olive oil may have some anti-atherogenic and anti-inflammatory properties, which could be useful in the maintenance of health.

Further evidence suggests that a MUFA-rich diet may indeed affect the process of cellular adhesion in humans. In an interesting study by Mata *et al.* (1996) healthy men and women living in a religious community were subjected to four consecutive dietary periods (isocaloric) differing in the fat content of SFA, MUFA and *n*-3 and *n*-6 PUFA. It was reported that LDL-induced monocyte adhesion to endothelial cells was lower during the MUFA period than each of the others and that resistance of LDL to oxidation was greatest during the MUFA period (Mata *et al.*, 1996). The authors suggested that the modulation of LDL fatty acid composition was responsible for the differences in adhesion and showed a significant negative correlation between monocyte adhesion to endothelial cells and the oleic acid content of LDL (Mata *et al.*, 1996). Expression of adhesion molecules by either cell type was not measured, but it is possible that

this too may have played some role in the decreased adhesion during the MUFA period. A similar study by Tsimikas *et al.* (1999) investigated the ability of oxidized LDL from Greek, American and Greek–American subjects to induce monocyte chemotaxis and adhesion to endothelial cells. They also demonstrated a strong negative correlation between the oleic acid content of LDL and both monocyte chemotaxis and adhesion, while there was a strong positive correlation between LDL PUFA content and monocyte adhesion (Tsimikas *et al.*, 1999).

5. Effects of Olive Oil on *in vivo* Immune Responses

The *ex vivo* studies described so far build an interesting picture of the possible influences of olive oil on immune function. However, once observations have been made *ex vivo*, it is important to extend these to *in vivo* studies. Mulrooney and Grimble (1993) attempted this by investigating the influence of dietary fatty acids on the inflammatory response to TNFα in rats. This situation mimics the invasion of the body by infective and inflammatory agents, which would result in the release of cytokines from cells of the immune system. The purpose of the released cytokines, apart from modulation of the immune system, is to bring about enhanced lipolysis, gluconeogenesis, muscle proteolysis and redistribution of tissue zinc in order to provide substrates for cells of the immune system and amino acids for the synthesis of acute-phase proteins. When weanling rats were fed for 8 weeks on diets containing 100 g/kg fat in the form of corn, fish, coconut oils or butter (rich in oleic acid) before an intraperitoneal injection of recombinant human TNFα, the increase in hepatic zinc concentration normally observed in the ensuing response did not occur in animals fed on the fish oil or butter diets (Mulrooney and Grimble, 1993) and the increase in plasma caeruloplasmin was smaller in the butter-fed animals than those fed on the other diets (Mulrooney and Grimble, 1993). There was also no increase in the rate of protein synthesis in response to TNFα in the livers of animals fed the butter diet, whereas animals fed on the corn oil and coconut oil diets demonstrated the normal increase in protein synthesis associated with the acute phase response (Mulrooney and Grimble, 1993). In a subsequent study, it was demonstrated that diets containing 50 or 100 g/kg butter or olive oil completely suppressed the increases in tissue zinc content, liver protein synthesis and serum caeruloplasmin levels in response to subcutaneous *Escherichia coli* endotoxin, when compared with a maize oil diet or standard laboratory chow (Besler and Grimble, 1995). In both studies, the butter and olive oil diets decreased the intensity of anorexia induced by TNFα or endotoxin (Besler and Grimble, 1995), demonstrating clearly the diminished susceptibility to the lethal effects of both agents in experimental animals.

An alternative experimental model for *in vivo* immune responses is the 'graft versus host' (Gv.H) response, which can be elicited in rodents by injection of allogenic cells into the footpad of a host. The response primarily involves the polyclonal activation and proliferation of host B cells. The 'host versus graft' (Hv.G) response, on the other hand, is a T cell-mediated response, in which cytotoxic T lymphocytes of the host recognize MHC antigens on the injected cells. In both

cases the enlargement of the popliteal lymph nodes (more than 15-fold in the Gv.H response and 4-fold in the Hv.G response) is due largely to proliferation of activated host cells within the lymph node, although there is also recruitment of cells from the bloodstream (see Fig. 12.1). Using this assay, Sanderson *et al.* (1995b) demonstrated that feeding rats a diet containing 200 g/kg fish oil suppressed the Gv.H response compared with feeding a low fat diet or diets containing 200 g/kg coconut oil, safflower oil or evening primrose oil; feeding a diet containing 200 g/kg olive oil had a similar effect, although the response was depressed only compared with the low fat and evening primrose oil diets (Sanderson *et al.*, 1995b) (Table 12.3). The expression of the adhesion molecules LFA-1 and ICAM-1 on lymphocytes from popliteal lymph nodes following a Gv.H response was significantly lower in animals fed the olive oil or fish oil diets compared with those fed the low fat or coconut oil diets (Sanderson *et al.*, 1995b). It was speculated that the smaller popliteal lymph node size in animals fed the fish oil or olive oil diets may result from a suppression of both the activation of cells within the node and of the movement of cells from the bloodstream into the nodes (Sanderson *et al.*, 1995b). Interestingly, while the fish oil diet had a similar suppressive effect on the Hv.G response as that on the Gv.H response, the olive oil diet had no effect on the Hv.G response (Sanderson *et al.*, 1995b). It appears, therefore, that in this model, olive oil is able to modulate *in vivo* responses involving B cells, but not those involving cytotoxic T lymphocytes.

In rodent models of mammary carcinogenesis, it has been demonstrated that maternal feeding of a diet containing 15% olive oil to mice before pregnancy resulted in *stimulation* of most areas of the white pulp responsible for the production of T and B lymphocytes in the spleens of the offspring (Zusman *et al.*, 2000; Kossoy *et al.*, 2002). In the lymph nodes, olive oil increased the areas of

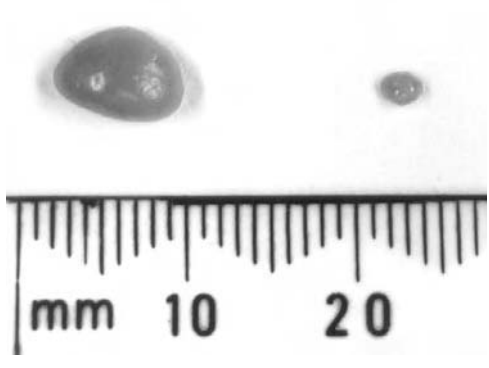


Fig. 12.1. The popliteal lymph node assay – a graft versus host model. This *in vivo* graft *vs* host model is based on the subcutaneous injection of lymph node lymphocytes from adult male Lewis rats into the footpad of weanling male DA/Lewis rats, with the control leg for each rat being injected with saline. The animals were sacrificed 7 d after injection and the popliteal lymph nodes dissected and weighed. The popliteal lymph node from the experimental leg increases dramatically in size and weight due to accumulation of immune cells from the circulation, increasing from a few mg in weight to approximately 100 mg. Photograph kindly supplied by Professor P. Calder and reproduced from Yaqoob (2004), with permission.

Table 12.3. Effects of dietary fatty acids on the graft versus host response.

Diet	Popliteal lymph node weight (mg)
Low fat	102.7 ± 8.2
Coconut oil	101.8 ± 14.9
Olive oil	77.3* ± 7.5
Safflower oil	92.3 ± 6.3
Fish oil	67.8* ± 5.7

*Denotes a significant difference compared with the low fat diet. The basis of the assay is illustrated in Fig. 12.2. Data are taken from Sanderson *et al.* (1995b) and reproduced from Yaqoob (2004), with permission.

the cortical and mantle layers (the main lymphocyte-producing regions), but decreased the area of the medulla, compared with corn oil (Kossoy *et al.*, 2002). However, these experiments were performed in a setting where the offspring were exposed to a carcinogenic agent and therefore reflect the influence of dietary fat on immune responses to tumours, which may be different from that in other settings. Compared with corn oil, the olive oil diet suppressed the appearance of chemically-induced mammary tumours in the offspring (Kossoy *et al.*, 2002). This was suggested to be due to enhanced apoptosis of tumour cells, stimulation of the production of lymphocytes in lymphoid tissues and an increase in the infiltration of lymphocytes into the tumour as a result of feeding olive oil (Kossoy *et al.*, 2002).

Although there are no published human studies which have set out to examine the effects of olive oil on *in vivo* immune responses, at least one study investigating the effects of fish oil supplements on immunological parameters (including the systemic humoral response to tetanus toxoid) in healthy volunteers has used olive oil as a placebo treatment (Virella *et al.*, 1991). The authors claim to demonstrate an immunosuppressive effect of fish oil compared with olive oil, but the protocol is far from satisfactory. Six volunteers were involved in the study, only two of whom received the olive oil treatment, and if the data are scrutinized it is clear that a larger number of subjects may have produced different results (Virella *et al.*, 1991), particularly since measurements of human immune responses are prone to substantial inter-individual variation.

6. Olive Oil and Host Defence

If olive oil suppresses immune function, it is possible that it will have a detrimental effect on host defence. This was investigated by Wallace *et al.* (2000), who examined the influence of a range of dietary fatty acids on macrophage-mediated cytotoxicity towards two tumour cell lines (P815 and L929). Feeding olive oil significantly inhibited the killing of these tumour cells compared with a low fat diet, but other high-fat diets, including those containing safflower oil and coconut oil,

had similar effects, so it is not clear whether the effect of the olive oil diet was in fact due to the amount of fat (Wallace *et al.*, 2000). In the same study, the olive oil diet decreased the *ex vivo* production of tumour necrosis factor a and nitrite by macrophages compared with the low fat diet, but once again the effect may have been due to amount of fat (Wallace *et al.*, 2000). Only a fish oil-containing diet appeared to have a specific effect on the responses described above (Wallace *et al.*, 2000). Puertollano *et al.* (2002) examined the effects of feeding a low-fat diet or high-fat diets containing 20% (by weight) hydrogenated coconut oil, olive oil or fish oil to Balb/c mice on *in vitro* cellular responses to *Listeria monocytogenes*. Feeding olive oil did not affect spleen lymphocyte proliferation, but it enhanced the cytotoxicity of the pathogen towards splenic cells compared with the low-fat and the hydrogenated coconut oil diets, suggesting a potentially detrimental effect of olive oil (Puertollano *et al.*, 2002). However, feeding olive oil did not affect the ability of *Listeria monocytogenes* to adhere to or invade the cells *in vitro* (Puertollano *et al.*, 2002). Also, the same group investigated the effects of hydrogenated coconut oil, sunflower oil and olive oil diets on phagocytic activity in Balb/c mice and demonstrated that the olive oil diet enhanced phagocytic activity and production of interleukin-1 relative to the other groups (de Pablo *et al.*, 1998b). Thus, the impact of olive oil on host defence is not yet clear.

7. Olive Oil-containing Emulsions in Clinical Nutrition

There has been considerable interest in manipulation of the fatty acid composition of lipid emulsions used in nutritional support for critically ill and surgical patients. The basis for the use of emulsions containing lipids other than the traditional soybean oil is that due to its high content of linoleic acid, soybean oil might promote the generation of arachidonic acid-derived eicosanoids and exaggerate the inflammatory responses seen in clinical situations involving stress or trauma. Whether this phenomenon does in fact occur *in vivo* and whether it affects clinical outcome is disputed. Nevertheless, immunomodulatory interventions aiming to ameliorate the systemic inflammatory response syndrome (SIRS), which is associated with sepsis and multiple organ failure, have focused on the replacement of some of the n-6 PUFA with other fatty acids, including MUFA. Proponents of emulsions containing olive oil suggest that it offers an immunologically neutral alternative to soybean oil for use in parenteral nutrition, with the potential benefit of some mild anti-inflammatory effects. Granato *et al.* (2000) compared the effects of an olive oil-based emulsion (ClinOleic) with two soybean oil-based emulsions (Intralipid and Ivelip) on a number of immune parameters. Both Intralipid and Ivelip suppressed lymphocyte proliferation and IL-2 production, whereas ClinOleic had no effect, which could be interpreted to suggest that olive oil-based emulsions offer a neutral alternative to soybean oil-based emulsions with respect to their effects on immune function (2000). A similar hypothesis was adopted in a study where rats were subjected to total parenteral nutrition (TPN) in the form of ClinOleic or Ivelip for 6d; in this study there were no significant differences in spleen lymphocyte proliferation, although the response tended to be higher in the ClinOleic group, and the

expression of the IL-2 receptor was significantly higher after TPN with ClinOleic (Moussa *et al.*, 2000). The neutral nature of olive oil-containing emulsions is also demonstrated in a study by Garnacho-Montero *et al.* (2002), where rats received saline, a soybean oil-based emulsion, a medium-chain triglyceride (MCT)/soybean oil emulsion or an olive oil-containing emulsion prior to challenge with *E. coli*. Both the soybean oil and MCT-containing emulsions diminished bacterial clearance, while the olive oil-containing emulsion did not. However, survival rates were not significantly different between groups (Garnacho-Montero *et al.*, 2002). Nevertheless, the effect of olive oil is potentially important, since impairment of phagocytosis and bacterial clearance is an undesirable outcome commonly associated with the use of intravenous lipid emulsions (Waitzberg *et al.*, 2002).

8. Olive Oil and Inflammatory Disease

Linos *et al.* (1991, 1999) compared the relative risk of development of rheumatoid arthritis (RA) in relation to lifelong consumption of olive oil in a Greek population, demonstrating that the risk of developing RA decerased significantly with increased consumption of olive oil, such that individuals in the highest category of olive oil consumption had an OR of 0.39 (95% CI 0.19, 0.82) when compared with those in the lowest category of consumption. This translates as a 2.5 times higher risk of developing RA in individuals with the lowest consumption of olive oil compared with those with the highest consumption. The study, although of great interest, has several limitations, including the fact that it is retrospective and the lifelong assessment of dietary patterns is likely to be very difficult to perform accurately. Nevertheless, it has been noted that the prevalence of RA is greater in Anglo-Saxon populations than in the Mediterranean basin (Cimmino *et al.*, 1998) and that RA seems to be less severe in Mediterranean populations (Drosos *et al.*, 1992). While genetics may play a role, only a small proportion of the difference in prevalence has so far been attributed to polymorphisms in HLA-DR (Boki *et al.*, 1992), suggesting that a role for diet is still possible.

One further piece of evidence suggests that olive oil may have beneficial effects relating to RA. In a study by Kremer *et al.* (1990) examining the effects of fish oil supplementation on the severity and progression of RA, olive oil was used as a placebo treatment, but clinical evaluations and immunologic tests showed it to have some mild beneficial effects. A total of 5 out of 45 clinical measures were significantly improved compared with baseline in the olive oil group, 8 out of 45 in a low-dose fish oil group and 21 out of 45 in a high-dose fish oil group (Kremer *et al.*, 1990). Production of interleukin-1 by macrophages was decreased in the olive oil group, although not to the same extent as either of the fish oil groups (Kremer *et al.*, 1990). The authors concluded that 'dietary supplementation with olive oil is also associated with certain changes in immune function, which require further investigation.' However, some caution needs to be applied in the interpretation of these results, since it is well known that clinical trials inevitably involve a 'placebo effect'. It could be argued that both the olive oil treatment and the low dose fish oil treatment represent placebo effects and

that the only genuine treatment effect is that of the high dose fish oil. It is difficult to justify how supplementation of a background diet (likely to contain appreciable quantities of oleic acid) with 9 g/d olive oil could bring about improvement in clinical symptoms in a chronic inflammatory disorder and changes in immune function when substantial alteration of the diet with MUFA failed to affect most parameters of immune function in healthy middle-aged men (Yaqoob *et al.*, 1998).

Olive oil has been demonstrated to ameliorate symptoms associated in rats with experimentally-induced ulcerative colitis, a form of inflammatory bowel disease (Nieto *et al.*, 2002). In this study, feeding olive oil to rats for only 2 weeks resulted in significant improvement in both macroscopic and microscopic scores in colon specimens, although, as with most other animal studies, the effects of olive oil were less dramatic than those of fish oil (Nieto *et al.*, 2002). These observations have not yet been extended to human studies.

Finally, there has been a long-standing interest in the potential protective effects of the Mediterranean diet on cardiovascular disease. Since atherosclerosis is increasingly being recognized to have a chronic inflammatory component, it is possible that at least part of the protective effect of olive oil could be manifested through effects on immune and inflammatory responses which are involved in atherogenesis. In this context, it is interesting to note that dietary olive oil decreased the expression of scavenger receptors in murine macrophages (Miles *et al.*, 2001) and reduced circulating levels of a number of markers of endothelial activation in humans (Perez-Jimenez *et al.*, 1999). A supplementation study comparing the effects of olive oil and fish oil demonstrated that both treatments lowered plasma fibrinogen levels in women with high baseline fibrinogen, which is a risk factor for cardiovascular disease (Oosthuizen *et al.*, 1994). However, as with the study by Kremer *et al.* (1990) in patients with RA, it is not clear how such a low dose of olive oil (6 g/d) would bring about this effect given the appreciable quantities of MUFA in the background diet. Thus the nature of the effect of olive oil in inflammatory diseases in general is poorly understood and requires clarification.

9. Are the Effects of Olive Oil on Immune Function Due to Oleic Acid or to Non-lipid Components?

Since olive oil contains a number of antioxidants, sterols, hydrocarbons and alcohols (Gunstone *et al.*, 1994), it is important to consider whether its effects on immune function, at least in animal studies, are due to oleic acid or to some other component of the oil. This was investigated by Jeffery *et al.* (1996), who compared the effects of feeding a diet containing high-oleic sunflower oil with a low fat, olive oil or safflower oil diet on lymphocyte proliferation and natural killer cell activity in rats. Feeding either the olive oil or the high-oleic sunflower oil diet significantly decreased the proliferation of spleen lymphocytes compared with feeding the low fat or safflower oil diets; the effects of the olive oil and high-oleic sunflower oil diets were not significantly different from one another (Jeffery *et al.*, 1996). Similarly, NK cell activity was significantly lower for spleen lympho-

cytes from rats fed the olive oil or the high-oleic sunflower oil than for those from rats fed the low fat or the safflower oil diets and the effects of the olive and high-oleic sunflower oils were not significantly different from one another (Jeffery *et al.*, 1996) (Fig. 12.2). This suggests that the effects of the olive oil diet in these animal studies were likely to be due to oleic acid rather than other components of olive oil. Moreover, most animal studies have employed refined olive oil, which would be stripped of most of its polyphenols. However, a human study comparing the effects of extra virgin olive oil with high-oleic sunflower oil on lipid peroxidation and eicosanoid production demonstrated that these parameters were higher in individuals consuming high-oleic sunflower oil, which suggests that the non-lipid component of olive oil may exert antioxidant effects *in vivo* (Oubina *et al.*, 2001). Furthermore, increasing the amounts of olive oil phenolic extracts administered to human volunteers dose-dependently decreased their excretion of 8-iso-PGF2α, a marker for oxidative stress (Visioli *et al.*, 2000).

Evidence that polyphenolic compounds may have direct effects on immune function (either dependent or independent of their antioxidant activities) has been published in the form of studies which have investigated the effects of single olive oil-derived polyphenols on in vitro immune responses. Oleuropein and hydroxytyrosol are the principal polyphenolics in olive oil and are potent scavengers of superoxide anion generation and have been demonstrated to inhibit neutrophil respiratory burst (Visioli *et al.*, 1998a). However, oleuropein enhances nitric oxide production by murine macrophages, by increasing both

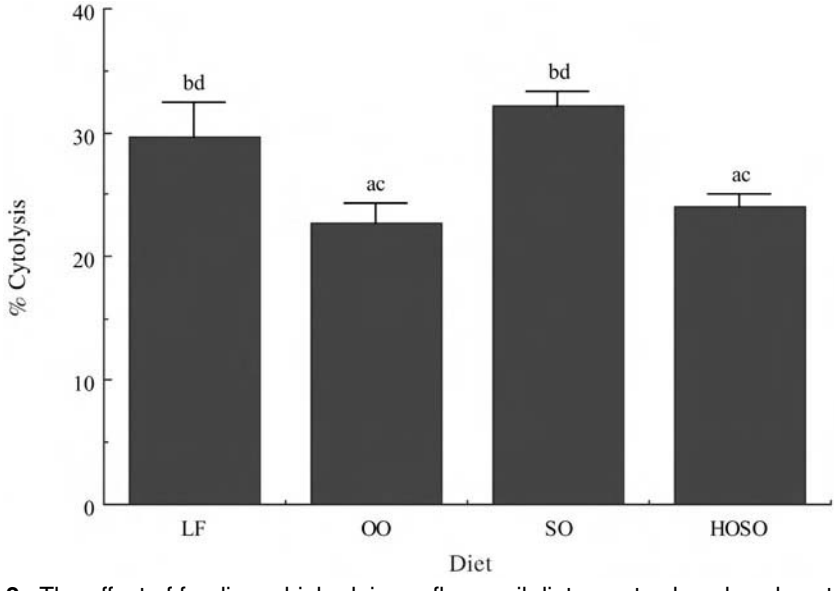


Fig. 12.2. The effect of feeding a high-oleic sunflower oil diet on rat spleen lymphocyte natural killer cell activity. Cytolysis of YAC-1 (target) cells by rat spleen lymphocytes (effector cells) was measured by release of ^{51}Cr by pre-loaded YAC-1 cells at a ratio of 100:1 effector:target cells. Results are expressed as % Cytolysis. Statistical significance is indicated as: [a]*vs* low fat (LF); [b]*vs* olive oil (OO), [c]*vs* safflower oil (SO); [d]*vs* high-oleic sunflower oil (HOSO). From Yaqoob (1998), with permission.

the activity and expression of the inducible form of nitric oxide synthase (Visioli *et al.*, 1998b). This could be interpreted as a pro-inflammatory action of oleuropein, although it does not appear to be a general feature of flavonoids, since a number of flavonoids inhibit nitric oxide production by macrophages (Kim *et al.*, 1999). Both oleuropein and hydroxytyrosol have recently been demonstrated to reduce the adhesion of monocytes to endothelial cells and to reduce the expression of VCAM-1 by endothelial cells *in vitro* (Carluccio *et al.*, 2003).

Since olive oil-derived polyphenolics are essentially hydrophilic, they are abundant in olive oil waste waters (at a concentration about ten times higher than in the oil phase), which are generated during olive oil production and discarded. Visioli *et al.* (1999) have demonstrated that olive mill waste-water extracts inhibit human LDL oxidation and scavenge superoxide anions and hypochlorous acid at concentrations of 20 ppm. In addition, two of the three extracts used (differing in their degree of refinement) inhibited the production of leukotrienes by human neutrophils and it was suggested that low molecular weight components, such as hydroxytyrosol, were responsible for the effect (Visioli *et al.*, 1999). This is consistent with the observation by De la Puerta *et al.* (1999) that phenolics from virgin olive oil inhibit the leukocyte 5-lipoxygenase. The same group tested the effects of single polyphenolics present in the non-lipid fraction of virgin olive oil in a murine model of inflammation and oedema in the ear. In this model, inflammation was induced by application of arachidonic acid or a phorbol ester and olive oil phenolics were applied topically to the ear (De la Puerta *et al.*, 2000). The extent of swelling was decreased by between 33% and 45% by oleuropein, hydroxytyrosol, tyrosol and caffeic acid and each of these compounds inhibited the enzyme myeloperoxidase, suggesting reduced infiltration of neutrophils into the inflamed tissue (De la Puerta *et al.*, 2000). Finally, a rat model of both acute and chronic arthritis compared the effects of a control diet and diets containing high-oleic sunflower oil, virgin olive oil, palm olein, virgin olive oil supplemented with 600 ppm polyphenols and fish oil (Martinez-Dominguez *et al.*, 2001). In the model of acute arthritis, the virgin olive oil and polyphenol-supplemented olive oil treatments significantly decreased the inflammation index compared with the control diet, while the other diets had no effect (Martinez-Dominguez *et al.*, 2001). In the chronic model, both the polyphenol-supplemented olive oil and fish oil prevented the development of inflammation to some degree, although the supplemented olive oil had a greater effect (Martinez-Dominguez *et al.*, 2001). Thus, these studies demonstrate that the phenolic components of olive oil have some anti-inflammatory activities. It is conceivable, therefore, that oleic acid modulates some aspects of immune function when fed at high levels and that phenolic components of olive oil modulate other functions; in other words, the effects of the lipid and non-lipid fractions of olive oil may be separate and distinct. Whether this is the case in humans *in vivo* remains to be determined.

10. Conclusion

Animal studies, depending on the protocol, species and type of measurement, generally support the idea that olive oil is capable of modulating functions of

cells of the immune system. The effects appear to be similar to, albeit weaker than, those seen following feeding of diets containing fish oils. There is some evidence that the effects of olive oil on immune function in animal studies are due to oleic acid, but there is also evidence that the polyphenolic components of olive oil have some anti-inflammatory and immunomodulatory properties. Animal models of inflammation, in particular, support this concept.

In contrast, consumption of a MUFA-rich diet by humans does not appear to bring about a general suppression of immune cell functions. The effects of this type of diet in humans are limited to decreasing the expression of adhesion molecules on PBMNC and decreasing LDL-induced adhesion of monocytes to endothelial cells. The lack of a clear effect of MUFA in humans may be attributable to the higher level of monounsaturated fat used in the animal studies; however, it is ultimately of importance to consider the effects of intakes of olive oil which are in no way extreme and can readily be achieved through consumption of meals which use olive oil as the primary cooking fat. A further problem with the interpretation of human studies is that it is extremely difficult to determine conclusively whether the effects observed are indeed due to an increased level of MUFA or to a concomitant (and unavoidable) decrease in the level of SFA.

Human studies investigating the influence of dietary olive oil on immune function have tended to focus on its MUFA content, but potential effects of the non-lipid components of olive oil on immune and inflammatory responses cannot be excluded, particularly since olive oil-derived phenolic compounds are associated with potent antioxidant activity and inhibition of lipoxygenase activity.

11. References

Berger, A., German, J.B., Chiang, B.L., Ansari, A.A., Keen, C.L., Fletcher, M.P. and Gershwin, M.R. (1993) Influence of feeding unsaturated fats on growth and immune status of mice. *Journal of Nutrition* 123, 225–233.

Besler, H.T. and Grimble, R.F. (1995) Comparison of the modulatory influence of maize and olive oils and butter on metabolic responses to endotoxin in rats. *Clinical Science* 88, 59–66.

Boki, K.A., Panayi, G.S., Vaughan, R.W., Drosos, A.A., Moutsopoulos, H.M. and Lanchbury, J.S. (1992) HLA class II sequence polymorphisms and susceptibility to rheumatoid arthritis in Greeks: the HLA-DRb shared-epitope hypothesis accounts for the disease in only a minority of Greek patients. *Arthritis Rheumatoid* 35, 749–755.

Calder, P.C., Yaqoob, P., Thies, F., Wallace, F.A. and Miles, E.A. (2002) Fatty acids and lymphocyte functions. *British Journal of Nutrition* 87, S31–S48.

Carluccio, M.A., Massaro, M., Bonfrate, C., Siculella, L., Maffia, M., Nicolardi, G., Distante, A., Storelli, C. and De Caterina, R. (1999) Oleic acid inhibits endothelial activation. A direct vascular antiatherogenic mechanism of a nutritional component in the Mediterranean diet. *Arteriosclerosis Thrombosis and Vascular Biology* 19, 220–228.

Carluccio, M.A., Siculella, L., Ancora, M.A., Massaro, M., Scoditti, E., Storelli, C., Visioli, F. Distante, A. and De Caterina, R. (2003) Olive oil and red wine antioxidant polyphenols inhibit endothelial activation. *Arteriosclerosis Thrombosis and Vascular Biology* 23, 622–629.

Chapman, P.T. and Haskard, D.O. (1995) Leukocyte adhesion molecules. *British Medical Bulletin*, 51, 296–311.

Cimmino, M.A., Parisi, M., Moggiana, G., Mela, G.S. and Accardo, S. (1998) Prevalence of rheumatoid arthritis in Italy: the Chivari study. *Annals of Rheumatic Disease* 57, 315–318.

Cleland, L.G., French, J.K., Betts, W.H., Murphy, G.A. and Elliot, M.J. (1988) Clinical and biochemical effects of dietary fish oil supplements in rheumatoid arthritis. *Journal of Rheumatology (Canada)*, 15, 1471–1475.

Cronstein, B.N. (1994) Adhesion molecules in the pathogenesis of rheumatoid arthritis. *Current Opinions in Rheumatology* 6, 300–304.

De Caterina, R., Cybulsky, M.I., Clinton, S.K., Gimbrone, M.A., Jr. and Libby, P. (1994) The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. *Arteriosclerosis and Thrombosis* 14, 1829–1836.

De la Puerta, R., Ruiz-Gutierrez, V. and Hoult, J.R.S. (1999) Inhibition of leukocyte 5-lipoxygenase by phenolics from virgin olive oil. *Biochemical Pharmacology* 57, 445–449.

De la Puerta, R., Martinez-Dominguez, E., Ruiz-Gutierrez, V. (2000) Effect of minor components of virgin olive oil on topical antiinflammatory assays. *Zeitschrift fur Naturforschung C* 55, 814–819.

De Pablo, M.A., Ortega, E., Gallego, A.M., Alvarez, C., Pancorbo, P.L. and Alavrez de Cienfuegos, G. (1998a) Influence of diets containing olive oil, sunflower oil or hydrogenated coconut oil on the immune response of mice. *Journal of Clinical Biochemistry and Nutrition* 25, 11–23.

De Pablo, M.A., Ortega, E., Gallego, A.M., Alvarez, C., Pancorbo, P.L. and Alvarez de Cienfuegos, G. (1998b) The effect of dietary fatty acid manipulation on phagocytic activity and cytokine production by peritoneal cells from Balb/c mice. *Journal of Nutritional Science and Vitaminology* 44, 57–67.

Drosos, A.A., Lanchbury, J.S., Panayi, G.S. and Moutsopoulos, H.M. (1992) Rheumatoid arthritis in Greek and British patients. *Arthritis Rheumatoid* 35, 745–748.

Ferro-Luzzi, A. and Branca, F. (1995) The Mediterranean diet, Italian style: prototype of a healthy diet. *American Journal of Clinical Nutrition* 61 (suppl), 1338S–45S.

Garnacho-Montero, J., Ortiz-Leyba, C., Garnacho-Montero, M.C., Garcia-Garmendia, J.L., Perez Paredes, C., Moyano-Del Estad, M.R., Barrero-Almodovar, A. and Jimenez-Jimenez, F.J. (2002) Effects of three intravenous lipid emulsions on the survival and mononuclear phagocyte function of septic rats. *Nutrition* 18, 751–754.

Granato, D., Blum, S., Rossle, C., Le Boucher, J., Malnoe, A., Dutot, G. (2000) Effects of parenteral lipid emulsions with different fatty acid composition on immune cell functions in vitro. *Journal of Parenteral and Enteral Nutrition* 24, 113–118.

Gunstone, F.D., Harwood, J.L. and Padley, F.B. (1994) *The Lipid Handbook*, 2nd edn. Chapman & Hall, London, pp.79–82.

Gurr, M.I. (1983) The role of lipids in the regulation of the immune system. *Progress in Lipid Research*, 22, 257–287.

Jeffery, N.M., Yaqoob, P., Newsholme, E.A. and Calder, P.C. (1996) The effects of olive oil upon rat serum lipid levels and lymphocyte functions are due to oleic acid. *Annals of Nutrition and Metabolism*, 40, 71–80.

Jeffery, N.M., Cortina, M., Newsholme, E.A. and Calder, P.C. (1997) Effects of variations in the proportions of saturated, monounsaturated and polyunsaturated fatty acids in the rat diet on spleen lymphocyte functions. *British Journal of Nutrition* 77, 805–823.

Kim, H.K., Cheon, B.S., Kim, Y.H., Kim, Y. and Kim, H.P. (1999) Effects of naturally occurring flavonoids on nitric oxide production in the macrophage cell line RAW 264.7 and their structure-activity relationships. *Biochemical Pharmacology* 58, 759–765.

Kossoy, G., Stark, A., Tendler, Y., Ben-Hur, H., Beniashvili, D., Mada,r Z. and Zusman, I. (2002) Transplacental efffects of high fat diets on functional activity of the spleen and lymph nodes, cell kinetics and apoptosis in mammary gland tumours in female rat offspring. *International Journal of Molecular Medicine* 10, 773–778.

Kremer, J.M., Lawrence, D.A., Jubiz, W., DiGiacomo, R., Rynes, R., Bartholomew, L.E. and Sherman, M. (1990) Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. *Arthritis and Rheumatism* 33, 810–820.

Languino, L.R., Duperray, A., Joganic, K.J., Fornaro, M., Thornton, G.B. and Altieri, D.C. (1995) Regulation of leukocyte-endothelium interaction and leukocyte transendothelial migration by intercellular

adhesion molecule 1-fibrinogen recognition. *Proceedings of the National Academy of Sciences USA*, 92, 1505–1509.

Linos, A., Kaklamanis, E., Kontomerkos, A., Koumantaki, Y., Gazi, S., Vaiopoulos, G., Tsokos, G.C. and Kaklamanis, P.H. (1991) The effect of olive oil and fish consumption on rheumatoid arthritis – a case control study. *Scandinavian Journal of Rheumatology 1991*, 20, 419–426.

Linos, A., Kaklamani, V.G., Kaklamani, E., Koumantaki, Y., Giziaki, E., Papazoglou, S. and Mantzoros, C.S. (1999) Dietary factors in relation to rheumatoid arthritis: a role for olive oil and cooked vegetables? *American Journal of Clinical Nutrition* 70, 1077–1082.

Martinez-Dominguez, E., De la Puerta, R. and Ruiz-Gutierrez, V. (2001) Protective effects upon experimental inflammation models of a polyphenol-supplemented virgin olive oil diet. *Inflammation Research* 50, 102–106.

Mata, P., Alonso, R., Lopez-Farre, A., Ordovas, J.M., Lahoz, C., Garces, C., Caramelo, C., Codoceo, R., Blazquez, E. and de Oya, M. (1996) Effect of dietary fat saturation on LDL oxidation and monocyte adhesion to human endothelial cells in vitro. *Arteriosclerosis Thrombosis and Vascular Biology*, 16, 1347–1355.

Miles, E.A., Wallace, F.A. and Calder, P.C. (2001) An olive oil-rich diet reduces scavenger receptor mRNA in murine macrophages. *British Journal of Nutrition* 85, 185–191.

Moussa, M., Le Boucher, J., Tkaczuk, J., Ragab, J., Dutot, G., Ohayon, E., Ghisolfi, J. and Thouvenot, J..P (2000) In vivo effects of olive oil-based lipid emulsion on lymphocyte activation in rats. *Clinical Nutrition* 19, 49–54.

Mulrooney, H.M. and Grimble, R.F. (1993) Influence of butter and of corn, coconut and fish oils on the effects of recombinant human tumour necrosis factor-a in rats. *Clinical Science*, 84, 105–112.

Munro, J.M. (1993) Endothelial-leukocyte adhesive interactions in inflammatory diseases. *European Heart Journal*, 14 (suppl K), 72–77.

Nieto, N., Torres, M.I., Rios, A. and Gil, A. (2002) Dietary polyunsaturated fatty acids improve histological and biochemical alterations in rats with experimental ulcerative colitis. *Journal of Nutrition* 132, 11–19.

Oosthuizen, W., Vorster, H.H., Jerling, J.C., Barnard, H.C., Smuts, C.M., Silvis, N., Kruger, A. and Venter, C.S. (1994) Both fish oil and olive oil lowered plasma fibrinogen in women with high baseline fibrinogen levels. *Thrombosis and Haemostasis* 72, 557–562.

Oubina, P., Sanchez-Muniz, F.J., Rodenas, S., Cuesta, C. (2001) Eicosanoid production, thrombogenic ratio and serum and LDL peroxides in normo- and hypercholesterolaemic post-menopausal women consuming two oleic acid-rich diets with different content of minor components. *British Journal of Nutrition* 85, 41–47.

Perez-Jimenez, F., Castro, P., Lopez-Miranda, J., Paz-Rojas, E., Blanco, A., Lopez-Segura, F., Velasco, F., Marin, C., Fuentes, F. and Ordovas, J.M. (1999) Circulating levels of endothelial function are modulated by dietary monounsaturated fat. *Atherosclerosis* 145, 351–358.

Poston, R.N., Haskard, D.O., Coucher, J.R., Gall, N.P. and Johnson-Tidy, R.R. (1992) Expression of intercellular adhesion molecule-1 in atherosclerotic plaques. *American Journal of Pathology*, 140, 665–673.

Puertollano, M.A., de Pablo, M.A. and Alvarez de Cienfuegos, G. (2002) Relevance of dietary lipids as modulators of immune functions in cells infected with Listeria monocytogenes. *Clinical and Diagnostic Laboratory Immunology* 9, 352–357.

Sanderson, P. and Calder, P.C. (1998) Dietary fish oil diminishes lymphocyte adhesion to macrophage and endothelial monolayers. *Immunology* 94, 79–90.

Sanderson, P., Yaqoob, P. and Calder, P.C. (1995a) Effects of dietary lipid manipulation upon rat spleen lymphocyte functions and the expression of lymphocyte surface molecules. *Journal of Nutritional and Environmental Medicine*, 5, 119–132.

Sanderson, P., Yaqoob, P. and Calder, P.C. (1995b) Effects of dietary lipid manipulation upon graft vs host and host vs graft responses in the rat. *Cellular Immunology*, 164, 240–247.

Tsimikas, S., Philis-Tsimikas, A., Alexoppulos, S., Sigari, F., Lee, C. and Reaven, P.D. (1999) LDL isolated from Greek subjects on a typical diet or from American subjects on an oleate-supplemented diet induces less monocyte chemotaxis and adhesion when exposed to

oxidative stress. *Arteriosclerosis, Thrombosis and Vascular Biology* 19, 122–130.

Virella, G. Fourspring, K., Hyman, B., Haskill-Stroud, R., Long, L., Virella, I., La Via, M., Gross, A.J. and Lopes-Virella, M. (1991) Immunosuppressive effects of fish oil in normal human volunteers: correlation with the in vitro effects of eicosapentaenoic acid on human lymphocytes. *Clinical Immunology and Immunopathology* 61, 161–176.

Visioli, F., Bellomo, G. and Galli, C. (1998a) Free radical-scavenging properties of olive oil polyphenols. *Biochemical and Biophysical Research Communications* 247, 60–64.

Visioli, F., Bellosta, S. and Galli, C. (1998b) Oleuropein, the bitter principle of olives, enhances nitric oxide production by mouse macrophages. *Life Science* 62, 541–546.

Visioli, F., Romani, A., Mulinacci, N., Zarini, S., Conte, D., Vincieri, F.F. and Galli, C. (1999) Antioxidant and other biological activities of olive mill waste. *Journal of Agriculture and Food Chemistry* 47, 3397–3401.

Visioli, F., Caruso, D., Galli, C., Viappiani, S., Galli, G. and Sala, A. (2000) Olive oils rich in natural catecholic phenols decrease isoprostane excretion in humans. *Biochemical and Biophysical Research Communications* 278, 797–799.

Waitzberg, D.L., Lotierzo, P.H., Logullo, A.F., Torrinhas, R.S.M., Pereira, C.C.A. and Meier, R. (2002) Parenteral lipid emulsions and phagocytic systems. *British Journal of Nutrition* 87, S49–S57.

Wallace, F.A., Neely, S.J., Miles, E.A. and Calder, P.C. (2000) Dietary fats affect macrophage–mediated cytotoxicity towards tumour cells. *Immunology and Cell Biology* 78, 40–48.

Yaqoob, P. (1998) Monounsaturated fat and immune function. *Proceedings of the Nutrition Society* 57, 511–520.

Yaqoob, P. (2004) Fatty acids and the immune system: from basic science to clinical applications. *Proceedings of the Nutrition Society* 63, 89–104.

Yaqoob, P., Newsholme, E.A. and Calder, P.C. (1994a) The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation. *Immunology* 82, 603–610.

Yaqoob, P., Newsholme, E.A. and Calder, P.C. (1994b) Inhibition of natural killer cell activity by dietary lipids. *Immunology Letters* 41, 241–247.

Yaqoob, P., Newsholme, E.A. and Calder, P.C. (1995a) The effect of fatty acids on leukocyte subsets and proliferation in whole blood. *Nutrition Research* 15, 297–287.

Yaqoob, P., Newsholme, E.A. and Calder, P.C. (1995b) Influence of cell culture conditions on diet-induced changes in lymphocyte fatty acid composition. *Biochimica et Biophysica Acta* 1255, 333–340.

Yaqoob, P., Knapper, J.A., Webb, D.H., Williams, C.M., Newsholme, E.A. and Calder, P.C. (1998) The effect of olive oil consumption on immune functions in middle-aged men. *American Journal of Clinical Nutrition* 67, 129–135.

Zusman, I., Ben-Hur, H., Budovsky, A., Geva, D., Berman, V., Gurevich, P., Tendler, Y., Lavee, S., Stark, A. and Madar, Z. (2000) Transplacental effects of maternal feeding high fat diets on lipid exchange and response of the splenic lymphoid system in mice offspring exposed to low doses of carcinogen. *International Journal of Molecular Medicine* 6, 337–343.

13 Olive Oil and Regulation of Gastrointestinal Function

MARIANO MAÑAS, MARÍA DOLORES YAGO AND EMILIO MARTÍNEZ-VICTORIA

Institute of Nutrition and Food Technology, University of Granada, Granada, Spain

1. Introduction

The eating habits of people in developed countries have been changing a great deal in recent years. The new eating habits involve diets rich in saturated fat and animal protein, and low in fibre and complex carbohydrates. These bad habits are producing an increase in chronic diseases. For this reason, many governments are promoting dietary recommendations and guidelines for healthy eating. In order to help people follow the recommendations and achieve a good balance of nutrients they have developed food guide pyramids. However, there is a great disparity between different versions of the food guide pyramids. This disparity concerns the kind of oil people use to prepare meals and the position that culinary oils occupy within the pyramids.

Northern Europeans use vegetable oil to cook (sunflower, for example). This oil, rich in polyunsaturated fatty acids (linoleic acid), is located at the top of their food guide pyramid. In fact, the apex of the Northern European pyramid is not a food group but rather a caution to use fats, oils and sweets sparingly. Meanwhile, populations living around the Mediterranean sea use olive oil rich in oleic acid, a monounsaturated fatty acid. The Mediterranean diet pyramid recommends the use of olive oil, virgin if possible, every day (Fig . 13.1).

The Mediterranean diet (which contains up to 40% of calories as fat) is characterized by a high intake of olive oil, in close association with low rates of chronic diseases among the population. Habitual consumption of dietary fats with different degrees of unsaturation appears to have different physiological consequences and effects on health. Nowadays there is substantial evidence supporting the notion that olive oil exhibits numerous biological functions that are beneficial for the state of health. As an example, it appears to have important roles in preventing cardiovascular disorders. In fact, it is known that Mediterranean countries have a lower prevalence of cardiovascular diseases compared with Northern Europe and other developed countries.

Fig. 13.1. The Mediterranean diet pyramid.

In this chapter we will study the effect of this dietary fat on gastrointestinal function and factors involved in its regulation. In the first section, we discuss gastrointestinal function and its regulation, including the digestion and absorption of the dietary fat. In the second section we describe current research about the role of the olive oil and its components on gastrointestinal physiology.

2. Physiology of the Gastrointestinal Tract

The gastrointestinal system includes the gastrointestinal tract (GIT) and the accessory glands (AG) (salivary glands, exocrine pancreas and liver). The major structures in the GIT are the mouth, pharynx, oesophagus, stomach, small intestine, large intestine (colon), rectum and anus (Johnson, 1994; McPhee *et al.*, 1997; Berne *et al.*, 2004) (Fig. 13.2).

The overall function of the gastrointestinal system is to process ingested foods into molecular forms that can be transferred from the external environment to the bloodstream. The digestive process is largely determined by the composition of food ingested. This fact determines the importance of the food and thus the diet, in most aspects of the physiology of the gastrointestinal system, including its regulation.

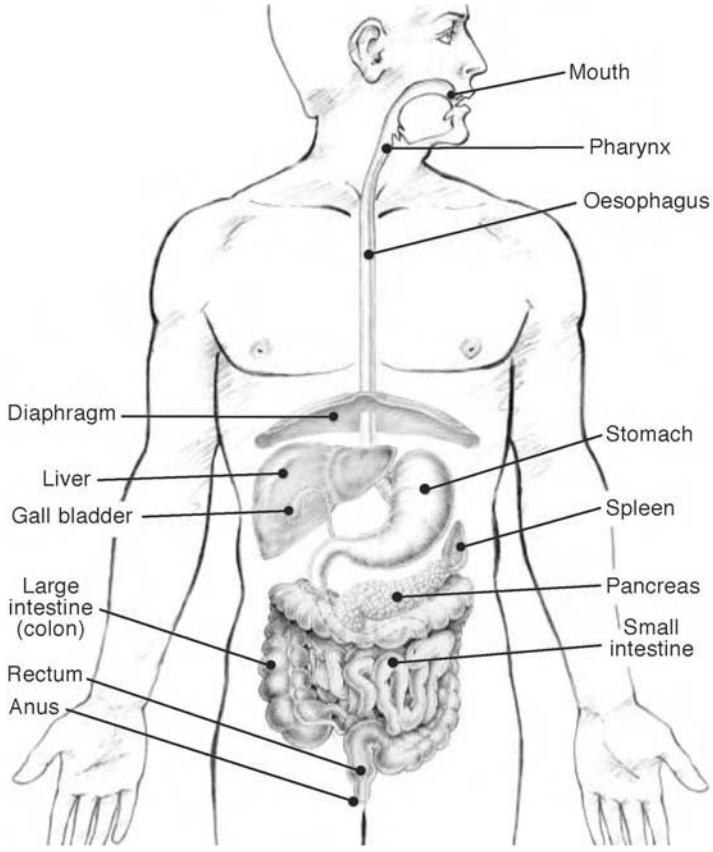


Fig. 13.2. The gastrointestinal system.

2.1. Structure of the gastrointestinal tract wall

The segments of the gastrointestinal tract have a wall consisting of a general layered structure. It has four layers, which are, from inside to outside (Fig. 13.3):

1. Mucosa. Consists of an epithelial layer, the epithelium, an outer connective layer, the lamina propria and a thin layer of longitudinal and circular smooth muscle, the muscularis mucosae.
2. Submucosa. Beneath the mucosa is this second loose connective-tissue layer with collagen and elastin fibers. In some segments of the GIT exocrine glands are present. There are also blood and lymphatic vessels whose branches penetrate into both overlying mucosa and underlying muscular layers. In this layer there is a dense network of nerve cells, which are highly interconnected, the submucosal plexus or Meissner's plexus, a part of the enteric nervous system.
3. Muscularis externa. This has two layers of smooth muscle cells, the inner thick circular layer (muscular cell oriented in a circular pattern) and the thinner outer longitudinal layer (muscular cell oriented in a longitudinal pattern). Between both, there is another highly interconnected nervous network, the

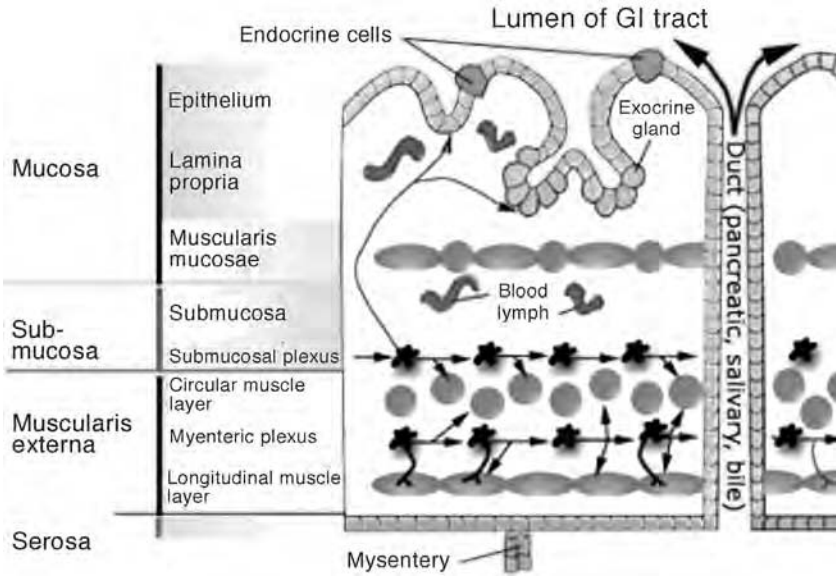


Fig. 13.3. Structure of the gastrointestinal tract wall.

myenteric or Auerbach plexus, the second component of the enteric nervous system. Both nervous plexuses constitute the intrinsic innervation of the GIT.

4. Serosa. The outer layer, consisting mainly of connective tissue and mesothelial cells.

2.2. Overall functions

The physiology of the gastrointestinal system includes four general processes: motility, secretion, digestion and absorption (Fig. 13.4).

The motor function of the GIT includes contractions of smooth muscle of the gastrointestinal wall. This process has two objectives: First, breakdown of the larger particles of ingested food and mixing of the luminal content with the digestive secretions. Second, movement of the luminal content through the tract from oral cavity to anus. The components of motility are: chewing, swallowing, gastric motility and emptying, intestinal motility, gallbladder motility and defecation.

Secretion is a process carried out by glands lining the gastrointestinal wall (gastric and intestinal juices) and by the accessory glands (saliva, pancreatic juice and bile). These secretions contribute to the breakdown of food into smaller molecules.

The ingested food consists of large particles containing macromolecules such as proteins and polysaccharides, which are unable to cross the wall of the GIT. The digestion includes both dissolving and breaking down these foods components (mechanical and chemical digestion).

The molecules produced by digestion then move from the lumen of the GIT, across the epithelial cells of the mucosa and enter the blood or lymph. This process is named absorption.

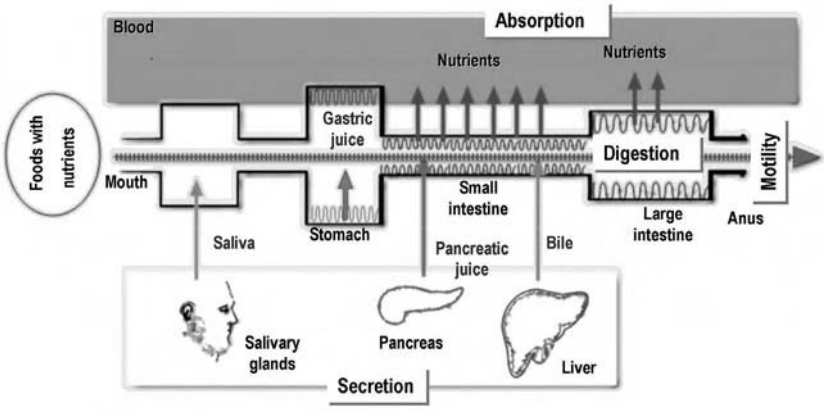


Fig. 13.4. General processes of the gastrointestinal system.

2.3. Regulation

All of these processes are controlled by two basic mechanisms, neural and hormonal.

Unlike other control systems that regulate variables in the internal environment (circulatory or respiratory systems), the control mechanisms of the gastrointestinal system regulate conditions in the lumen of the tract (external environment). With few exceptions, the stimuli that start this control system are the volume and composition of the luminal content. The major luminal stimuli are the distension of the gastrointestinal wall, the chyme acidity and the chyme concentration of the digestion product of macronutrients (monosaccharides, fatty acids, peptides, amino acids, etc.). These stimuli act on the receptor located in the gastrointestinal wall (mechanoreceptors, chemoreceptors and osmoreceptors) to trigger reflexes, neural or hormonal, that influence the effectors (smooth muscle, exocrine or endocrine secretor cells, etc.).

The neural regulation is carried out by the enteric nervous system (intrinsic regulation) and by the autonomic nervous system (sympathetic and parasympathetic divisions) (extrinsic regulation). The intrinsic regulation uses two types of neural reflex arc: short reflexes, from receptors of the gastrointestinal wall through the intrinsic nerve plexuses to effector cells (smooth muscle, secretor, vascular and immune cells), and long reflexes from receptor on the gastrointestinal tract to the central nervous system by way of afferent nerves and back to the intramural nervous plexuses and effector cells by way of the autonomic nerve fibres. Most of these reflexes are initiated by signals within the tract (luminal signals) and also by other external signals, such as the sight and smell of food.

The hormonal regulation is carried out by hormones secreted by cells scattered throughout the epithelium of the GIT and not clustered into discrete organs like other endocrine glands (for example the thyroid gland). The mechanisms involved can be endocrine, paracrine and neurocrine.

Today, we know several gastrointestinal hormones secreted in different regions of the gastrointestinal tract (listed in Table 13.1). The well-established

Table 13.1. Gastrointestinal hormones.

Localization of endocrine cells	Hormone
Stomach	Gastrin
	Somatostatin
	CGRP
	Ghrelin
Duodenum-jejunum	Secretin
	Cholecistokinin (CCK)
	Motilin
	GIP (Gastric Inhibitory Polypeptide)*
	Somatostatin
	VIP
Pancreatic islets	Insulin
	Glucagon
	Pancreatic polypeptide
	Somatostatin
	Enteroglucagon (glucagons-like peptides)
Ileum-colon	Enteroglucagon (glucagons-like peptides)
	PYY
	Neurotensin
	Somatostatin

*Glucose-dependent insulinotropic polypeptide.

hormones are: secretin, cholecystokinin (CCK), gastrin, GIP (gastric inhibitory peptide or glucose-dependent insulinotropic peptide) and motilin. Gastrin stimulates gastric acid secretion and proliferation of gastric epithelium. Secretin stimulates secretion of water and bicarbonate from the pancreas and bile ducts. CCK stimulates secretion of pancreatic enzymes, and contraction and emptying of the gall bladder and relaxes the sphincter of Oddi. GIP inhibits gastric secretion and motility and potentiates release of insulin from beta cells in response to elevated blood glucose concentration. Motilin apparently is involved in stimulating housekeeping patterns of motility (migrating motor complex) in the stomach and small intestine. There are other candidate hormones, such as PYY, PP, enteroglucagon, etc.

In summary, the food ingested determines the gastrointestinal function not only in health but also in disease. Then, foods can have a preventive and therapeutic role in different pathologic disturbances of the gastrointestinal tract. This has been demonstrated in epidemiologic, clinical and experimental studies.

2.4. Digestion and absorption of the dietary fat

Olive oil is a vegetable oil that is mainly ingested as added fat in the diet of the Mediterranean countries. Unlike vegetable seed oils or fish oil, which have a high content of n-6 and n-3 polyunsaturated fatty acids (PUFAs) respectively, or animal fat from ruminants and some vegetable fats (e.g. coconut or palm oil) that are rich in saturated fatty acids, olive oil has a high content of monounsaturated

fatty acid (oleic acid, C18:1 n-9). Moreover, virgin olive oil, in its insaponificable fraction has α-tocopherol (with vitamin E activity), β-carotene (with vitamin A activity) and a number of phenolic compounds with antioxidant activity.

The triacylglycerols, the major component of the dietary fat, are insoluble in water and form larger lipid droplets. Because lipases are hydrophilic enzymes that act only at the surface of the lipid droplet, for an adequate rate of fat digestion it is necessary to increase the surface area through the emulsification of the larger droplets to smaller ones with a diameter of 1 mm. This process requires the mechanical disruption of the large droplets into smaller droplets and an emulsifying agent that prevents the reaggregation of the smaller droplets. The mechanical disruption is provided by the motility of the pyloric antrum of the stomach and the motor pattern of the small intestine. The major emulsifying agents are food phospholipids and the phospholipids and bile salt present in the secreted bile. Both molecules are amphipathic, since they contain both hydrophobic (lipid soluble) and polar (hydrophilic) faces. These molecules have a detergent action towards particles of dietary fat, which causes fat globules to become emulsified into minute, microscopic droplets. Emulsification is not digestion *per se*, but is of importance because it greatly increases the surface area of fat, making it available for digestion by lipases, which cannot access the inside of lipid droplets (Fig. 13.5).

Fats are digested by catalytic hydrolysis. Pancreatic lipase hydrolyses triacylglycerols to give free fatty acids and 2-monoglycerides. Phospholipases (A_2) hydrolyse phospholipids to 1-lysophospholipids and fatty acids. Cholesterol esterase hydrolyses cholesterol esters to cholesterol and fatty acids.

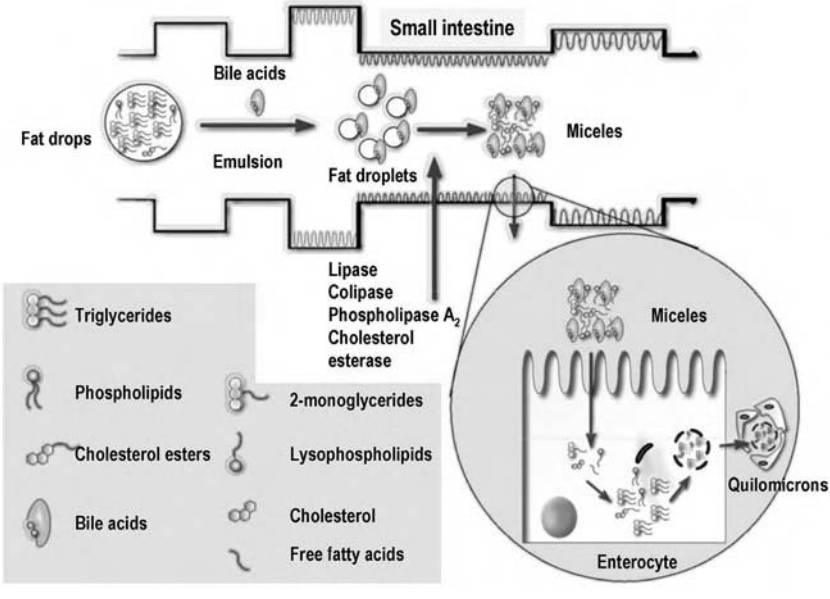


Fig. 13.5. Digestion and absorption of dietary fats.

Free fatty acids and monoglycerides are not water soluble either. A further action of bile is the formation of micelles. The micelle consists of aggregations of free fatty acids, mono-acylglycerols in the middle with the polar ends of the bile salts and phospholipids enabling solution in water. The micelles serve the function of 'shuttling' products of fat digestion from the site of digestion to the brush border, where they can be absorbed into the enterocyte. This serves two purposes. First it removes the products of fat digestion so that they do not inhibit the action of the lipase (product inhibition) and secondly it transports the insoluble digestion products to the cell membrane where they can diffuse directly into the cell (Fig. 13.5).

Inside the intestinal epithelial cells, in the vesicles of the smooth endoplasmic reticulum, the lipids are reprocessed and the 2-monoglycerides are re-esterified, lysophospholipids are reconverted to phospholipids and most of the cholesterol is re-esterified. The processed lipids, along with those that are synthesized *de novo*, combine with proteins to form chylomicrons that enter the bloodstream at the thoracic vena cava, via lymphatic draining the gut. A large body of evidence suggests that olive oil influences the formation of chylomicrons and postprandial lipaemia (see Chapter 8).

3. Olive Oil and the Digestive System

While the protective effect of olive oil against cardiovascular diseases is well documented, few reports exist about the possible favourable effects of olive oil on the gastrointestinal system.

We found that there was a lack of scientific support for a well known phenomenon of Mediterranean people, the health and therapeutic benefits of olive oil on gastrointestinal system mentioned by Hippocrates in ancient times. Moreover, there were no comparative studies investigating the influence of different types of dietary fat on this system. It was for this reason that we decided to initiate our research in this field. At the Institute of Nutrition and Food Technology in Granada, our group has been working on this issue since 1980, particularly on two aspects: First, the plasma profile of gastrointestinal peptides after long-term adaptation to dietary fats of different degrees of saturation, which in turn influences gastric, biliary and pancreatic secretion. Second, the adaptation of pancreatic acinar cell membranes to the type of dietary fat and the subsequent effects on signal transduction pathways and cellular responses (enzyme release) evoked by secretagogues such as CCK and ACh.

3.1. Olive oil and gastrointestinal secretions

Over the past years, the general population has been encouraged to reduce the consumption of food items with a high content of saturated fat in the hope of minimizing the risk of several degenerative diseases. When examining the biological effects of dietary fat, a first level to consider is the gastrointestinal (GI) tract. Investigations performed in our laboratory throughout the past decade in

human and animal models show that, compared with other dietary fats, habitual consumption of virgin olive oil affects the circulating levels of several gut peptides, including gastrin, cholecystokinin (CCK), pancreatic polypeptide (PP), secretin, somatostatin and peptide YY (PYY), both at fasting state and in response to meal ingestion. Given the role of these hormones in the regulation of gastric, pancreatic and biliary secretion, the existence of clear functional effects was an expected finding.

3.1.1. Studies in animal models

3.1.1.1. PANCREATIC AND BILE SECRETION IN DOGS. This work was carried out in mongrel dogs of both sexes weighing 15–25 kg. The animals were weaned and randomly assigned to one of two experimental groups, an olive oil (O) group and sunflower oil (S) group. They were fed for 8 months on diets in which the source of fat was olive oil and sunflower oil, respectively. The diets were isoenergetic and isonitrogenous and thus differed only in their fatty acid composition. After the 8-month adaptation period, the exocrine pancreatic secretion was studied according to the experimental design shown in Table 13.2 and Fig. 13.6.

The results of this work (Ballesta *et al.*, 1990a) showed that the exocrine pancreatic secretory activity in response to food was greater in dogs fed on a diet rich in sunflower oil than in animals given the same diet with virgin olive oil. Parallel experiments undertaken by our group (Ballesta *et al.*, 1991, 1990b) indicated not only that there were no differences between groups O and S in digestive utilization of dietary fat and protein, but that in some cases the indices of nutritive utilization were actually higher in dogs fed the virgin olive oil diet. Such adaptation seems to be a key advantage in terms of exocrine pancreatic economy, at least in this species.

The different responses to food ingestion may be explained in terms of balance between those factors that stimulate and inhibit postprandial pancreatic secretion. It is known that oleic acid (the major fatty acid in the diet given to group O) is a potent stimulant of CCK and secretin release, both of which are strong pancreatic secretagogues. However, this monounsaturated fatty acid is also the most effective releaser of gastrointestinal peptides that inhibit exocrine pancreatic secretion, including PYY and PP. This suggests that reduced secretion of pancreatic juice in the animals of group O may have resulted from the balance between the stimulating (CCK and secretin) and inhibiting (PYY and PP) effects of oleic acid on pancreatic secretion. Confirmation of this point came

Table 13.2. Fatty acid composition of experimental diets.

Fatty acid (%)	Olive oil diet	Sunflower seed oil diet
16:0 (palmitic acid)	11.7	9.1
18:0 (stearic acid)	2.4	3.5
18:1*n*-9 (oleic acid)	60.0	25.5
18:2*n*-6 (linoleic acid)	15.3	56.3
18:3*n*-3 (linolenic acid)	0.8	2.3

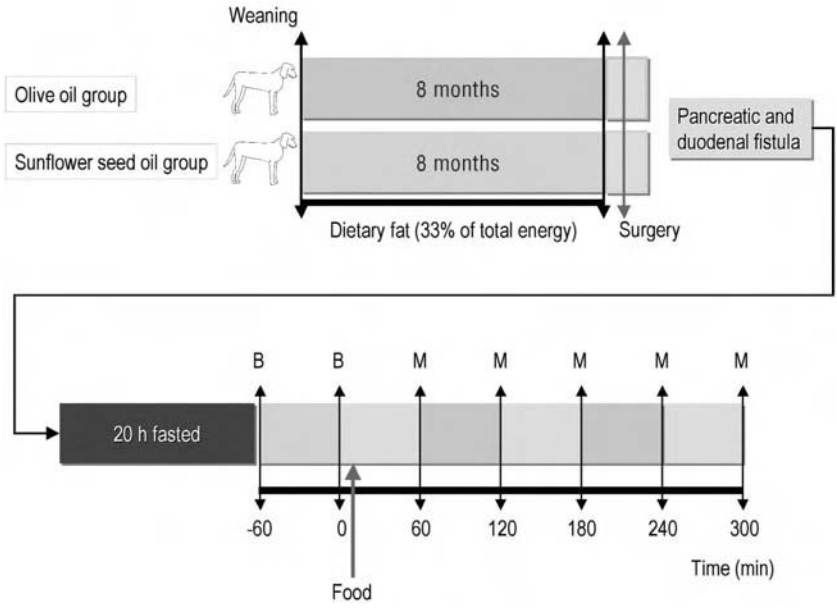


Fig. 13.6. Experimental design of the studies in dogs.

later, when we found higher blood concentration of the above inhibitory hormones in dogs previously submitted to a 6-month feeding period on olive oil diets as compared with animals that received sunflower oil (Yago *et al.*, 1997a).

According to these results, it seems that adaptation of the pancreas to the type of dietary fat is associated with the existence of differences in the circulating levels of several gastrointestinal hormones, either at rest or in response to food ingestion, which in turn may act on the synthesis or the secretion of enzymes and other constituents of the juice.

In an attempt to study further this phenomenon, we examined whether or not a similar adaptation period with lower levels of fat (sunflower and olive oil) exerted the same effects described above, i.e. if the changes were related not only to the type but also to the amount of dietary fat (Yago *et al.*, 1997b). The results suggested that there could be a direct relationship between the amount of oleic acid in the diet and the plasma concentration of both inhibitory peptides PP and PYY.

Concerning the study of biliary adaptation to dietary fats (Ballesta *et al.*, 1992), our purpose was to determine whether the long-term intake of high-fat diets differing in the degree of unsaturation influenced bile flow and the content of cholesterol and bile acids in the bile secreted.

Chronic intake of either olive oil or sunflower oil evoked different effects on *in vivo* secretory activity. The time-course changes of bile flow, bile acid concentration and bile acid output after the ingestion of food showed a different pattern in each group. A greater involvement of the gallbladder in animals fed on the olive oil diet was suggested, as most clearly shown by the marked rise in bile acid concentration and output after eating. This pronounced elevation over basal was likely responsible for the prolonged bile flow in this group, because a greater pool of bile acids

entering the small intestine would raise the efficiency of the enterohepatic circulation of these anions. That the type of dietary fat can affect gallbladder emptying in response to food is a logical finding if we consider that oleic acid, the major fatty acid in olive oil, is the most potent CCK releaser known to date.

The gastrointestinal peptide CCK causes gallbladder contraction and relaxation of the sphincter of Oddi, thus allowing concentrated bile (rich in bile acids) to flow into the duodenum, emulsify the fat and form micelles. There is a positive linear relationship between CCK release and gallbladder contraction. Higher CCK release in response to food has been associated with stronger gallbladder emptying. In addition, decreased postprandial gallbladder emptying has been suggested to play a key role in the development of gallstones. Therefore, dietary fat may be important in the pathogenesis of vesicular stasis.

Polyunsaturated fatty acids have been shown to raise the biliary secretion of cholesterol, a finding that may be related to their presumed hypocholesterolaemic effect. In our experimental conditions, some differences were found between the diet rich in polyunsaturated fatty acids and that rich in monounsaturated fatty acids with respect to cholesterol content and output. However, the biliary cholesterol content in response to food intake was higher throughout the experiments in dogs given olive oil as the source of dietary fat. Thus, our results indicated a hypocholesterolaemic effect of olive oil compared with sunflower oil.

3.1.1.2. PANCREATIC SECRETION IN RATS. *IN VIVO* AND *IN VITRO* STUDIES. The mechanisms of pancreatic adaptation to the type of dietary fat are unclear. According to our data, it seems to be associated with the existence of differences in the circulating levels of several gastrointestinal hormones thereby influencing the synthesis or secretion of enzymes and other constituents. A second possibility is that dietary fat composition can change the responsiveness of the pancreas to circulating secretagogues.

It has been demonstrated in different tissues that the lipid profile of the diet can influence the fatty acid composition of cell membranes, evoking a modification of cell function. Regarding the exocrine pancreas information on this topic is very limited. To our knowledge, no-one except for Beaudoin *et al.* (1989) has examined in the rat the effects of feeding different dietary fats on *in vivo* pancreatic responses. The fats employed by these authors are seldom used for cooking purposes in our geographical area. For this reason it was pertinent to confirm if different types of lipids could affect exocrine pancreatic secretion in anaesthetized rats (Díaz *et al.*, 2003).

We decided to compare the effects of virgin olive oil, a typical component of the Mediterranean diet and a good source of monounsaturated fatty acids (MUFAs), with those of sunflower oil, which is rich in polyunsaturated fatty acids (PUFAs). Both oils compete in Southern European markets for consumers' preference. To achieve our objective, two separate groups of weaning rats were fed the experimental diets for 8 weeks. We then examined resting and CCK-8-stimulated exocrine pancreatic secretion in the cannulated anaesthetized animal. We also analysed total protein and amylase content of the pancreas. Furthermore, in order to demonstrate a direct effect of the type of dietary fat, the fatty acid composition of pancreatic membranes was determined after feeding the respective diets.

This investigation showed that chronic intake of diets differing only in the type of fat added (olive oil or sunflower oil) influences the fatty acid profile of pancreatic cell membranes. Rats fed the olive oil diet showed higher levels of oleic acid, whereas those fed sunflower oil had increased linoleic acid and PUFA n-6 content (Table 13.3).

Exocrine pancreatic secretion in anaesthetized animals was also affected (Diaz *et al.*, 2003). The differences between dietary treatments concerned (i) the magnitude of the secretion of fluid and amylase in resting conditions; (ii) the time-course of changes in all major secretory parameters evoked by a continuous intravenous infusion of CCK-8 (Fig. 13.7). Undoubtedly, much work is required to determine the mechanism by which changes in the composition of dietary fat modulates exocrine pancreatic secretion in the whole animal. However, the possibility exists that these *in vivo* data are reflecting a distinct modulating effect of the type of dietary fat on the secretory activity at the cellular level.

This notion is reinforced by the results of recent investigations (Yago *et al.*, 2004) using viable pancreatic acinar cells isolated from rats kept on an identical dietary protocol. We isolated pancreatic plasma membranes and determined their fatty acid composition. In addition, amylase secretion and cytosolic concentration of free calcium $[Ca^{2+}]c$ were measured in freshly prepared pancreatic acini. It was shown that membrane fatty acids were profoundly affected by the diets; the rats fed the olive oil diet had higher levels of 18:ln-9 (42.86%) and total MUFA compared with the animals fed the sunflower oil diet (25.37%). Reciprocally, the sunflower oil diet resulted in greater levels of total and n-6 PUFA than the olive oil diet. The most striking effect was observed for 18:2n-6 (group S: 17.88%; group O: 4.45%), although the levels of 20:4n-6 were also different. The proportion of total saturated fatty acids was similar in both groups, and there was only a slight, not significant, effect on the unsaturation index.

Table 13.3. Fatty acid composition of pancreatic cell membranes in rats fed diets containing different dietary fats.

Fatty acid	Olive oil group	Sunflower oil group
16:0	25.04 ± 0.50	26.39 ± 1.22
16:1*n*-7	5.09 ± 0.45	4.17 ± 0.43
18:0	6.87 ± 0.65*	10.21 ± 0.92
18:1*n*-9	43.09 ± 2.19***	24.90 ± 1.08
18:2*n*-6	4.58 ± 0.65***	18.58 ± 1.20
18:3*n*-3	0.40 ± 0.05	0.32 ± 0.08
SFA	39.12 ± 2.49	39.66 ± 2.04
MUFA	48.30 ± 2.52***	29.20 ± 1.41
PUFA	12.58 ± 1.49***	31.14 ± 2.04
PUFA*n*-6	10.89 ± 1.34***	29.29 ± 1.99
UI	2.43 ± 0.22	3.15 ± 0.30

Results are expressed as percentage of total fatty acid content. Values are mean ± SEM. By row, values with asterisks are significantly different *vs* sunflower oil group: *$P < 0.05$; **$P < 0.005$; ***$P < 0.001$ (Student's *t*-test). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UI, unsaturation index, calculated according to: UI = [sum(fatty acid per cent)*(number of double bonds)]/saturated fatty acids per cent.

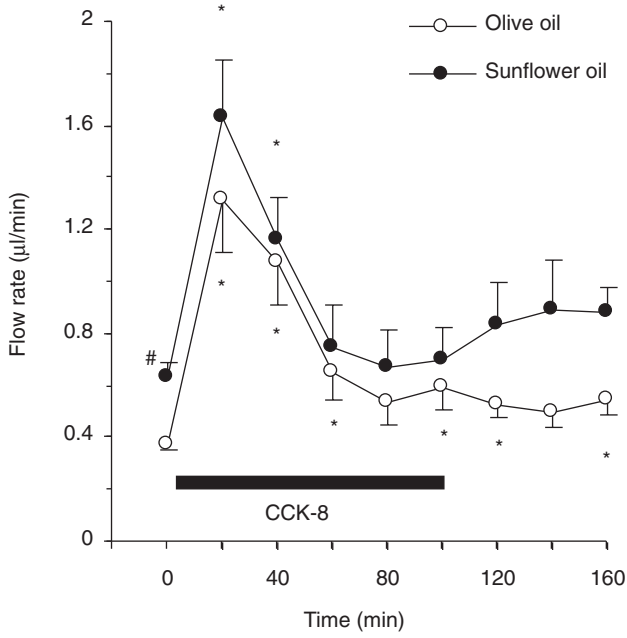


Fig. 13.7. Time-course changes in CCK-8-evoked pancreatic flow rate in anaesthetized rats fed diets containing different dietary fats. Time O represents the baseline situation. *$P < 0.05$ as compared with the respective baseline value. #$P < 0.05$ between the two dietary groups.

A very interesting finding was that, compared with the olive oil group, acinar cells from rats fed sunflower oil secreted more amylase at rest but less in response to CCK-8, and this was paralleled by reduced $[Ca^{2+}]c$ responses to the secretagogue.

The dose–response curve for CCK-8-induced amylase release in pancreatic acinar cells is typically bell-shaped, reaching a maximum at around 0.1 nM. Our results were consistent with those in the literature, since the strongest secretory effect was observed in our study at 0.1 nM CCK-8, and a characteristic decrease occurred after the addition of higher concentrations. This pattern was followed in cells from both the olive oil and sunflower seed-oil groups (Fig. 13.8). Quantitatively, however, marked differences were revealed between the two dietary groups. Thus, values for basal (unstimulated) amylase release in acini from rats fed the olive oil diet were similar to those reported by most authors employing chow-fed rats whereas release in cells from the sunflower oil-fed rats was markedly higher. The reason for this effect is unknown. High basal values of amylase release have been found in acinar cells from pancreatitic rats and the authors related this finding to increased $[Ca^{2+}]c$. In our study, basal $[Ca^{2+}]c$ values were comparable in acini from both groups, so they can not account for the differences in amylase release in unstimulated conditions. It is possible that different membrane composition is modifying the permeability for amylase.

In contrast to the observations in basal conditions, net amylase secretion in response to all concentrations of CCK-8 was drastically reduced after sunflower-oil feeding (Fig. 13.8). This diminished secretory activity may be explained, with-

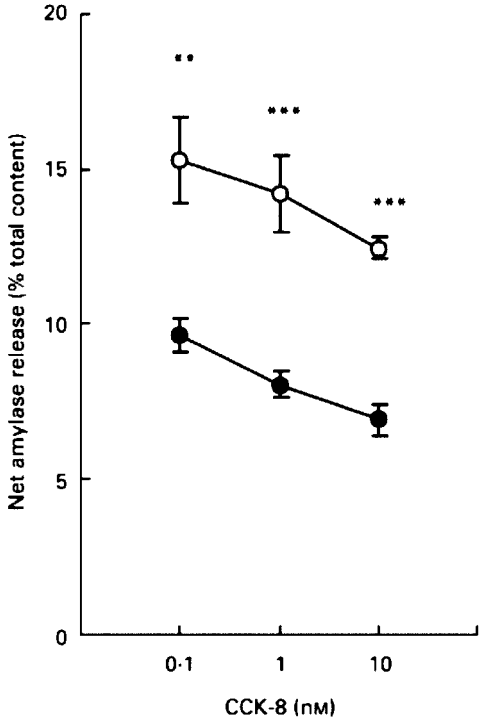


Fig. 13.8. Net amylase release (increase above basal) stimulated by cholecystokinin octapeptide (CCK-8) in pancreatic acini isolated from rats fed diets containing either virgin olive oil (-○-) or sunflower seed oil (-●-) as the fat source. Mean values were significantly different between dietary groups: $^{**}P < 0.05$, $^{***}P < 0.005$.

out excluding other components of the secretory pathway, by the attenuation of CCK-8-evoked $[Ca^{2+}]c$ responses in the sunflower seed-oil group (Fig. 13.9). Moreover, the fact that not only the absolute value of the $[Ca^{2+}]c$ peak but also the peak increase over basal is lower in cells from the sunflower-oil fed rats suggests a reduction in the filling state of CCK-8-releasable Ca^{2+} pools and/or a limitation in the production or effectiveness of the mediators that participate in the Ca^{2+} mobilization pathways.

The differences we found in acinar secretory activity and intracellular Ca^{2+} mobilization (Yago *et al.*, 2004) were most probably related to the dietary-induced changes in cell membrane composition. Lipids are emerging as potent regulators of cell function. At the level of CCK-8-evoked transduction pathways in acinar cells, this regulatory action of lipids could potentially involve many steps of the cascade because of the properties they confer on biological membranes or due to their performance as intracellular mediators themselves:

1. Dietary fats differing in the degree of unsaturation have been shown to modify insulin binding in rat adipocytes and the activity of membrane-associated enzymes in liver, heart and submandibular glands. Many steps of the stimulus-secretion coupling process in acinar cells are membrane-dependent. Although

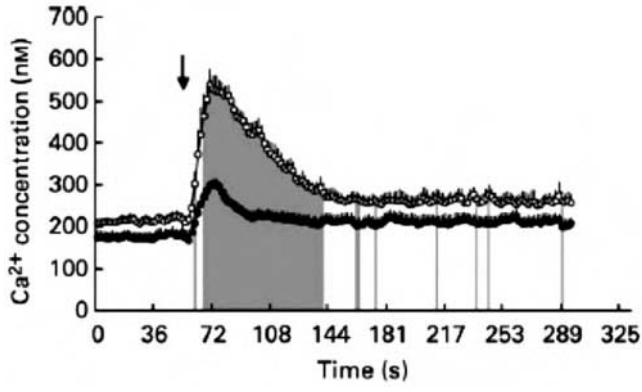


Fig. 13.9. Time-course changes in intracellular free Ca^{2+} concentration evoked by 0·1 nM, 0·1 nM-cholecystokinin octapeptide (CCK-8) in suspensions of fura-2-loaded pancreatic acinar cells isolated from rats fed diets containing either virgin olive oil (-○-) or sunflower seed oil (-●-) as the fat source. The arrow indicates the point of CCK-8 addition. Shaded areas represent significant differences ($P < 0.005$) between groups.

our results do not support gross modifications of whole-membrane fluidity, differential enrichment in certain fatty acids may influence the accessibility of the CCK receptor, the interaction with G proteins or the functionality of such enzymes as phospholipases and protein kinase C which are known to interact with cell membranes during their activation.

2. Apart from their structural role, membrane fatty acids participate themselves as mediators in signal transduction. The CCK receptor in rat pancreatic acinar cells can display two binding (high and low) affinity states. Moreover, CCK occupancy of high and low affinity sites is thought to be related to the initiation of different intracellular events and consequent biological responses.

The moderately high concentrations of CCK-8 used in our study probably stimulated low-affinity sites and initiated a route linked to both phospholipase C (PLC) and phospholipase D. PLC activation involves the hydrolysis of phosphatidylinositol bisphosphate and subsequent production of inositol trisphosphate (IP3), which initiates the Ca^{2+} signal, and diacylglycerol. The membrane modifications after olive oil and sunflower oil intake could reasonably have involved an alteration in the phosphoinositide turnover and a change in the supply of inositol lipid precursors of IP3. Reduced production of IP3 in acini from rats fed sunflower oil might explain the diminished Ca^{2+} peaks in response to CCK-8, since the initial rise in Ca^{2+} transients is mainly due to Ca^{2+} released from IP3-sensitive internal stores. Alternatively, it is tempting to speculate that diacylglycerol, abundantly generated via PLC and phospholipase D, and possibly with different acyl moieties as a consequence of the changes in the membrane, may have resulted in differential activation of protein kinase C, a crucial modulator of the secretory machinery of acinar cells. This is strongly supported by the finding in guinea-pig epidermis that diacylglycerol with a 18:2n-6 metabolite at the 2-position inhibited protein kinase C isozymes compared with 1,2-dioleylglycerol.

In conclusion, our results indicate that the type of dietary fat strongly influences the fatty acid composition of rat pancreatic cell membranes and this is associated with a change in the secretory activity and intracellular Ca^{2+} mobilization stimulated by CCK-8 in viable pancreatic acini.

More recently, we have confirmed the effects of the olive oil by using an inverted fluorescence microscope attached to a continuous perfusion system to study cellular Ca^{2+} homeostasis in single cells isolated from rats fed a diet containing virgin olive oil. A group fed a commercial chow was used as control (Martínez *et al.*, 2004). Feeding diets rich in virgin olive oil did not significantly alter the resting $[Ca^{2+}]c$ values or basal amylase secretion. However, both the Ca^{2+} oscillations and the large Ca^{2+} transients in response, respectively, to low (physiologic) and high concentrations of CCK-8 were significantly enhanced by the olive oil diet compared with the control one. These effects on Ca^{2+} mobilization correlated, to a great extent, with CCK-8-evoked amylase secretory activity.

3.1.2. Human studies

There have been very few studies to examine the effects of diets of different composition on the human exocrine pancreas, and those available have focused on the influence of quantity (not quality) of the three major nutritional components in the diet.

Patients were divided into two experimental groups, an olive oil group and a sunflower oil group, according to their dietary habits and specifically to the type of dietary fat habitually consumed before the study. This information was gained from a dietary history interview performed at the beginning of the study.

The subjects from both groups were told at the start of the study to consume their habitual diets for the 30-day period immediately before surgery, with these requirements: (i) the only source of dietary fat used to prepare their meals had to be olive oil (group O) or sunflower oil (group S); (ii) subjects had to avoid eating food items high in saturated fat (butter, sausages, etc.). Compliance with the diets was checked by completion of four 7-day dietary records. After the 30-day adaptation period we studied the plasma profile of gastrointestinal peptides and examined pancreatic, biliary and gastric secretions before and after the administration of liquid meals that included the corresponding oil.

The test meals (pH 6.33; 294 mosm/l; 1 kcal/ml) were composed of 17% energy as protein, 30% as fat, and 53% as carbohydrate, in addition to vitamins and minerals. The meals were isoenergetic and isonitrogenous, contained no measurable amounts of cholesterol and phospholipids, and were prepared by mixing the separate modular components lactalbumin, maltodextrins, and a vitamin-mineral mixture. Olive oil was added to the meal given to the olive oil group and sunflower oil to the sunflower oil group (Table 13.4).

Each subject was studied on 2 consecutive days and after at least 8 hours of fasting. Duodenal samples were taken immediately before, and at 30, 60, 120 and 180 min after beginning the ingestion of the liquid meal (200 ml ingested over 30 min). The complete feeding and sampling procedure was repeated on the second experimental day, each individual receiving the same meal as the day before (Fig. 13.10).

Table 13.4. Fatty-acid composition of the liquid test meals, expressed as g/100 g total fatty acids.

	Group S	Group O
18:1*n*-9	29.03 ± 0.66[2]	61.89 ± 2.00
18:2*n*-6	42.16 ± 1.70[2]	5.06 ± 0.12
Total monounsaturated	29.67 ± 0.66[2]	63.08 ± 1.95
Total polyunsaturated	44.62 ± 1.55[2]	8.19 ± 0.14
Total saturated	25.93 ± 1.93[NS]	29.03 ± 1.86
Total unsaturated/total saturated	2.99 ± 0.30	2.51 ± 0.23

Results are mean ± SE. [2] Significant differences ($P < 0.05$) between the two diets; [NS] not significant.

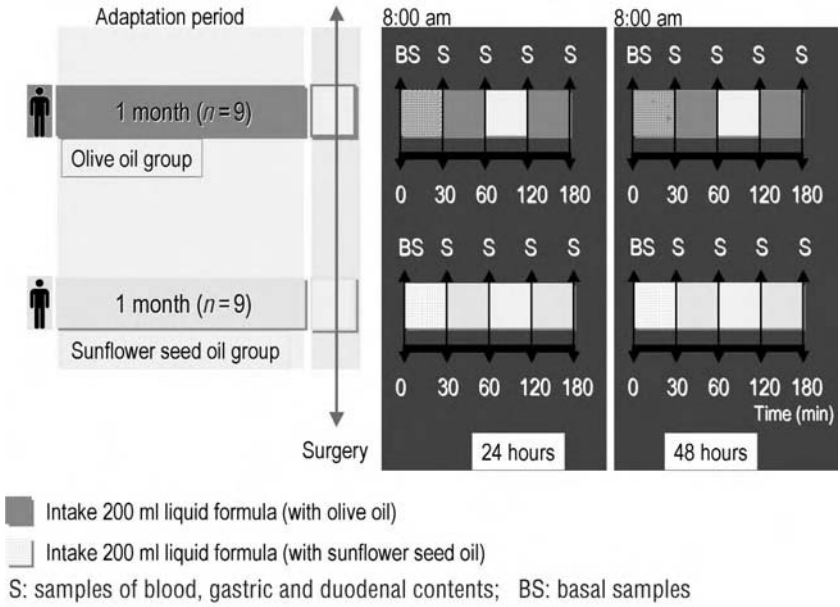


Fig. 13.10. Experimental design of the studies in humans.

3.1.2.1. OLIVE OIL AND GASTROINTESTINAL HORMONES. We observed no differences between the two groups in the plasma level of secretin and somatostatin, neither at rest nor after the administration of the liquid meal (Yago *et al.*, 1998). In contrast, meal-stimulated CCK levels were higher in the olive oil group than in the sunflower oil one throughout the entire postprandial period (Yago *et al.*, 1997c). The type of dietary fat therefore affects postprandial CCK release in response to food (Fig. 13.11), which agrees with the above-mentioned observation that oleic acid, the major fatty acid in olive oil, is one of the most potent stimulants for CCK release. Greater plasma concentrations of PYY and PP were also noted in the subjects given the olive oil diet (Fig. 13.11) (Yago *et al.*, 1997c; Serrano *et al.*, 1998).

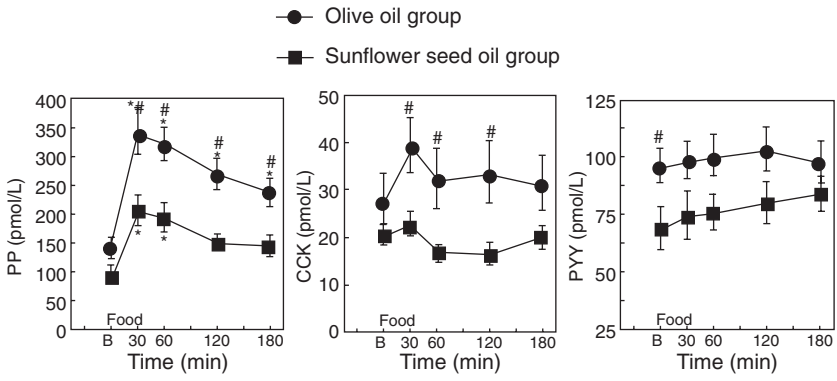


Fig. 13.11. Plasma levels of PP, CCK and PYY in humans, in response to intake of the olive oil and sunflower seed oil rich liquids diets. * Statistically significant differences versus basal values; # statistically differences between experimental groups.

In the dog, PYY has been shown to inhibit CCK release and in dogs and rats it seems that postprandial release of PYY is mediated by CCK. These reports propose an interesting auto regulatory feedback loop whereby CCK-stimulated PYY release would serve to inhibit the secretagogue effect of postprandially released CCK. In our study (Yago *et al.*, 1997c), the plasma PYY concentration in Group O subjects was consistently higher than in Group S, both in the fasting state and after food ingestion. In addition, we were able to demonstrate in Group O, but not in Group S, a positive correlation between CCK and PYY. This suggests that, also in humans, postprandial PYY release is mediated at least in part by CCK.

Information about this link between CCK and PYY in humans is very scarce. Besides, no studies have dealt with the fact that the degree of unsaturation of dietary fat (olive oil – rich in monounsaturated fatty acids, or sunflower oil – a fat source of high polyunsaturated fatty acid content) could have quite different effects on the release of these hormones.

Nevertheless, a direct stimulatory effect of fat on the PYY endocrine cell in the ileocolonic mucosa cannot be discarded, given that distal perfusions with oleic acid can evoke PYY release. In fact, the correlation CCK-PYY was non-existent in fasting conditions: plasma PYY concentration, in contrast to that of CCK, was significantly higher in Group O than in Group S. We also found higher plasma PYY levels in fasted dogs previously fed over 6 months on diets containing olive oil as the fat source, compared with the group of animals given sunflower oil (Yago *et al.*, 1997a).

Thus, the main conclusions of this part of our work are: (i) dietary fats of different degrees of saturation affect plasma CCK and PYY levels in humans and (ii) postprandial PYY release in olive oil group is mediated, at least in part, by CCK as has been previously described in dogs and rats.

Gastrointestinal function depends on a complex interplay between nerves and hormones. Among the latter, pancreatic polypeptide (PP) is believed to have important regulatory roles at multiple levels. Although PP release is governed by a number of factors, the presence of fat in the intestine has been demonstrated to increase the plasma concentration of the hormone. Indeed, our study in humans (Serrano *et al.*, 1998), revealed that administration of the liquid meals caused a

high initial rise followed by a prolonged secondary phase, with hormone levels significantly higher in Group O throughout the entire postprandial period as compared with Group S. The meals were identical except for their fatty acid profile, so there must be some influence of the type of dietary fat upon the plasma concentrations of PP. This is consistent with the reported effects of polyunsaturated and monounsaturated fats as weak and strong stimulants, respectively, for PP secretion in human intestinal perfusion studies. Again, the fact that postprandial levels of both CCK and PP were significantly higher in the subjects given the meal containing olive oil (Group O) is compatible with the evidence of the role of CCK as the most important hormonal mediator in the intestinal phase of PP release.

Our observation that the type of fat differentially affects the secretion of a number of gastrointestinal hormones in humans could be of use in designing enteral diets for diverse gastrointestinal diseases.

3.1.2.2. OLIVE OIL AND SECRETION OF PANCREATIC ENZYMES. We investigated in human subjects previously kept on diets that contained olive oil (group O) or sunflower oil (group S) as the main source of dietary fat for 30 days whether or not the ingestion of liquid meals that included the corresponding oil led to differences in pancreatic enzyme activities in the duodenum (Yago *et al.*, 1997d).

Duodenal enzyme activities (lipase, colipase, amylase, trypsin and chymotrypsin) in resting conditions were similar in the two dietary groups. No significant differences were revealed in postprandial enzyme activities, except for lipase activity.

The presence of food in the digestive tract in group O induced an increase in the lipase activity of the duodenal content, peaking 30 min after starting the meal and remaining significantly elevated until the first hour. In group S, lipase activity followed a similar pattern although an acute decrease was observed after the first 30 min, and values remained low until the end of the study. As a consequence, duodenal lipase activity was significantly higher in group O than in group S at 60 min after the ingestion of the test-meal (Fig. 13.12). We believe that this difference may be related to the higher postprandial levels of CCK observed in group O. The greater suppression of meal-stimulated lipase output by the CCK receptor antagonist, loxiglumide, in relation to other pancreatic enzymes, such as trypsin, observed in human acute experiments seems to be in keeping with the former idea.

Taken together, our experiments did not reveal a great influence of olive oil on pancreatic enzymes (except for lipase activity) in human duodenal contents. An explanation could be that longer periods of time are needed for the human pancreas to be affected by dietary fat.

3.1.2.3. OLIVE OIL AND BILIARY SECRETION. The overall impact of the type of dietary fat on bile lipids and lithogenesis in humans is unclear, although epidemiological, clinical and animal studies indicate an important role of this dietary component. A key limitation of animal studies, however, is the existence of marked differences in biliary lipid homeostasis compared with humans, i.e., most animal species readily convert any excess cholesterol to bile acids so biliary cholesterol concentration seldom approaches the saturation point.

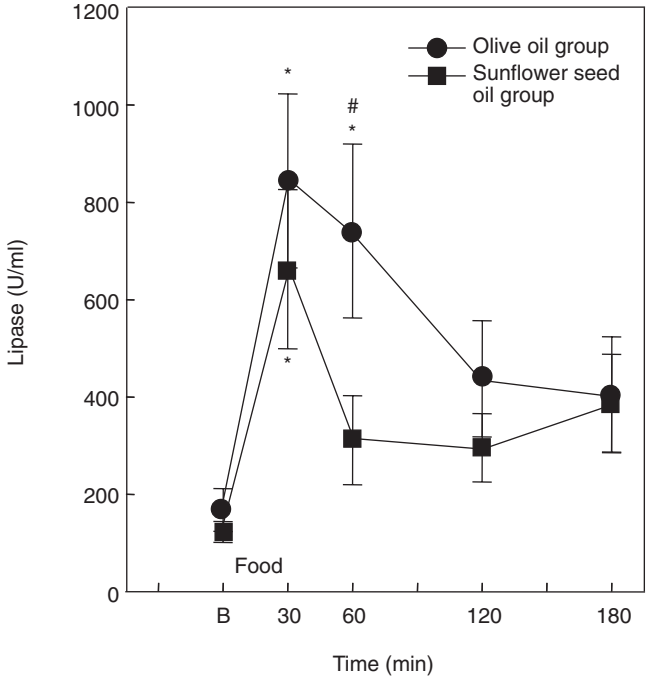


Fig. 13.12. Lipase activity in duodenal content in response to liquid formula in both experimental groups. *Statistically significant differences versus basal values; #statistically differences between experimental groups.

As for the human studies, the investigation of the effect of dietary fats on biliary composition has yielded equivocal results. One reason for this is the small number of available studies, possibly because of the difficulty and risks of sample collection, and a second reason concerns the variety of dietary protocols, including differences in the amount of dietary fat and in the duration of the treatment.

While the effects of polyunsaturated fat rather than the typical Western diet have been investigated in a number of studies, the influence of monounsaturated fats has been less explored. Therefore, we aimed to compare the effects of two dietary oils that differ markedly in their fatty acid profile (virgin olive oil and sunflower oil) on biliary lipid composition in human subjects (Yago *et al.*, 2005). These oils are used preferentially in our geographical area. Furthermore, olive oil is a major component of the Mediterranean diet, and its role in human health is being actively debated at present. The study group, which comprised 18 gallstone female patients referred to elective cholecystectomy, was kept on the experimental diets for 30 days prior to surgery. This design made it possible to collect fasting gallbladder bile and stones (at surgery). In addition, fasting and postprandial hepatic bile (post-cholecystectomy) was examined in order to rule out the possibility that the dietary intervention was masked by the occurrence of preexisting gallstones.

Despite marked differences in the absolute concentration of biliary lipids and total lipid content, chronic manipulation of dietary fat ingestion did not influence the cholesterol saturation or the profile of individual bile acids in gallbladder bile obtained from gallstone patients.

After cholecystectomy, cholesterol saturation index (CSI) of hepatic bile in fasted patients was similar in both dietary groups and indicative of supersaturation. In response to the test meals, CSI dropped significantly in patients given the olive oil diet, reaching values lower than 1 at 120 min postprandially. In contrast, hepatic bile secreted by sunflower oil-fed patients appeared supersaturated (CSI over 1.5) throughout the whole experiment (Fig. 13.13).

Overall, the results indicate that the type of dietary fat habitually consumed (olive oil *vs* sunflower oil) can influence bile composition in humans with established cholelithiasis. In gallbladder, this influence was mainly noted in the presence of more concentrated bile in the olive oil group, although this was not translated into a modification of cholesterol saturation. This is likely due to the fact that cholesterol gallstones were already present by the time the dietary intervention started. After cholecystectomy, cholesterol saturation of hepatic bile dropped typically in response to a test meal only in patients given the olive oil diet and remained unchanged – supersaturated – in those given sunflower oil. Further research is required to define the precise mechanisms of the reported effects and to confirm whether a similar effect occurs in healthy subjects.

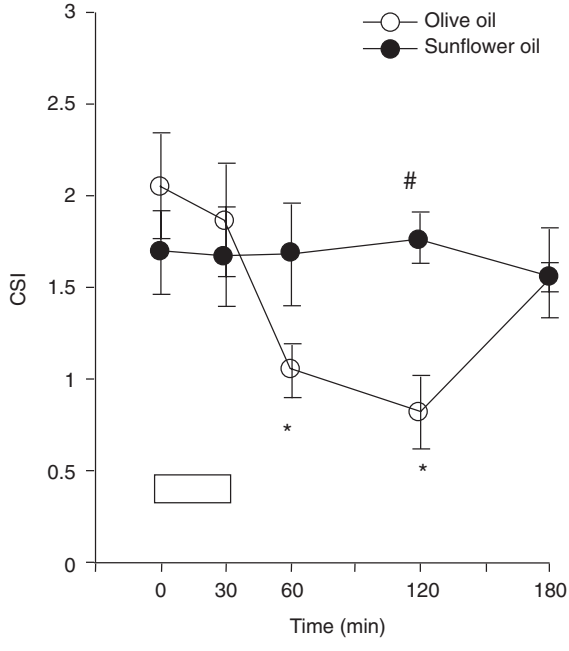


Fig. 13.13. Time course of changes in the cholesterol saturation index (CSI) in duodenal samples from cholecystectomized patients after administration of a liquid meal. Subjects were kept on diets that contained olive oil or sunflower seed oil as the main source of dietary fat for 30 d before surgery and were given meals that included the corresponding oil. Zero time denotes fasting values. Meals were ingested within 30 min (open bar). #Significantly different ($P < 0.05$) than olive oil group. *Significantly different ($P < 0.05$) from the respective fasting value.

3.1.2.4. OLIVE OIL AND GASTRIC SECRETION. Since 1886, when Ewald and Boas noted that addition of olive oil to a test meal of gruel depressed acid secretion in human subjects, numerous investigations have confirmed that the presence of fat at different segments of the gastrointestinal tract inhibits acid secretion, with olive oil (or oleic acid) being used in most of them. In human studies, this response was found during acute experiments and under non-physiological conditions. Comparative studies investigating the influence of different fats on gastric acid secretion were lacking. It is for this reason that we examined in man (by using the above mentioned protocol) the effects of dietary fat type (olive oil and sunflower oil) upon gastric acid secretion and the release of the hormones gastrin, CCK, somatostatin and PYY (Serrano *et al.*, 1997). Somatostatin is produced by endocrine D cells in the stomach, PYY is released in ileum and colon mucosa and CCK cells are located in the duodenum mucosa. The three hormones have been reported to be released by dietary fat and they all seem to be involved in the mechanism by which fat inhibits gastric acid secretion. Gastric acid secretion was estimated by measuring intragastric pH and the concentration of hydrogen ion.

In our experimental conditions, the pH values during the basal (fasting) period were similar in both groups. Ingestion of the meals resulted in an immediate rise in gastric pH in both groups. However, the return to pre-meal values was completed in group S within 60 min, and this was followed by a further decline to values below the fasting ones at the end of the experiment. In contrast, the pH decrease in group O subjects was markedly attenuated. Accordingly, the concentration of hydrogen ions in group S was significantly enhanced over basal and remained high during the entire postprandial period while the meal-induced changes in group O were modest (Fig. 13.14).

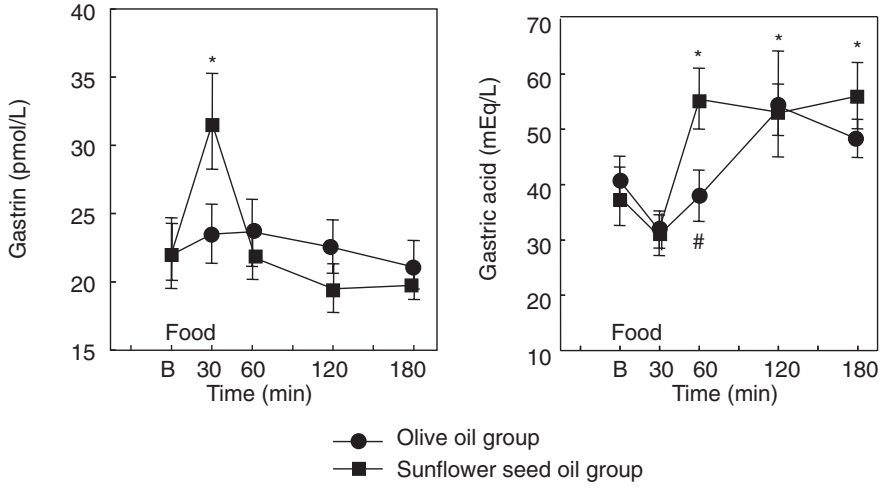


Fig. 13.14. Plasma levels of gastrin and gastric acid content in humans in response to liquid meals rich in olive or sunflower seed oils. *Significantly different ($P < 0.05$) from the respective fasting value. #Significantly different ($P < 0.05$) from the sunflower oil group.

The fasting concentration of serum gastrin was comparable in both groups. The meal induced a significant rise in gastrin levels in the sunflower group that peaked at 30 min postprandially. Gastrin concentration returned to basal values at 60 min after the meal was started. No significant changes were found in group O throughout the study period (Fig. 13.14).

Plasma somatostatin values before and after the administration of the test meals were similar in the experimental groups. In contrast, PYY levels in those subjects that received the olive oil meal remained consistently higher than in those that received sunflower oil.

Human intake of diets containing olive oil results in an attenuated gastric acid secretion in response to a meal when compared with those containing sunflower oil, rich in polyunsaturated fatty acids. These results complement and explain the finding of others who had observed before in patients with gastric and duodenal ulcers that the substitution of animal fat for olive oil in the diet was associated with a significant reduction in the size of the ulcers and an important increase in the percentage of healing.

According to our data, the effect of olive oil on gastric acid secretion involves the suppression of serum gastrin. If we consider the normal stimuli for the release of this hormone, a similar increase in serum levels should be expected in both groups after the ingestion of two meals with the same volume, pH, organoleptic properties, and identical quantity and type of protein. Liquid meals differed only in the type of fat.

The lack of gastrin response in group O may be due to the release of some factor capable of inhibiting gastrin secretion from G cells. A putative one could be somatostatin since it has been shown that intravenous administration of this hormone inhibits gastrin release and acid secretion. However, plasma somatostatin levels were similar in our study groups so circulating somatostatin is unlikely to mediate the suppression of serum gastrin and the decrease of gastric acid secretion found in the subjects fed olive oil.

A role for somatostatin as paracrine mediator of the inhibition should not, however, be excluded. Somatostatin inhibits gastrin release at a local level. Then, there is the possibility that oleic acid can result in a greater local concentration of somatostatin around G cells, thus abolishing the postprandial gastrin. Rather than directly, this effect may involve the action of CCK, a potent releaser of gastric somatostatin that remained significantly higher in blood from olive oil patients for the duration of the postrandial period.

Another candidate for the action of olive oil on gastric secretion is PYY, and two mechanisms could account for it. On the one hand, this peptide has been shown to reduce markedly pentagastrin- and vagally-induced human gastric secretion. The existence of higher PYY levels in our group O compared with group S is compatible with the hypothesis that PYY is involved in the suppression of the acid secretory response in those patients. On the other hand, PYY is known to inhibit gastric emptying so our finding that the increase in the postprandial intragastric pH lasted for longer in group O subjects could be explained not only by a reduced gastric acid secretion but also to a slower gastric emptying of the meal. PYY would modulate the delivery of chyme to duodenum so the stomach does not empty more chyme than the small intestine can process. In this way, olive oil may exert additional beneficial effects upon the absorption of nutrients.

Taken together, these results (Serrano *et al.*, 1997) may be of use in deciding the most appropriate nutritional therapy for patients recovering from a number of gastrointestinal diseases where attenuation of acid secretion is a key therapeutic goal.

4. References

Ballesta, M.C., Mañas, M., Mataix, F.J., Martinez-Victoria, E. and Seiquer, I. (1990a) Long-term adaptation of pancreatic response to dietary fats of differents degrees of saturation: olive oil and sunflower oil. *British Journal of Nutrition* 64, 487–496.

Ballesta, M.C., Martinez-Victoria, E., Mañas, M., Mataix, F.J. Seiquer, I. and Huertas, J.R. (1990b) Fat digestibility in dogs after long-term adaptation to diets varying in the degree of lipid saturation (virgin olive oil and sunflower oil) *Medical Science Research* 18, 517–519.

Ballesta, M.C., Martinez-Victoria, E., Mañas, M., Mataix, F.J., Seiquer, I. and Huertas, J.R. (1991) Protein digestibility in dog. Effect of the quantity and quality of dietary fat (virgin olive oil and sunflower oil). *Die Nahrung-food* 35, 161–167.

Ballesta, M.C., Mañas, M., Martinez de Victoria, E., Seiquer, I., Huertas, J.R. and Mataix, F.J. (1992) Adaptation of biliary response to dietary olive oil and sunflower seed oil in dogs. *British Journal of Nutrition* 68, 175–182.

Beaudoin, A.R., Begin, M.E., Ells, G., St-Jean, P., Laforest, L., Proulx, J. and Vachereau, A. (1989) Type of dietary lipids exerts a major influence on the secretory activity of the exocrine pancreas: medium-term studies. *Pancreas* 4(4), 418–412.

Berne, R.M., Levy, M.P., Koeppen, B.M. and Stanton, B.A. (2004) Physisology. 5th edn. Mosby, San Louis.

Díaz, R.J., Yago, M.D., Martínez-Victoria, E., Naranjo, J.A., Martínez, M.A. and Mañas, M. (2003) Comparison of the effects of dietary sunflower oil and virgin olive oil on rat exocrine pancreatic secretion in vivo. *Lipids* 38, 1119–1126.

Johnson, L.R. (1994) *Physiology of the Gastrointestinal Tract*. 3rd edn. Raven Press, New York.

Lamrani, A., Tulliez, M., Chauvelot-Moachon, L., Chaussade, S., Mauprivez, C., Hagnere, A.M. and Vidon, N. (1999) Effects of octreotide treatment on early TNF-alpha production and localization in experimental chronic colitis. *Alimentary Pharmacology and Therapeutics* 13(5), 583–94.

Martínez, M.A., Lajas, A.I., Yago, M.D., Redondo, P.C., Granados, M.P., Gonzalez, A., Rosado, J.A., Martınez-Victoria, E., Mañas, M. and Pariente, J.A. (2004) Dietary virgin olive oil enhances secretagogue-evoked calcium signaling in rat pancreatic acinar cells. *Nutrition* 20, 536–541.

McPhee, S.J., Lingappa, V.R., Ganong, W.F. and Lange, J.D. (1997) *Pathophysiology of Disease*. 2nd edn. Appleton and Lange, Stanford.

Serrano, P., Yago, M.D., Mañas, M., Calpena, R., Mataix, J. and Martinez-Victoria, E. (1997) Influence of type of dietary fat (olive and sunflower oil) upon gastric acid secretion and release of gastrin, somatostatin and peptide YY in man. *Digestive Diseases and Sciences* 42, 626–633.

Serrano, P., Yago, M.D., Martinez de Victoria, E., Medrano, J., Mataix, J. and Mañas, M. (1998) Influence of the type of dietary fat upon the plasma levels of secretin and pancreatic polypeptide in cholecystectomized humans. *Biogenic Amines* 14, 313–330.

Yago, M.D., Martinez-Victoria, E., Mañas, M., Martinez, M.A. and Mataix, J. (1997a) Plasma peptide YY and pancreatic polypeptide in dogs after long-term adaptation to dietary fats of different degrees of saturation: olive and sunflower oil. *Journal of Nutritional Biochemistry* 8, 502–507.

Yago, M.D., Martinez de Victoria, E., Huertas, J.R. and Mañas, M. (1997b) Effects of the amount and type of dietary fat on exocrine pancreatic secretion in dogs after different periods of adaptation. *Archives of Physiology and Biochemistry* 105, 78–85.

Yago, M.D., Mañas, M., Gonzalez, M.V., Martinez-Victoria, E., Perez, M.T. and Mataix, J. (1997c) Plasma levels of cholecystokinin and peptide YY in human: response to dietary fats of different degrees of unsaturation (olive and sunflower oil). *Biogenic Amines* 13, 319–331.

Yago, M.D., Gonzalez, M.V., Martinez-Victoria, E., Mataix, J., Medrano, J., Calpena, R., Perez, M.T. and Mañas, M. (1997d) Pancreatic enzyme secretion in response to test meals differing in the quality of dietary fat (olive and sunflower seed oils) in human subjects. *British Journal of Nutrition* 78, 27–39.

Yago, M.D., Serrano, P., Mañas, M., Mataix, J., Medrano, J., Calpena, R. and Martinez-Victoria, E. (1998) Release of secretin and somatostatin after test meals with different fatty-acid composition in cholecystectomized humans. *Journal of Nutritional Biochemistry* 9, 186–192.

Yago, M.A., Díaz, R.J., Ramirez, R., Martinez, M.A., Mañas, M. and Martinez-Victoria, E. (2004) Dietary-induced changes in the fatty acid profile of rat pancreatic membranes are associated with modifications in acinar cell function and signalling. *British Journal of Nutrition* 91, 227–234.

Yago, M.D., González, V., Serrano, P., Calpena, R., Martínez, M.A., Martínez-Victoria, E. and Mañas, M. (2005) Effect of the type of dietary fat on biliary lipid composition and bile lithogenicity in humans with cholesterol gallstone disease. *Nutrition* 21, 339–347.

14 The Effect of Olive Oil on Inflammatory Bowel Disease

XAVIER LLOR, EDUARD CABRÉ AND MIQUEL A. GASSULL

Gastroenterology Department, Hospital Universitari Germans Trias i Pujol, Badalona, Barcelona, Spain

1. Introduction

The potential role of dietary fat on IBD (inflammatory bowel disease) was suggested a decade ago, by our group, in a study geared at establishing a potentially positive effect of polymeric enteral nutrition to achieve and maintain clinical remission in Crohn's disease patients (González-Huix *et al.*, 1993). In that study, patients with active Crohn's disease who were treated with a polymeric enteral formula achieved a similar rate of remission to the prednisone-treated patients. Furthermore, short-term relapse rate was similar in both groups. These results were in line with some previously reported studies confirming a favourable effect of elemental diets on Crohn's disease (O'Morain *et al.*, 1984; Saverymuttu *et al.*, 1985; Jones, 1987). While the enteral diet used in our study had a very high fat content (33% of the total calories), the other mentioned studies had a very low fat content (between 0.6 and 1.3% of total calories). On the other hand, a different study published by Giaffer *et al.* (1990) also using a high fat content (36% of total calories), did not show a positive effect. An important difference though, between the latest study and ours was that while our patients' diet had a high monounsaturated fatty acid content in the form of oleic acid, Giaffer's diet was high in the n-6 polyunsaturated fatty acid, linoleic acid. Thus, remission-inducing diets would be either those with a very low fat content and low α-linoleic acid or those with high monounsaturated fatty acid content. A potential explanation would be that excessive presence of n-6 fatty acids would favour the synthesis of arachidonic acid-derived eicosanoids (LTB_4, TxA_2, PGE_2) with a high pro-inflammatory activity, whereas diets in which α-linoleic content would be low or replaced by monounsaturated fat would attenuate the inflammatory response.

This theory could, at least partially, explain the observed increase in Crohn's disease incidence in Scandinavia after the introduction and generalization of consumption of n-6 fatty acid-rich seed oils substituting saturated fat (mostly

 Olive Oil and Health
(eds J.L. Quiles, M.C. Ramirez-Tortosa and P. Yaqoob)

from butter) that took place in the late 1960s to prevent coronary heart disease. The same trend was seen after a major dietary change from n-3 to n-6 fatty acids occurred in Japan (Shoda *et al.*, 1996) or a partial substitution of olive oil by seed oils took place in the Mediterranean countries (unpublished data).

2. Oleic Acid in the Cell

Oleic acid (18:1n-9), as opposed to linoleic acid and α-linolenic acid, is not an essential fatty acid. Once ingested, like the rest of 18-carbon fatty acids, oleic acid is desaturated and elongated to a 20-carbon fatty acid, eicosatrienoic acid (ETA, 20:3n-9) (James *et al.*, 2000). For this process to occur, oleic acid would have to compete with dietary linoleic acid for the desaturase and elongase enzymes (Lands *et al.*, 1990) and because such competition is negligible, there is very little eicosatrienoic acid in cell membranes. In fact, ETA is virtually absent from plasma lipids, unless severe essential fatty acid deficiency exists.

The main competition for these enzymes is between linoleic acid (LA, 18:2n-6), which humans consume in considerable amounts due to its widespread abundance in the diet, and α-linolenic acid (α-LNA, 18:3n-3), which is consumed in far lesser amounts. The former is converted to arachidonic acid and the latter to eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Both, n-3 and n-6 fatty acids are structural phospholipids of the cell membrane and, as mentioned, they compete for their metabolic conversion through desaturase and elongase enzymes that are common to both pathways. These enzymes have a greater affinity for n-3 fatty acids (Rose and Connolly, 1999), therefore, an increase in their intake translates into a decrease in linoleic acid metabolization and a subsequent decrease in arachidonic acid concentration. This would decrease the production of arachidonic acid-derived eicosanoids that are considered pro-inflammatory.

Although there is little eicosatrienoic acid in cell membranes, when ETA is included in the diets of linoleic acid-sufficient rats, it also gets incorporated into cell membranes (Cleland *et al.*, 1996). Unlike n-3 PUFA, however, the incorporation of n-9 into cell membranes is much slower and significant changes in membrane composition may not be seen until 12 weeks of olive oil supplementation (Hillier *et al.*, 1991). In any case, as it happens with eicosapentaenoic acid, an increase in dietary intake of ETA could also alter the balance of eicosanoids produced by leukocytes towards a less inflammatory set. In fact, ETA antagonizes the conversion of arachidonic acid to pro-inflammatory LTB_4. This is accomplished because ETA is a substrate of 5-lipoxygenase which results in the formation of LTA_3, which is a good inhibitor of leukotriene-A_4 hydrolase, one of the enzymes, along with arachidonate 5-lipoxygenase, necessary for LTB_4 synthesis (Jakschik *et al.*, 1983; Evans *et al.*, 1985). Much less clear, however, is the effect of ETA on cyclooxygenase (COX), the enzyme that catalyses the synthesis of pro-inflammatory prostaglandin E_2 (PGE_2) from arachidonic acid. ETA lacks the n-6 double bond necessary for prostanoid formation, but it may inhibit PGE_2 formation through inhibition of endothelial PGI_2 production (Lerner *et al.*, 1995).

3. Oleic Acid on Lymphocyte Function

Several studies using significant amounts of oleic acid in animal models and cell lines have shown the capacity of oleic acid to modulate the activity of lymphocytes. Thus, oleic acid was capable of partially inhibiting lymphocyte proliferation, IL-2 production, the expression of IL-2 receptor and adhesion molecule and the activity of NK cells that play an important role in graft rejection (Calder, 1995, 1996, 1998a,b). Results from human studies have not been so impressive. No changes in lymphocyte proliferation, NK cell activity, or production of TNF-α, IL-1α, IL-1β, IL-2 or IFN-γ by mononuclear cells were seen after a 12-week period of daily supplementation with 9 g of olive oil. In the same study there was no effect of fish oil on these parameters (Yaqoob e *et al.*, 2000). Another study in which healthy middle-aged males increased their oleic acid intake from 11% to 18% of dietary energy at the expense of saturated fatty acids for 2 months showed no changes in NK cell activity or lymphocyte proliferation, but there was a significant decrease in the expression of intercellular adhesion molecule 1 by peripheral blood mononuclear cells (Yaqoob *et al.*, 1998).

Another mechanism that could also be determinant in regulating the activity of lymphocytes is induction of apoptosis. In fact, a growing number of studies suggest that a deregulated activation of T lymphocytes plays an important role in the pathogenesis of Crohn's disease (Elson *et al.*, 1995; Sartor, 1995). Apoptosis is a crucial process in the maintenance of the immune system's homeostasis (Thompson, 1995). An alteration in this mechanism could imply an accumulation of T cells in the tissue and, as a consequence, an inflammatory reaction (Sprent and Tough, 2001). In Crohn's disease, mucosal T cells become resistant to different apoptotic stimuli. At the same time, these cells have a decrease in the pro apoptotic protein Bax, resulting in an imbalance between Bax and the anti apoptotic protein Bcl-2 in the inflamed mucosa (Boirivant *et al.*, 1999; Ina *et al.*, 1999; Itoh *et al.*, 2001). Thus, it seems reasonable to think that the elimination of T cells would help in controlling inflammation. In fact, in some animal models of colitis, the induction of T cell apoptosis (through IL-12 antibodies, the blockade of IL-6 signalling or the deletion of CD44v7+) resulted in a dramatic improvement (Fuss *et al.*, 1999; Atreya *et al.*, 2000; Wittig *et al.*, 2000). Furthermore, the anti TNF-α agent Infliximab could, at least partially, exert its beneficial effect on Crohn's disease patients by inducing T cell apoptosis (ten Hove *et al.*, 2002).

The three studies that have addressed a potential pro apoptotic effect of oleic acid on T cells have reported rather inconsistent results. All used the human T lymphocyte cell line Jurkat. In the first one, while DHA induced apoptosis of these cells, similar concentrations of arachidonic acid or oleic acid had little effect (Siddiqui *et al.*, 2001). In the second study, arachidonic acid supplementation caused a decrease in the expression of some anti-apoptotic genes, but while oleic acid inhibited the proliferation of Jurkat cells, it did not affect apoptosis (Verlengia *et al.*, 2003). Finally, in the third study, oleic acid was able to induce apoptosis (although to a much lesser extent than linoleic acid) through changes in mitochondrial trans membrane potential and over expression of p53 and c-myc (Cury-Boaventura *et al.*, 2004). It may be important to note that gut mucosal T-lymphocytes in inflammatory bowel disease are in a very high state of activation and studies using appropriately activated cells are limited.

4. Human Trials of Olive Oil and Oleic Acid on Inflammatory Bowel Disease

One of the important drawbacks in evaluating the effects of olive oil or oleic acid on patients with inflammatory bowel disease stems from the fact that olive oil has often been used in trials as a placebo *vs* the n-3 fatty acids, for which a therapeutic effect was being sought. In fact, it was assumed by the authors that there were no anti-inflammatory effects of olive oil and therefore that it served as a suitable, neutral, placebo. One of the first studies that indirectly evaluated the effect of oleic acid on inflammatory bowel disease was a controlled randomized trial to determine the efficacy and safety of polymeric enteral nutrition (PEN), in comparison with steroids, for active Crohn's disease patients. This study showed that the enteral nutrition treatment was as effective as steroids in inducing remission (González-Huix *et al.*, 1993). The polymeric enteral diet had a high fat content of which a very significant percentage was the monounsaturated fatty acid oleic acid. The study included Crohn's disease patients with a Van Hees activity index (VHAI) greater than 120 and symptoms plus lab indices compatible with active disease. The two groups were homogeneous regarding activity level and disease location (only colonic disease *vs* small bowel with or without colon involvement). The steroid treatment group received 1 mg/kg/day of prednisone and the PEN group was administered 2800 kcal/day. The PEN formula contained 22% of proteins, 46% of carbohydrates and 32% of lipids (41% monounsaturated, 28% saturates, 18% polyunsaturated and 13% medium chain triglycerides). Clinical remission (as defined as ≤3 stools/day, absence or mild abdominal pain, no fever and heart rate ≤90 b/min, 10% weekly reduction in VHAI with a final VHAI <120) was achieved in a majority of patients in both groups (88.2% in the steroid group and 80% in the PEN group), with a mean time to achieve remission of 2.0 weeks in the former group and 2.4 weeks in the PEN group. Relapse rate at 1 year was 66.6% in the steroid group and 41.6% in the PEN group (Fig. 14.1). Tolerance of the PEN diet was excellent.

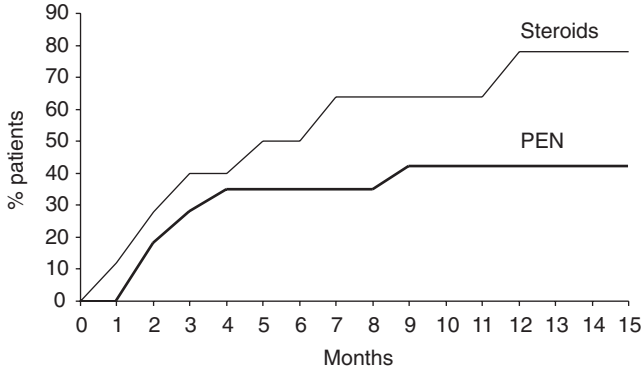


Fig. 14.1. Cumulative probability of relapse during follow up in both groups (log rank test, p = 0.21). Both groups were taking mesalazine (1.5 g/day).

In light of the above results and other data on enteral nutrition for Crohn's disease patients (O'Morain *et al.*, 1984; Saverymuttu *et al.*, 1985; Jones, 1987; Giaffer *et al.*, 1990), we hypothesized that diets with either very low total fat or high in monounsaturated fatty acids would be particularly effective in Crohn's disease. Thus, we designed one of the very few studies with the goal of specifically evaluating the effect of oleic acid on Crohn's disease (Gassull *et al.*, 2002). In this multi-centre, randomized study, patients were assigned to either receive 1 mg/kg/day of oral prednisone (steroid group), polymeric enteral diet 1 (PEN 1 group) with 22% of proteins, 46% of carbohydrates and 32% of lipids (79% oleate, 6.5% linoleate), or PEN 2 group with the same percentage of protein, carbohydrates and lipids but the latest containing 45% linoleate and only 28% oleate. Inclusion criteria as well as clinical remission assessment were similar to those established in the previous study. Treatment failure was considered when remission was not achieved 4 weeks after starting treatment. Surprisingly, the study was terminated early after 62 patients had been included and the stopping rule was fulfilled: failure to achieve a remission rate >33% in any of the arms (the PEN 1 in this case) when at least 15 patients per arm had been included, and with highly significant differences among groups justified stopping the trial. Indeed, the PEN 1 group performed the worst with overall remission rates, by intention to treat, of 20% *vs* 52% for PEN 2 and 79% for the steroids group. After exclusion of non-compliant patients during the first week, remission rates were 27%, 63% and 79% respectively (Fig. 14.2). Overall, the response to the enteral diet high in oleate was very poor.

Why were the results so different between the two studies? The groups were homogeneous at entry in the two studies and inclusion and response criteria were practically identical. One potential explanation could be the source of oleic acid provided in the diets. In the latter study, synthetic trioleate was the source of oleic acid while olive oil had been used in the former. Olive oil, besides oleic acid, contains other components such as phenols and antioxidants that could be accountable for a positive effect of whole oil *vs* oleic acid itself.

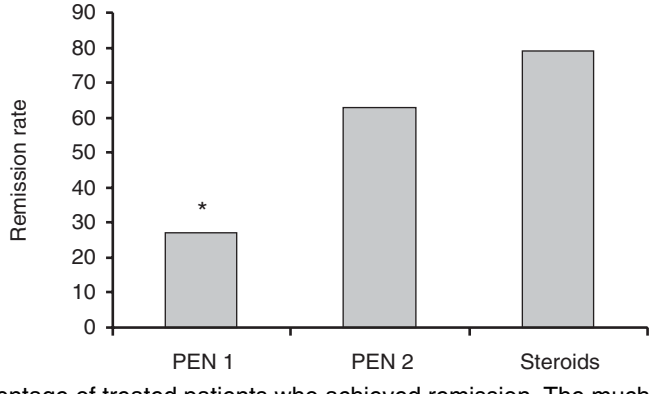


Fig. 14.2. Percentage of treated patients who achieved remission. The much lower remission rate of PEN 1 group prompted the early termination of the trial.

Another and rather more complex potential explanation is that the anti-inflammatory effects of fatty acids could depend on the relative ratios of different fatty acids to each other, as some *in vitro* studies have suggested, rather than on than concentrations of individual fatty acids (Karsten *et al.*, 1994; Sadeghi *et al.*, 1999).

A different potentially positive effect of oleic acid was evaluated regarding its role in slowing gastrointestinal transit and delaying defecation in patients with ulcerative colitis after ileal pouch-anal anastomosis (Soper *et al.*, 1990). In this small, preliminary and limited study, eight subjects were studied on 2 consecutive days. On day 1, saline was infused into the ileal pouch and on day two, emulsified oleic acid was infused. In comparison with the saline infusion, oleic acid slowed gastric emptying and small-bowel transit and delayed the time to defecation. It is unclear, though, if this could potentially be useful to reduce stool output in this population and a clinical trial is still lacking.

As mentioned, several studies (involving both ulcerative colitis and Crohn's disease patients) included olive oil as a placebo treatment given in the control arm of trials testing a potentially anti-inflammatory effect of n-3 fatty acids. However, it is impossible to ascertain whether olive oil had any effect because there was no 'true' placebo-treated group in these studies.

The study that included the largest number of patients showed that, in ulcerative colitis patients with active disease who received 4.5 g of EPA as triacyl glycerol for 1 year, this fatty acid had a significant steroid-sparing effect in comparison with patients who received olive oil, even though it failed to prevent clinical relapse in the group of patients enrolled while their disease was in remission (Hawthorne *et al.*, 1992). A smaller study showed that a daily administration of 5.4 g of n-3 fatty acids as triacylglycerol to ulcerative colitis patients was unable to show a steroid-sparing effect in comparison to the olive oil treated group (Stenson *et al.*, 1992). Finally, a study that included both ulcerative colitis (with active disease) and Crohn's disease patients (in different stages of disease activity) was able to show only a small clinical benefit of n-3 fatty acids in ulcerative colitis patients *vs* olive oil treated patients (Lorenz *et al.*, 1989).

5. References

Atreya, R., Mudter, J., Finotto, S., Mullberg, J., Jostock, T., Wirtz, S., Schutz, M., Bartsch, B., Holtmann, M., Becker, C., Strand, D., Czaja, J., Schlaak, J.F., Lehr, H.A., Autschbach, F., Schurmann, G., Nishimoto, N., Yoshizaki, K., Ito, H., Kishimoto, T., Galle, P.R., Rose-John, S. and Neurath, M.F. (2000) Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in Crohn disease and experimental colitis in vivo. *Nature Medicine* 6, 583–588.

Boirivant, M., Marini, M., Di Felice, G., Pronio, A.M., Montesani, C., Tersigni, R. and Strober, W. (1999) Lamina propria T cells in Crohn's disease and other gastrointestinal inflammation show defective CD2 pathway-induced apoptosis. *Gastroenterology* 116, 557–565.

Calder, P.C. (1995) Fatty acids, dietary lipids and lymphocyte functions. *Biochemistry Society Transactions* 23, 302–309.

Calder, P.C. (1996) Effects of fatty acids and dietary lipids on cells of the immune system. *Proceedings of the Nutrition Society* 55, 127–150.

Calder, P.C. (1998a) Dietary fatty acids and lymphocyte functions. *Proceedings of the Nutrition Society* 57, 487–502.

Calder, P.C. (1998b) Dietary fatty acids and the immune system. *Nutrition Reviews* 56, S70–83.

Cleland, L.G., Neumann, M.A., Gibson, R.A., Hamazaki, T., Akimoto, K. and James, M.J. (1996) Effect of dietary n-9 eicosatrienoic acid on the fatty acid composition of plasma lipid fractions and tissue phospholipids. *Lipids* 31, 829–837.

Cury-Boaventura, M.F., Pompéia, C. and Curi, R. (2004) Comparative toxicity of oleic acid and linoleic acid on Jurkat cells. *Clinical Nutrition* 23(4), 721–732.

Elson, C.O., Sartor, R.B., Tennyson, G.S. and Riddell, R.H. (1995) Experimental models of inflammatory bowel disease. *Gastroenterology* 109, 1344–1367.

Evans, J.F., Nathaniel, D.J., Zamboni, R.J. and Ford-Hutchinson, A.W. (1985) Leukotriene A3. A poor substrate but a potent inhibitor of rat and human neutrophil leukotriene A4 hydrolase. *Journal of Biological Chemistry* 260, 10966–10970.

Fuss, I.J., Marth, T., Neurath, M.F., Pearlstein, G.R., Jain, A. and Strober, W. (1999) Anti-Interleukin 12 Treatment Regulates Apoptosis of Th1 Cells in Experimental Colitis in Mice. *Gastroenterology* 117, 1078–1088.

Gassull, M.A., Fernandez-Banares, F., Cabre, E., Papo, M., Giaffer, M.H., Sanchez-Lombrana, J.L., Richart, C., Malchow, H., Gonzalez-Huix, F. and Esteve, M. (2002) Fat composition may be a clue to explain the primary therapeutic effect of enteral nutrition in Crohn's disease: results of a double blind randomised multicentre European trial. *Gut* 51, 164–168.

Giaffer, M.H., North, G. and Holdsworth, C.D. (1990) Controlled trial of polymeric versus elemental diet in treatment of active Crohn's disease. *Lancet* 335, 816–819.

González-Huix, F., de León, R., Fernández-Bañares, F., Esteve, M., Cabré, E., Acero, D., Abad-Lacruz, A., Figa, M., Guilera, M., Planas, R. and Gassull, M.A. (1993) Polymeric enteral diets as primary treatment of active Crohn's disease: a prospective steroid controlled trial. *Gut* 37, 778–782.

Hawthorne, A.B., Daneshmend, T.K., Hawkey, C.J., Belluzzi, A., Everitt, S.J., Holmes, G.K., Malkinson, C., Shaheen, M.Z. and Willars, J.E. (1992) Treatment of ulcerative colitis with fish oil supplementation: a prospective 12 month randomised controlled trial. *Gut* 33, 922–928.

Hillier, K., Jewell, R., Dorrell, L. and Smith, C.L. (1991) Incorporation of fatty acids from fish oil and olive oil into colonic mucosal lipids and effects upon eicosanoid synthesis in inflammatory bowel disease. *Gut* 32, 1151–1155.

Ina, K., Itoh, J., Fukushima, K., Kusugami, K., Yamaguchi, T., Kyokane, K., Imada, A., Binion, D.G., Musso, A., West, G.A., Dobrea, G.M., McCormick, T.S., Lapetina, E.G., Levine, A.D., Ottaway, C.A. and Fiocchi, C. (1999) Resistance of Crohn's disease T cells to multiple apoptotic signals is associated with a Bcl-2/Bax mucosal imbalance. *Journal of Immunology* 163, 1081–1090.

Itoh, J., de La Motte, C., Strong, S.A., Levine, A.D. and Fiocchi, C. (2001) Decreased Bax expression by mucosal T cells favours resistance to apoptosis in Crohn's disease. *Gut* 49, 35–41.

Jakschik, B.A., Morrison, A.R. and Sprecher, H. (1983) Products derived from 5,8,11-eicosatrienoic acid by the 5-lipoxygenase-leukotriene pathway. *Journal of Biological Chemistry* 258, 12797–12800.

James, M.J., Gibson, R.A. and Cleland, L.G. (2000) Dietary polyunsaturated fatty acids and inflammatory mediator production. *American Journal of Clinical Nutrition* 71, 343S–348S.

Jones, V.A. (1987) Comparison of total parenteral nutrition and elemental diet in induction of remission of Crohn's disease. Long-term maintenance of remission by personalized food exclusion diets. *Digestive Disease Science* 32, 100S–107S.

Karsten, S., Schafer, G. and Schauder, P. (1994) Cytokine production and DNA synthesis by human peripheral lymphocytes in response to palmitic, stearic, oleic, and linoleic acid. *Journal of Cell Physiology* 161, 15–22.

Lands, W.E., Morris, A. and Libelt, B. (1990) Quantitative effects of dietary polyunsaturated fats on the composition of fatty acids in rat tissues. *Lipids* 25, 505–516.

Lerner, R., Lindstrom, P., Berg, A., Johansson, E., Rosendahl, K. and Palmblad, J. (1995) Development and characterization of essential fatty acid deficiency in human endothelial cells in culture. *Proceedings of the Natural Academy of Science of the USA* 92, 1147–1151.

Lorenz, R., Weber, P.C., Szimnau, P., Heldwein, W., Strasser, T. and Loeschke, K. (1989) Supplementation with n-3 fatty acids from fish oil in chronic inflammatory bowel disease – a randomized, placebo-controlled, double-blind cross-over trial. *Journal of Internal Medicine Supplements* 225, 225–232.

O'Morain, C., Segal, A.W. and Levi, A.J. (1984) Elemental diet as primary treatment of acute Crohn's disease: a controlled trial. *British Medical Journal (Clinical Research Edition)* 288, 1859–1862.

Rose, D.P. and Connolly, J.M. (1999) Omega-3 fatty acids as cancer chemopreventive agents. *Pharmacology Therapie* 83, 217–244.

Sadeghi, S., Wallace, F.A. and Calder, P.C. (1999) Dietary lipids modify the cytokine response to bacterial lipopolysaccharide in mice. *Immunology* 96, 404–410.

Sartor, R.B. (1995) Current concepts of the etiology and pathogenesis of ulcerative colitis and Crohn's disease. *Gastroenterology Clinic of North America* 24, 475–507.

Saverymuttu, S., Hodgson, H.J. and Chadwick, V.S. (1985) Controlled trial comparing prednisolone with an elemental diet plus non-absorbable antibiotics in active Crohn's disease. *Gut* 26, 994–998.

Shoda, R., Matsueda, K., Yamato, S. and Umeda, N. (1996) Epidemiologic analysis of Crohn disease in Japan: increased dietary intake of n-6 polyunsaturated fatty acids and animal protein relates to the increased incidence of Crohn disease in Japan. *American Journal of Clinical Nutrition* 63, 741–745.

Siddiqui, R.A., Jenski, L.J., Neff, K., Harvey, K., Kovacs, R.J. and Stillwell, W. (2001) Docosahexaenoic acid induces apoptosis in Jurkat cells by a protein phosphatase-mediated process. *Biochimica et Biophysica Acta* 1499, 265–275.

Soper, N.J., Chapman, N.J., Kelly, K.A., Brown, M.L., Phillips, S.F. and Go, V.L. (1990) The 'ileal brake' after ileal pouch-anal anastomosis. *Gastroenterology* 98, 111–116.

Sprent, J. and Tough, D.F. (2001) T cell death and memory. *Science* 293, 245–248.

Stenson, W.F., Cort, D., Rodgers, J., Burakoff, R., DeSchryver-Kecskemeti, K., Gramlich, T.L. and Beeken, W. (1992) Dietary supplementation with fish oil in ulcerative colitis. *Annals of Intern Medicine* 116, 609–614.

ten Hove, T., van Montfrans, C., Peppelenbosch, M.P. and van Deventer, S.J. (2002) Infliximab treatment induces apoptosis of lamina propria T lymphocytes in Crohn's disease. *Gut* 50, 206–211.

Thompson, C.B. (1995) Apoptosis in the pathogenesis and treatment of disease. *Science* 267, 1456–1462.

Verlengia, R., Gorjao, R., Kanunfre, C.C., Bordin, S., de Lima, T.M. and Curi, R. (2003) Effect of arachidonic acid on proliferation, cytokines production and pleiotropic genes expression in Jurkat cells – a comparison with oleic acid. *Life Science* 73, 2939–2951.

Wittig, B.M., Johansson, B., Zoller, M., Schwarzler, C. and Gunthert, U. (2000) Abrogation of experimental colitis correlates with increased apoptosis in mice deficient for CD44 variant exon 7 (CD44v7). *Journal of Experimental Medicine* 191, 2053–2064.

Yaqoob, P., Knapper, J.A., Webb, D.H., Williams, C.M., Newsholme, E.A. and Calder, P.C. (1998) Effect of olive oil on immune function in middle-aged men. *American Journal of Clinical Nutrition* 67, 129–135.

Yaqoob, P., Pala, H.S., Cortina-Borja, M., Newsholme, E.A. and Calder, P.C. (2000) Encapsulated fish oil enriched in alpha-tocopherol alters plasma phospholipid and mononuclear cell fatty acid compositions but not mononuclear cell functions. *European Journal of Clinical Investigation* 30, 260–274.

15 Olive Oil, and Other Dietary Lipids, in Cancer: Experimental Approaches

EDUARD ESCRICH, MONTSERRAT SOLANAS AND RAQUEL MORAL

Department of Cell Biology, Physiology and Immunology, Medical Physiology Unit, School of Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain

1. Cancer

Cancer is a health problem in all societies and is known from ancient times. However, it is in the last 100 years, after the decrease in mortality due to infectious diseases and the increase in the life span, when cancer appears as one of the most important causes of death in developed countries, together with cardiovascular illnesses, accidents, and, more recently, AIDS. Worldwide, approximately 10 million people annually are diagnosed with cancer and more than 6 million people die of the disease every year, cancer being the second cause of mortality after cardiovascular disease. Nevertheless, the seriousness of the cancer problem is that it is the most relevant if one considers the early deaths, taking the potential years of life lost as an indicator. The most common cancers worldwide (excluding skin cancers other than melanoma) are lung (12.3% of all cancers), breast (10.4%) and colorectal cancer (9.4%). In general, in men the most frequent cancer is that of the lung, followed by prostate, stomach, colon and rectum, and liver. However, in women the most frequent one is breast cancer, and then, those of cervix uteri, colon and rectum, lung and stomach. The highest mortality rates by cancer in men are those of lung and stomach, and in women, those of breast and lung (Fig. 15.1) (Parkin *et al.*, 2002; International Agency for Research on Cancer, 2003; Bingham and Riboli, 2004; Ferlay *et al.*, 2004; Jemal *et al.*, 2005).

A neoplasia is a cell growth disorder, characterized primarily by an excessive, altered and uncontrolled proliferation of cells without any relation to the physiological demands of the involved organ, resulting in an abnormal mass (tumour) of the original tissues. There exist a number of varieties in all types of tissues and the resulting tumours have remarkable differences in their biological

 Olive Oil and Health
(eds J.L. Quiles, M.C. Ramírez-Tortosa and P. Yaqoob)

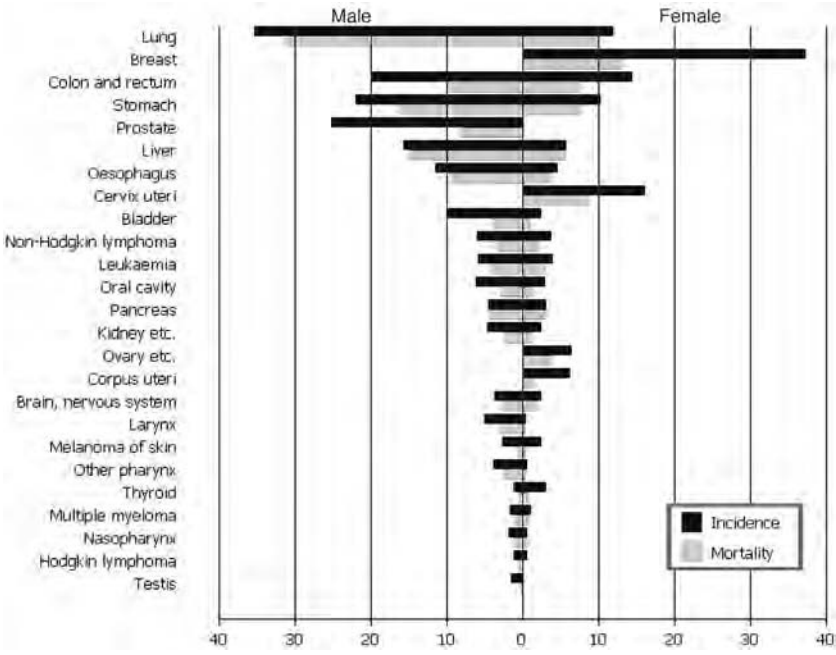


Fig. 15.1. Age-standardized rate per 100,000 (all ages) in all world (Ferlay *et al.*, 2004).

behaviour. The neoplasia can be benign or malignant, the latter being classified as cancer. The benign shows an expansive, slow and circumscribed growth, and the cells that form the tumour remain in the original location without body dissemination. Only when they affect essential structures (for instance, some brain tumours) during their growth, when they secrete some factors (such as hormones) or when they become malignant (as in colorectal adenomas), do they pose a threat to the bearing individual which could become fatal. On the contrary, the cancer is characterized by a fast growth, invasion and destruction of the adjacent tissues and dissemination along the whole body (metastasis), which in many cases provokes the death of the affected individual (Kumar *et al.*, 2004).

1.1. Molecular mechanisms of cancer

Living organisms are formed by cells that need to divide and to differentiate to constitute the different tissues and organs of the individual, or to die if they are not necessary or are altered. The proliferation, differentiation and programmed cell death or apoptosis processes are essential for the formation, functionality maintenance and repair of all the tissues and organs of the organism. In normal conditions, these processes are tightly regulated in response to distance produced signals from both the external and internal medium, as well as local signals from the extracellular matrix and the adjacent cells. This rigorous control ensures organ and tissue homeostasis. Different genes are involved in such regulation, mainly: protooncogenes, suppressor genes, cell cycle genes, DNA repair genes and apopto-

sis regulating genes (Darnell *et al.*, 1990; Evan and Vousden, 2001; Lowe *et al.*, 2004). The structural alterations of these genes (genetic mechanisms) as well as a variety of mechanisms that influence the behaviour, but not the structure, of DNA (epigenetic mechanisms) form the basis of the molecular mechanisms of cancer.

The series of events by which the cell proliferates, giving two daughter cells, is known as the 'cell cycle'. This process is regulated by the sequential activation or inactivation of proteins called cyclins and cyclin-dependent kinases (Cdk). It comprises four phases: G_1, S, G_2 and M. In the G_1-phase the cell is in full metabolic activity and the cell size and the extracellular conditions are controlled, in such a way that if these are not optimal the cell enters in G_0-phase (it stops growing transiently or permanently if it has reached the final development stage – for instance the neurons). During the S-phase (synthesis) DNA duplication occurs. In the G_2-phase the cell recovers its metabolic activity and its capability to enter mitosis. In the M-phase (mitosis) the molecular mechanisms that participate in cell size regulation and in protein production stop, and the cell divides into two daughter cells through the chromosomes and cytoplasm separation. It is estimated that the M-phase lasts for 1–2 hours, whereas the interphase between two cell divisions (G_1+S+G_2) has a reference duration of 12–24 hours, being variable depending on the cell circumstances. The protooncogenes and the growth factors, on the one hand, and the suppressor genes, on the other, are part of the signals that regulate the cell cycle, stimulating or slowing respectively the cell proliferation (Evan and Vousden 2001; Ponder, 2001).

The protooncogenes are highly conserved eukaryotic genes that codify proteins that regulate the normal cell proliferation or differentiation. Currently, around 100 of these genes are known and are classified according to the localization and the function of the encoded protein: growth factors (for instance, *sis*), membrane receptors (for instance, the receptor protein-tyrosine kinases – RPTKs – family, including *erbB* family members), mitogenic signal cytoplasmatic transducers (for instance, cytoplasmic protein-tyrosine kinases such as *src* and *abl*, the large family of small GTPases family including *ras* and *rho* families) and transcription factors (for instance, *myc*, *fos*, *jun*, *erb*A). The suppressor genes codify proteins that control the cell division cycle, slowing or inhibiting proliferation. They also participate in the DNA lesions repair, and favour apoptosis when DNA lesions are very serious. Around 30 suppressor genes have been described, among them *p53*, *Rb*, *BRCA*1, *BRCA*2 and the adenomatous polyposis coil *APC* gene (Futreal *et al.*, 2004). The mutation of the protooncogenes or their epigenetic alteration leads these normal cellular genes to become oncogenes and they are involved in the initiation of cancer because they trigger an excess of cell proliferation which is not subject to the usual controls. Therefore, the mutations in the protooncogenes are activating because they imply a gain of function (Volgestein and Kinzler, 2002). On the contrary, the mutations in the suppressor genes represent a loss of function because the alterations inactivate them. These alterations are also part of the mechanisms of cancer, because the ability to inhibit proliferation, to repair DNA lesions and the possibility of apoptosis are lost (Knudson, 1971; Harris, 1988). The oncogenes show a dominant phenotype at the cellular level as the activation of a single copy of the gene is enough to produce their cell growth stimulating effect. This is another of the characteristics

that distinguishes them from the suppressor genes, whose inactivation is usually recessive at the cellular level, as the absence of the normal protein is necessary to lose their effects. In general, the dominant mutations that lead to the activation of the protooncogenes are produced in somatic cells. The mutations in tumour suppressor genes may occur first in the germ line, conferring to the individuals a predisposition to tumour development, and the second mutational event, in somatic cells by loss of heterozygosity (LOH). However, in addition to the classical tumour suppressors, which have genetic and/or epigenetic alterations in both alleles (the Knudson's hypothesis of biallelic inactivation), there must be added the new identified 'haploinsufficient' tumour suppressor genes that show loss of function after only one of the alleles being altered (Bishop, 1991; Yarnold, 1996; Balmain *et al.*, 2003; Sherr, 2004).

It is estimated that in DNA a number of spontaneous mutations due to DNA polymerase errors during DNA replication are produced, as well as oxidative and carcinogen-induced lesions (Floid, 1990; Alberts *et al.*, 2002). In the normal genome a series of genes exist whose function consists in repairing these types of lesions, re-establishing the DNA normality. On the one hand, there are the mismatch repair (MMR) genes (*MSH*2, *MLH*1, *PMS*1, etc) that correct the replication mistakes and, on the other, the nucleotide-excision repair (NER) genes (for example, *XPA-XPG*) and the base-excision repair (BER) genes (different *glycosylase* genes), which are in charge of the repair of lesions induced by different genotoxic agents. However, if these repair genes get mutated, their inactivation removes their repairing function, and as a consequence, the spontaneous or induced mutations that can be produced in the proliferation regulating genes accumulate progressively, contributing to cancer development. For this reason, these genes belong to a class of cancer genes that have been called stability genes or caretakers. Other stability genes control processes involving large portions of chromosomes, such as those responsible for mitotic recombination and chromosomal segregation (for example, *BRCA*1, *BLM* and *ATM*). Stability genes keep genetic alterations to a minimum, and thus when they are inactivated, mutations in other genes occur at a higher rate, leading to the genetic instability characteristic of cancer cells (Hoeijmakers, 2001; Barnes and Lindahl, 2004).

In normal conditions, cells have a particular life time that is distinct depending on the cell type. Some cells reach a differentiation status that is very stable, for example neurons, which allows them to have a long life. Others, for example blood cells, have relatively short half lives. In some cases, the cells die prematurely due to necrosis (infection, hypoxia, chemical toxicity, etc.) or in a physiologically programmed way (apoptosis) (McConkey, 1998). Apoptosis is produced in a natural manner during the early embrionary and post-natal development in many tissues to remove structures with a medium functional value, but unnecessary in the mature final organ. It can also be triggered in old cells and in nonviable cells by accumulation of multiple alterations, being in this case a defence mechanism (Steller, 1995; Jacobson *et al.*, 1997). Apoptosis is caused by activation of intracellular proteases, known as caspases, that are responsible directly or indirectly for the morphologic and biochemical events that characterize the apoptotic cell. The numerous proteins that regulate these cell death proteases are involved in the different apoptosis phases: induction phase, in which the death

process starts; effector phase, when the need for the cell death is determined; and degradation phase, in which the irreversible structural alterations that will drive the cell towards cell death are generated (Hetts, 1998; McConkey, 1998). Two principal pathways for caspase activation have been recognized. One is triggered by engagement of so called 'death receptors' on the cell surface (extrinsic pathway). The other, of more ancient origin, is provoked by various forms of stress, including inadequate cytokine support and diverse types of intracellular damage (intrinsic pathway). During stress, the cell's decision on whether to invoke the suicide program – 'to be or not to be' – rests primarily within the Bcl-2 family. Its interacting opposing members, anti-apoptotic (Bcl-2, Bcl-x_L, etc.) and pro-apoptotic (Bax, Bcl-x_S, etc.), integrate developmental cues with the signals received from other cells and assess intracellular damage to determine whether to throw the caspase execution switch or to maintain the cell survival. Some suppressor genes as *p53* also participate in apoptosis. Thus, when alterations in the genome have been produced, *p53* induces the cell cycle arrest in G1-phase, allowing the repair genes to exert their function. However, in particular situations, such as the serious accumulation of multiple DNA lesions, its levels increase considerably and, in this case, the apoptosis mechanisms are activated through the regulation of some of its genes, such as *Bax*, as mentioned above. As a whole, it is claimed that the dysregulation of apoptosis is inextricably linked to cancer appearance (Kelekar and Thompson, 1998; Tsujimoto and Shimizu, 2000; Evan and Vousden, 2001; Hung and Chow, 2004).

Therefore, cancer is a disease of the stated genes and, probably, of others still unknown. In it, the normal homeostatic mechanisms mentioned above, that regulate cell behaviour, are upset. Due to the alterations that are produced, the cancerous cell acquires different capabilities that make it independent of the physiological control. The essential ones are: self-sufficiency in growth, insensitivity to antigrowth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis (Hanahan and Weinberg, 2000). A cancerous cell originates, when dividing, another cell that is also cancerous because it has inherited the progenitor alterations. But, in general, the mutations are produced in the somatic cell DNA, so the cancer suffered by a person is not inherited by his offspring. Only when the mutations appear in the germ line cells (spermatozoon or oocytes), can they be transmitted to the offspring (if they inherit the mutated allele) and increase notably the cancer risk. This situation occurs in some cancers, such as that of the breast, and represents between 3% and 10% of the totality of these cancers. In families with the most frequent type of hereditary breast cancer, the Lynch Syndrome, the high penetrance of the involved genes (*BRCA*) represents a risk of 60–85% of developing the disease up to 70 years old (Bishop, 1987; Narod and Foulkes, 2004). Clinically, the cancer natural history can pass from a diffuse and disordered growth (hyperplasia) to a precancerous lesions status (dysplasia) with cell abnormalities (atypia) from which a malignant tumour can develop. However, the nodular expression is often the way of debuting of cancer. Presumably, this will be initially localized (cancer *in situ*), until the progressive accumulation of the alterations leads the affected cells to infiltrate the neighbouring tissues and finally to the metastatic invasion, which will cause the individual's death (Kumar *et al.*, 2004).

1.2. Carcinogenesis stages

Several lines of evidence indicate that tumorigenesis is a multi-step process and that these steps reflect genetic alterations, described above, that drive the progressive transformation and evolution of normal cells into highly malignant derivates (Foulds, 1958; Hanahan and Weinberg, 2000). Its development is characterized by several events: sporadic mutations or exposure to the relevant agent/s; on occasion, metabolism of the agent/s; initiation due to an interaction between the agent/s and the cell constituents (especially the DNA); repair of the DNA damage, death of the cell or persistence and replication of a clone of abnormal cells within the tissue; growth of this abnormal clone into a definite focus of pre-neoplastic cells (promotion); growth of the tumour and its spread to other parts of the body (progression) (Hathway, 1986; Pitot, 1993; World Cancer Research Fund and American Institute for Cancer Research, 1997). The concepts of tumour initiation, promotion and progression come from the carcinogenesis assays with animal models (Rous and Kidd, 1941; Beremblum and Shubik, 1947) and, although they represent a simplification of the real process, they are very useful to understand the natural history of a cancer.

Initiation constitutes the starting-point of neoplastic transformation. Tumour initiation is caused by sporadic or inherited mutations induced by endogenous or exogenous genotoxic agents (chemical products – polycyclic aromatic hydrocarbons, aflatoxins, etc. -; physical radiations – UV, X, gamma -; or biological agents – human Papilloma virus (cervix cancer), hepatitis B virus (liver cancer), Epstein Barr virus (Burkitt lymphoma) etc. -) that activate or inactivate cellular genes involved in proliferation, differentiation or apoptosis, previously described, or by foreign genes introduced by viruses. The most important mutations are point mutation, gene amplification and clastogenic damage, which includes different changes such as deletion, rupture, translocation and chromosomal recombination. Epigenetic changes that modify gene expression, such as alterations in DNA methylation patterns, are also, possibly, involved (Pitot, 1993; Weinberg, 1994; Ponder, 2001). In any case, numerous data exist (long latency times after carcinogen exposure, properties of the immortalized cultured cells, exponential increase of the incidence and mortality rates, etc.) to suggest that the mere presence of a mutation in one gene is not enough for a cancer to be produced, but rather that the disease is the result of a number of mutations in several genes. The final biological aggressiveness of the tumour will depend on the type and the quantity of mutations, as well as on the importance of the affected genes. This fact explains the different clinical behaviour of apparently identical tumours and makes the establishment of the prognosis difficult. In the initiation moment, besides the carcinogens, the co-carcinogen agents may participate. These factors can act by enhancing or facilitating the genotoxic action of the carcinogens and/or interfering with DNA lesion repair (Moolgavkar and Knudson, 1981; Loeb and Loeb, 2000).

Tumour promotion is the process by which initially transformed cells can increase the genetic damage produced during the initiation and proliferate under

the action of different stimuli (hormones, growth factors, some dietary lipids, some drugs, phorbol esters, etc.), influencing the later growth and clonal expansion of abnormal cells. The promoters alter the expression of the cell genetic information through several mechanisms, including their interaction with membrane or intracellular receptors, or modifying other compounds (such as protein kinase C – PKC – which seems to play a major role in the development of tumour promotion) and cellular functions. Moreover, the proliferation increase that they favour increases the possibility of new mutations as the number of cell divisions increases (Pitot, 1993; Loeb and Loeb, 2000; Nowell, 2002).

Finally, the third phase, or tumour progression, occurs, in which the cell undergoes additional genetic alterations that lead to the expression of the malignant phenotype. During this phase, the cells show a marked genomic instability and acquire two of the essential characteristics of malignization: the ability to infiltrate adjacent tissues and metastasizing power (Nowell, 2002).

There is no clear separation between initiating factors and promoting factors. Thus, it may be possible for particular structural DNA alterations caused by genotoxic agents to have specific effects on gene expression, thereby altering the cell response to extracellular signals; likewise, the promoting effects of particular environmental factors could affect the mechanisms of DNA repair and apoptosis, modifying the mutation rate. Finally, taking into account, on the one hand, the increases of the population life expectancy and, on the other, the existence of latent cancers that have never been clinically aroused, it can be considered that in human cancer appearance the promotion and progression stages, which modulate the clinical course of the disease, have more relevance that the initiating causes that have provoked it. Bearing in mind that death is unavoidable, slowing the carcinogenesis process and delaying the clinical appearance of the tumour are valuable elements that should be considered as part of the prevention strategies (World Cancer Research Fund and American Institute for Cancer Research, 1997).

2. The Diet

Humans and animals need nutrients with which to build or repair their body tissues, and energy to function. All these needs are satisfied by feeding. Feeding, diet and nutrition are related concepts, but not strictly identical. Feeding is the entire process of obtaining and consuming food by an organism. The foods contain a number of substances from a natural (animal or vegetable) or artificial origin, called nutrients, which provide lipids, carbohydrates, proteins, vitamins, minerals and water. The word 'feeding' includes all aspects from food selection to cooking and ingestion. This process is dependent on the individual needs, resources, learning, culture, religion, socio-economical status, psychological aspects and geographic area. The pattern of meals and beverages regularly consumed by the individuals is the diet. The basic concepts regarding dietary composition vary according to the needs of the individuals of the population submitted to them. Initially, it is considered that the diet must be suitable or balanced in the unique sense of providing enough nutrients to assure the

individual's survival and covering their metabolic needs. In this sense, the essential is that the diet, besides being of sufficient quantity, to be varied enough to avoid nutritional deficiencies. Later, the concept of satisfying pleasantly the hunger and well-being feelings is added. Currently, in developed countries there is emphasis on the potential of foods to promote health and well-being and to decrease disease risk. With this last aim, the concept of functional foods appears (Diplock *et al.*, 1999; O'Carroll, 1999).

Finally, nutrition incorporates all processes by which an organism assimilates, transforms and uses the nutrients contained in foods. Nutrition, unlike feeding, is a physiological process independent of individual will and habits. The digestion process is of great efficacy, and also, simplicity since it permits the processing and transformation of a great variety of foods into a few essential nutrients which are those that can be assimilated and used by our cells. There exist innumerable foods, but there are only some tens of nutrients, generically classified as carbohydrates, fatty acids, aminoacids, minerals, vitamins and water. Feeding and nutrition are tightly interrelated. Good feeding guarantees correct nutrition. The achievement of correct nutrition that is, what our organism needs, should always be the aim (James *et al.*, 1990; Grande Covian, 1993; Campillo, 1997; Mahan and Escott-Stump, 2000).

When analysing the social changes produced in relation to the types of foods that humans obtain in their diet, it is observed that these have evolved, or are doing so, from scanty, in relation to the developed physical activity, and monotonous feeding, to a varied and abundant one. This evolution has gone from a gatherer–hunter system to a peasant–agricultural one, and, in developed countries, to an urban–industrial one. Current dietary patterns are associated with these changes. Currently, the most striking evident difference is that, in Africa and Asia (apart from high-income Asian countries, notably Japan), half or more of all total energy is supplied by cereals or, less often, starchy roots or fruits. By contrast, in Europe and North America, less than one quarter of total energy is supplied by cereals. Consumption of added fats, alcohol, meat, dairy products and sweeteners is generally reciprocal with consumption of starchy staples. This general tendency is establishing around the world. On a global basis, the increase in consumption of added fats is striking and increasing in all regions of the world, with the exception of Oceania. As a consequence, the diets contain a high proportion of total calories, mainly from the fats. On the other hand, lifestyle in the occidental societies tends towards the acquisition of sedentary habits with the consequent accumulation of body energy (Food and Agriculture Organisation and World Health Organization, 1991; World Cancer Research Fund and American Institute for Cancer Research, 1997).

The type of feeding is an important factor in relation to health. Nutritional imbalances constitute the origin of several diseases. Although the mechanisms by which the different alimentary factors participate in health or disease of individuals are not clear, the scientific evidence of a relationship between dietetic habits and health or particular illnesses are abundant enough to generate dietary recommendations within public health policies. Moreover, these alimentary factors will be of special relevance, among all the environmental ones,

given the continuous exposition or deficiency to which the individuals of a population are submitted from their dietetic habits. In the developed countries, innumerable diseases due to an excess of feeding are produced, whereas in the less developed countries the contrary is usually the case. In this sense, the pathologies that have been associated with dietary habits are diverse, among them cancer (James *et al.*, 1990). In Fig. 15.2 a simple summary of the pathologies most frequently related to diet are shown. Regarding cancer, it is estimated that nearly one-third of all cancers (or 3–4 million cases worldwide) are caused by inadequate diet and these could be reduced through individual and social actions (Colditz *et al.*, 2002).

The words 'healthy food' and 'harmful food' are frequently used. This is problematic, since these assertions have often not been based on scientific evidence and confusion arises as a result. Indeed, the illustrious and world-reknowned endocrinologist, Dr Gregorio Marañón, pointed out that, 'there is no part of medicine more changeable or with more shifting foundations than dietetic science; not a year passes without something fundamental changing' (Marañón, 1929). The fact that many aspects of nutrition and health still remain poorly understood supports this assertion and generates the need to contribute new data to the characterization of the biological activity of the usual nutrients in human feeding, which will allow the formulation of scientific opinions in relation to population health and the food security. To describe the current knowledge on diet and cancer, especially on the role of olive oil and that of other dietary lipids in this disease, is the main aim of this chapter.

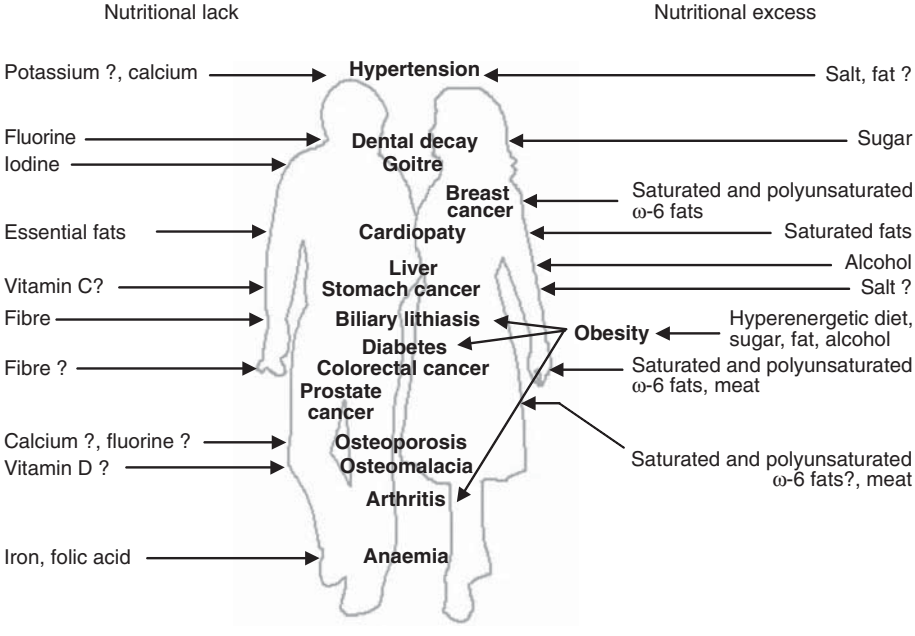


Fig. 15.2. Pathologies associated with nutritional imbalances (modified from James *et al.*, 1990).

3. Diet and Cancer

The incidence and mortality rates of cancer show different geographical distribution, with a marked overall difference in the total cancer burden between developed and developing countries (Parkin *et al.*, 2002; International Agency for Research on Cancer, 2003). A remarkable example of this fact occurs in some of the most frequent cancers, such as those of the breast, colon and rectum, and prostate (see geographical distribution for breast cancer in Fig. 15.3).

Fig. 15.3. Age-standardized incidence (A) and mortality (B) rates of breast cancer per 100,000 by area (Ferlay *et al.*, 2004).

This suggests that the degree of development/industrialization, environmental factors and lifestyle are strongly involved in this phenomenon. Among the environmental factors, diet is thought to be one of the most important contributing factors to cancer risk, although it is also linked to metabolic and genetic ones (Armstrong and Doll, 1975; Rogers and Longnecker, 1988; Willet, 2001a,b; Rock, 2003; Bingham and Riboli, 2004; Bray *et al.*, 2004). Two studies estimate that, on average, 32–35% of cancers could be attributed to nutrition, although the contribution of diet to specific types of cancer varies from as little as 10% for lung cancer to 80% for cancer of the large bowel (Doll and Peto, 1981; Willett, 1995).

Both macro- and micronutrients and other bioactive constituents of the diet have been shown to alter the various events of carcinogenesis. The nutrients and factors showing modulatory effects in experimental cancer and/or human epidemiological studies include: macronutrients (fat, carbohydrates and protein – positive association); fibre (negative association); vitamins (folic acid, riboflavin, β-carotene, retinol, α-tocopherol, C, E and vitamin B_{12} – negative association); minerals (selenium, zinc, magnesium and calcium – negative association -); total calories (positive association -), caloric restriction (negative association); and moderate intake of wine, and, recently, beer is also being suggested (negative association) (Kritchevsky and Klurfeld, 1987; Pariza, 1987; Birt *et al.*, 1992; World Cancer Research Fund and American Institute for Cancer Research, 1997; Kundu and Surh, 2004; Stevens and Page, 2004). Studies performed mainly experimentally have determined that some of these nutrients are involved in several carcinogenic processes. In this way, some vitamins (riboflavin, choline) and minerals (selenium) have shown the capacity to increase inactivation of certain carcinogens (Poirier, 1987; Schi *et al.*, 1994). Moreover, some phytochemical products have the ability to increase expression (enzyme induction) of critical detoxification enzymes (glutathione, glutathione transferase and glucoronyl transferase) that are responsible for the destruction of reactive electrophiles and oxidants into innocuous, excretable metabolites (Steinmetz and Potter, 1991). Food and nutrition also have a role in initiation, DNA repair, promotion and progression. Fat diet, casein and deficiency of folate and/or choline have been involved in the initiation of some tumours (World Cancer Research Fund and American Institute for Cancer Research, 1997). Dietary deficiency of methyl groups is in relation to increased expression and increased DNA strand breaks in *p53* suppressor gene. In addition, the increased expression of *ras* and *myc* oncogenes has been described in animals fed diets deficient in methyl groups, and may be reversed by later supplementation of the animals with methyl donors (Wainfan and Poirier, 1992; Christman *et al.*, 1993). Other nutrients such as vitamins A, D and B_{12}, iron, folic acid, zinc and glucose play a role in the control of cell cycle progression, modulating aberrant cell growth (Bohnsack and Hirsch, 2004). Recently, some evidence has been obtained about the potential role of some nutrients on the DNA repair processes (Moller and Loft, 2004; Oommen *et al.*, 2005). Although *in vivo* there are no established dietary agents that alter the apoptosis pathways, *in vitro* some dietary antioxidants have demonstrated to have the ability to induce it (Borek, 2004). Moreover, particular short-chain fatty acids (produced in the colon by the fermentation of fibre and complex carbohydrates) may induce apoptosis in colon cancer cell lines (Miller, 2004).

The effects of diet on experimental tumour promotion have largely been investigated in four organ systems: mouse skin, rat mammary gland, rat intestine, and rat and mouse liver. Findings from such investigations indicate that increased ingestion of fats and/or calories markedly enhances tumour promotion in most tissues examined (Kritchevsky and Klurfeld, 1987; Pariza, 1987; Birt *et al.*, 1992; World Cancer Research Fund and American Institute for Cancer Research, 1997; Solanas *et al.*, 2002b). It has also been described that high quantities of casein (20%) and deficiencies of the major dietary methyl donors, methionine and choline, enhance tumour formation. Nowadays, a large body of evidence suggests important roles of oxidative stress in promotion and progression, besides its known role in tumour initiation. Thus, oxygen free radicals have been involved in expansion of tumour clones and the acquisition of malignant properties (Dreher and Junod, 1996). The nutrients that have shown protective effects against tumour promotion are selenium, retinoids and vitamin D.

Obesity also seems to have a role in human cancers, such as those of the breast, endometrium, colon and kidney, but this effect has not been completely demonstrated. Physical activity, which maintains lean body mass as well as influencing several other systems in the body, especially endocrine and immunological function, is very consistently associated with a lower risk of colon cancer, and probably breast cancer (World Cancer Research Fund and American Institute for Cancer Research, 1997).

The role of food and nutrition, including accompanying carcinogens, in the progression stage of the carcinogenesis is not so clear. However, some data exist about the effect of some nutrients on invasion and metastasis. In a model of metastasic growth, vitamin C has been shown to act as an angiostatic factor, aiding host resistance to tumour growth and invasiveness (Borek, 2004). In human tumour xenografts, n-6 polyunsaturated fatty acids (PUFA) stimulated primary tumour growth and metastasis, whereas long-chain n-3 PUFA were inhibitory (Rose and Conolly, 1997). Epidemiological data and some experimental studies on smoking suggest that tobacco-associated carcinogens could act both early and late in the cancer process (Wogan *et al.*, 2004).

There are certainly numerous factors and nutrients playing a role in the relationship between nutrition and cancer. However, among them, dietary lipids constitute perhaps one of the most remarkable factors, since cancer risk is elevated among sedentary individuals and/or those consuming a high-fat diet (Cohen, 1992; Bartsch *et al.*, 1999). A great number of experimental and epidemiological studies have shown the relationship that exists between dietary lipids and some cancers, such as those of the breast, colon and rectum, and prostate. The fact that this relationship does not exist in all cancers, suggests a specific action of dietary lipids, beyond their caloric supply. By contrast, caloric restriction appears to affect all cancers in a general manner, normally leading to a certain degree of tumour regression. Nevertheless, the consistency and reproducibility of the studies of diet and cancer varies according to the type of study. In general, experimental studies in animal models provide clear and relatively reproducible results on this relationship. They have the great advantage that the study is performed under the same alimentary (complete diet) and physiological (ingestion, digestion, absorption and metabolism) conditions as those that occur

in the human species. They permit experimental modification of the dietary factors, segregating the variables that are the subject of study. Typical parameters of the disease (clinical behaviour, anatomopathology, molecular mechanisms, etc.) can also be determined. The problem with these experimental studies is that there is some limitation in the direct extrapolation of the results to the human situation. Cell culture studies have technical advantages; but they have the disadvantage of lacking appropriate physiological conditions and tissue architecture. This means that the autocrine and paracrine influences among cells and cell types are very different from those of the tumour cells *in vivo*. Finally, the epidemiological studies are controversial. Regarding the ecological studies (international and migration studies) a positive association between the *per capita* fat consumption and the mortality rates of the mentioned cancers is found. It is noteworthy that countries with a Mediterranean diet show middle values for these cancers. However, in case-control and cohort prospective studies the relationship is not so clear. Several explanations have been put forward to explain these discrepancies, among them the low number of effectives of these last mentioned studies in comparison with international studies. Meta-analysis techniques have permitted reanalysis of the data and, in some cases, a relationship between dietary lipids and cancer has been shown (Armstrong and Doll, 1975; Carroll and Khor, 1975; Armstrong *et al.*, 1982; Carroll, 1992; Willet, 1999; Lee and Lin, 2000).

Dietary lipids have been particularly associated with colorectal, breast and prostate cancers. Among them, breast cancer is, up to now, the most exhaustively studied.

4. Experimental Data on the Effects of Olive Oil and Other Dietary Lipids in Cancer

4.1. Breast cancer

Since 1942, when Tannenbaum demonstrated that spontaneous breast cancer incidence was higher in mice fed a fat-supplemented diet (Tannenbaum, 1942), a number of experimental and epidemiological studies have shown the relationship between dietary lipids and breast cancer. Despite some contradictory epidemiological studies, in general a positive association between *per capita* fat consumption and breast cancer mortality rates has been found. It is noteworthy that the Mediterranean diet countries show intermediate values (Rogers and Longnecker, 1988; Hunter and Willet, 1994; Assmann *et al.*, 1997; Lipworth *et al.*, 1997; Bartsch *et al.*, 1999; Rock, 2003).

The use of experimental models has demonstrated that the effect of dietary lipids on breast cancer depends on the type and the quantity of consumed fat, as well as on the particular critical phases of the carcinogenesis. In general, high-fat diets act mainly on the promotion stage of breast cancer, although diets with the same quantity of energy and total fat content differ in their capacity to modulate mammary tumour growth depending on their fatty acid composition (Rose

et al., 1993; Kaput and Rodríguez, 2004). Moreover, a minimal requirement of PUFA seems to exist, from which the effect of fat would depend on the total quantity of lipids in the diet. In particular, at an intake above 4–5% energy of PUFA, the yield of 7,12-dimethylbenz(α)anthracene (DMBA)-induced mammary carcinogenesis increases proportionally to the quantity of total fat of the diet, irrespective of its animal or vegetable origin (Carroll, 1992; Bartsch *et al.*, 1999). Nevertheless, the specific type of fatty acid predominant in fats constitutes an important factor. Thus, the n-6 PUFA, mainly linoleic acid (18:2n-6) – LA -, from vegetable oils, are the main promoters of carcinogenesis, as has been shown consistently in experimental studies (Ip, 1987; Bartsch *et al.*, 1999; Stoll, 2002). In the 14 experimental series developed by our group up to now, diets with a high content of n-6 PUFA (corn oil) consistently exert a stimulating effect on experimental mammary carcinogenesis. In animals fed this diet, tumours appear earlier, and the incidence, tumour content and volume are higher than in the other dietary groups (Escrich *et al.*, 1992, 2001; Moral *et al.*, 2003; Solanas *et al.*, 2000, 2001, 2002b). Representative results from two of these 14 experimental series are shown in Fig. 15.4.

Within the n-6 PUFA family, the γ-linolenic acid (18:3n-6) – GLA -, found in special oils such as that of the evening primrose, is an exception because it has antiproliferative properties (Bégin *et al.*, 1988; Kenny *et al.*, 2001). Moreover, conjugated linoleic acid (CLA), a term to include several geometrical and positional isomers of the LA, could have an inhibitory effect on breast cancer (Ip, 1997; Davis *et al.*, 1999). CLA is found in meat and ruminant-derived dairy products, as it is synthesized naturally by the microorganisms of the rumen of these animals (*Butyvibrio fibrisolvens*) (Davis *et al.*, 1999; Ip *et al.*, 2003; Khan and Heuvel, 2003). CLA also shows anti-diabetogenic, anti-carcinogenic and antioxidant properties (Moya-Camarena *et al.*, 1999; Khan and Heuvel, 2003).

n-3 PUFA α-linolenic acid (18:3n-3) – LNA -, found in low quantities in vegetable oils, red meat and dairy products, and the long chain n-3 PUFA eicosapentaenoic acid (20:5n-3) – EPA -, and docosahexaenoic acid (22:6n-3) – DHA -, from fish and fish oils, would be inhibitory of mammary tumour growth (Ip *et al.*, 1986; Bartsch *et al.*, 1999; Woutersen *et al.*, 1999) and metastasis (Cave, 1997).

In the experimental mammary cancer, saturated fats, mainly from animal origin, have a promoting role, but are less potent than the vegetable-origin n-6 PUFA (Welsch, 1992; Fay *et al.*, 1997). Also, they could act as co-carcinogens during the initiation stage (Kritchevski *et al.*, 1984). The *trans* fatty acids, most often produced during the manufacturing process for many vegetable oil products such as margarine, behave as the saturated fats (Ip *et al.*, 1986; Ip, 1997).

Regarding monounsaturated fatty acids (MUFA), fundamentally oleic acid (18:1n-9) – OA -, which is the main component of olive oil, the results are somewhat different depending on the studies. On the one hand, as noted above, several epidemiological studies have shown that the incidence rates of breast cancer in Mediterranean countries, where the consumption of olive oil is high, are lower than those of Northern European and American countries (Assmann *et al.*, 1997; Lipworth *et al.*, 1997). On the other hand, the experimental studies have yielded different results, some reporting a non-promoting effect, some a

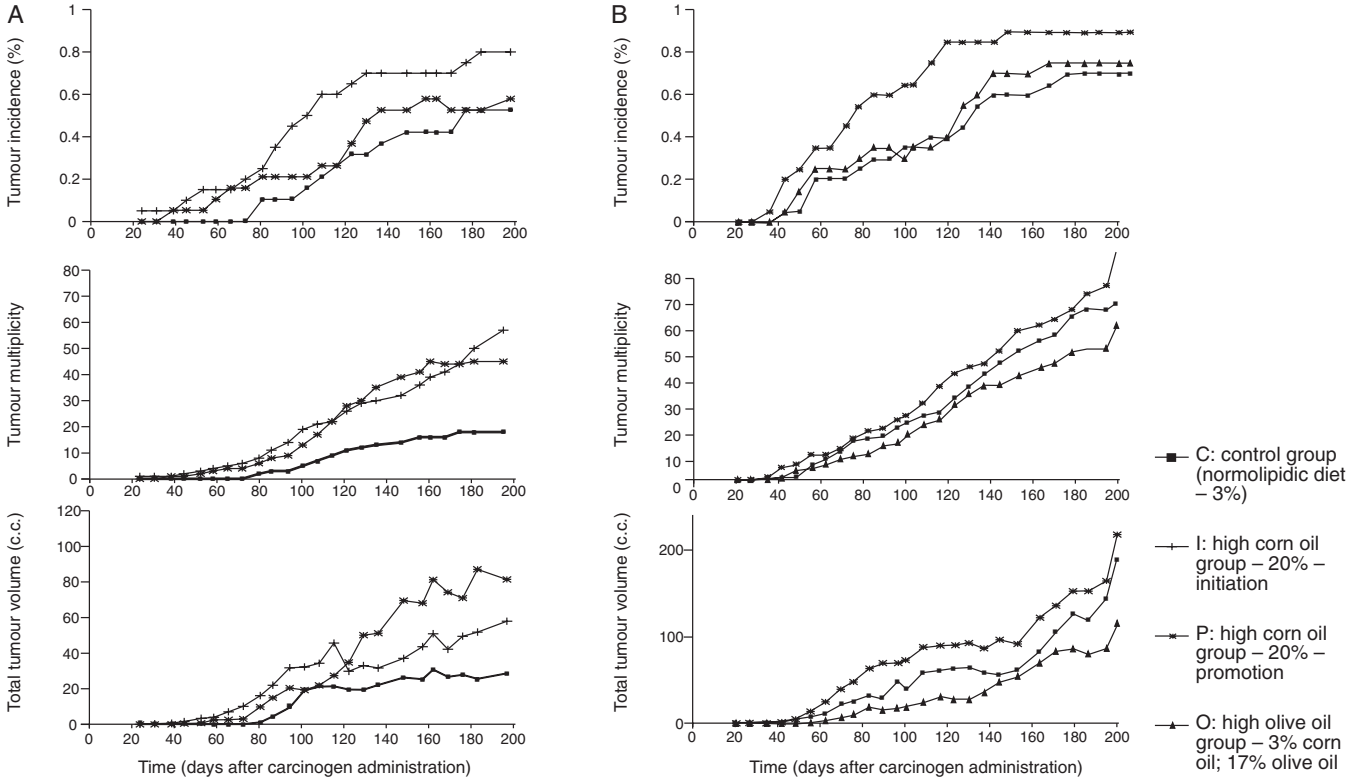


Fig. 15.4. Effects of dietary corn and olive oils on three representative parameters of the female Sprague-Dawley rat DMBA-induced mammary carcinogenesis (from a total of eight previously described – Escrich *et al.*, 1991; Ruiz de Villa *et al.*, 1999 -). A: series 6, B: series 14. Tumour incidence: percentage incidence of rats with palpable mammary tumours. Tumour multiplicity: cumulative total number of palpable mammary tumours. Total tumour volume: total volume of palpable mammary tumours. The differences observed between the different experimental groups and the control are in all cases statistically significant (Friedman's two factors test; $p < 0.05$).

weak promoting effect and some a protective one (Ip, 1997; Zusman *et al.*, 1997; Willett, 1999; Lee and Lin, 2000; Kushi and Giovannucci, 2002; Solanas *et al.*, 2002b). These differences are likely to be related to the distinct varieties of olive oil used in the experiments (Cohen *et al.*, 2000). In this sense, we have demonstrated the negative modulating role, possibly protective, of extra virgin olive oil in the appearance and the progression of experimental breast cancer. Thus, the groups of animals fed a high olive oil diet show a significantly higher latency time of the cancerous disease, a lower incidence of affected animals in comparison with a high corn oil diet group, although higher than that of the control group, and a lower tumour content and volume than the rest of the experimental groups, including the control one (Fig. 15.4). The analysis of the tumour regression showed that the negative modulating effect proposed for the high virgin olive oil diet would not be as potent as for regressing mammary tumours that have already appeared, either totally or partially, but also this effect would consist of a slow progression of the tumour growth rather than a real tumour regression. These results also suggest that the high olive oil diets effect would not be as potent as for using them as therapeutic agents, at least by themselves (Solanas *et al.*, 2002b).

In any case, it is necessary to consider that, although the main component of olive oil is OA, found at 72–84% of total fatty acids, this oil also contains other components, which have been proposed as accountable for the specific effects of virgin olive oil on breast cancer. In the first place, there are PUFA: 5–11% of LA, 0.3–0.8% of LNA; and saturated fatty acids: 13–16% of palmitic (16:0) and estearic (18:0) acids (Gerber, 1997; Visioli and Galli, 1998). Besides the fatty acids, virgin olive oil contains other components: α-tocopherol (vitamin E) and phenolic compounds (hydroxytyrosol, oleuropein and lignans), with antioxidant capability (Galli and Visioli, 1999); phytosterols (β-sitosterol and D5-avenasterol), flavonoids (rutin, luteolin) and triterpenic hydrocarbon squalene (Gerber, 1997; Rao *et al.*, 1998). Based on these components, several hypotheses have been proposed to explain the possible protective effect of olive oil, including its high content of OA, or its high antioxidant capacity, or both. Moreover, it has also been proposed that the protective effect could be due to a LA deficit, since a critical level of this fatty acid in the diet (4–5%) seems to be necessary to exert the promoting effect, as mentioned above. Also, the relative ratio of mono- to polyunsaturated fatty acids, rather than the absolute level of LA, could account for the protective role of olive oil (Cohen *et al.*, 1986a; Ip, 1997; Takeshita *et al.*, 1997; Zusman *et al.*, 1997; Smith *et al.*, 1999). Thus, the variety of olive oil used in the experiments is again an important factor.

Taking into account the importance of olive oil in the Mediterranean diet, it is apparent that the relationship between olive oil consumption and breast cancer is controversial, but it could have important repercussions in human health, especially among the female population (Martin-Moreno, 2000).

Regarding the effect of dietary energy, a positive association between the dietary caloric supply and cancer mortality rates has been demonstrated. Caloric restriction has an indiscriminate inhibitory effect on carcinogenesis, which suggests a non-specific mechanism of action (Kritchevsky, 1999). Therefore, a part of the stimulating effect of high-fat diets could be conferred by its high caloric con-

tent. Nevertheless, as discussed in the next section, specific mechanisms exist through which dietary lipids could influence breast cancer (Freedman *et al.*, 1990). As it has been pointed out, an argument for this conclusion is the fact that only certain types of tumour are stimulated by diets with a high content of fat.

The differential effect of different types of dietary lipids on experimental breast cancer has also been characterized morphologically by our group, for the first time, according to the available information, through an exhaustive histopathological analysis. High corn oil diets, mainly when they act on the promotion of the carcinogenesis, are associated with adenocarcinomas of a higher degree, stromal invasion and more prominent tumoral necrosis and prevalent cribiform pattern in comparison with control and high olive oil diets. The adenocarcinomas of the olive oil diet group exhibit a low histopathological grade, few invasive and necrotic areas, similar to the control situation, and show a higher percentage of papillary areas. Therefore, the tumours of the virgin olive oil group show a lower degree of morphological malignancy than those of the corn oil group, being more similar to control tumours (Fig. 15.5). This is compatible with their lower degree of clinical malignancy and with the non-promoting effect of the virgin olive oil on experimental mammary carcinogenesis (Costa *et al.*, 2001, 2002, 2004).

4.2. Colorectal cancer

Colorectal cancer is the second/third leading cause of cancer-related death in both men and women in Western countries (Ferlay *et al.*, 2004; Jemal *et al.*, 2005). Large regional differences, rapid increases in several countries and adaptation of migrant populations to the incidence of hosting areas suggest that environmental factors, mainly diet and lifestyle, play a crucial role. Fat seems to be one of the most important dietary components in colorectal cancer risk and the association of saturated and animal fat with this risk is quite strong (World Cancer Research Fund and American Institute for Cancer Research, 1997; Kushi and Giovannucci, 2002). International comparisons and animal studies have indicated a strong positive association of dietary fat with colorectal cancer risk. As with studies of breast cancer, analytic epidemiological studies generally fail to detect a positive association of total fat intake with risk of this neoplasia (Kushi and Giovannucci, 2002).

Several animal models based on the induction of colorectal tumours by chemical carcinogens, such as 1,2-dimethyllhydrazin (DMH), azoxymethane (AOM), methylnitrosourea (NMU), or methylnitrosoguanidine (MNNG), as well as transplantable models and transgenic mice have been used to study the link between dietary fats and colorectal tumorigenesis (Klurfeld and Bull, 1997). In these animal models it has been demonstrated that the effect of dietary fat depends on both the type and the amount of fat. In fact, the composition of ingested dietary fatty acids is more critical to colon cancer risk than is the total amount of fat. These types of study have also indicated that the influence of type and amount of dietary fat is exerted foremost during the postinitiation and

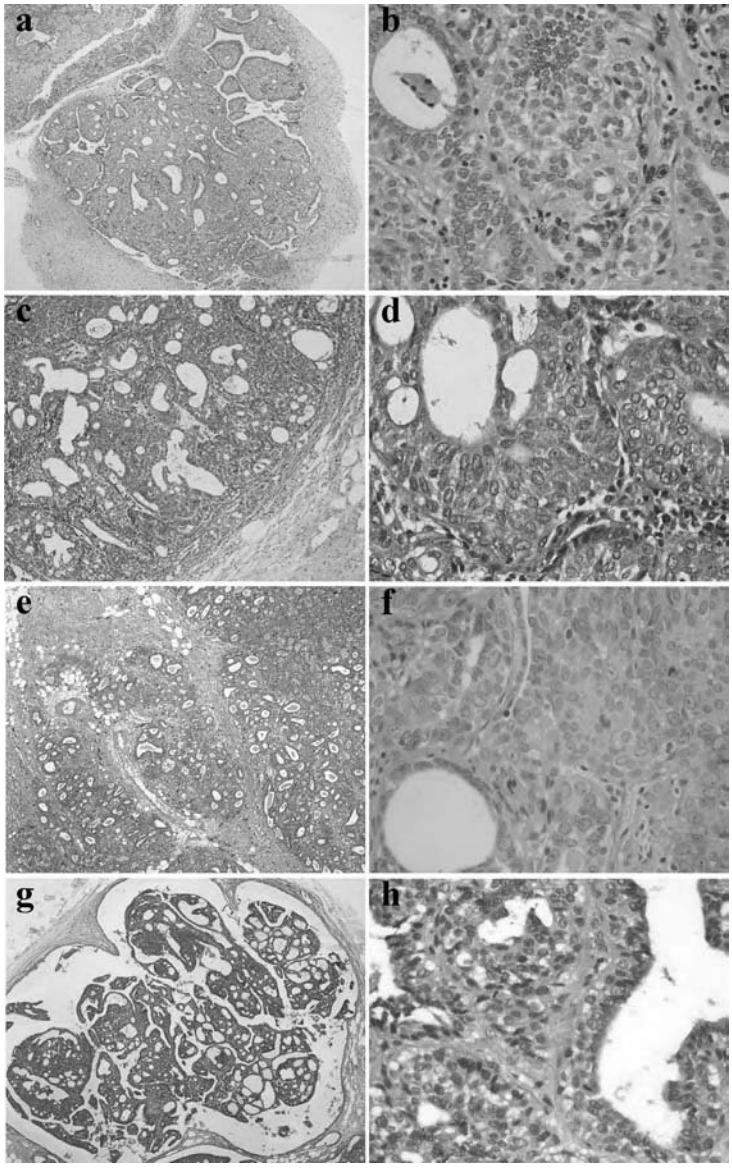


Fig. 15.5. Histopathologic features of rat DMBA-induced mammary adenocarcinomas from the different dietary groups. (a and b): most of the tumours from the low fat diet groups were low grade and non-invasive; displaying cribriform and papillary areas. (c and d): carcinomas from high corn oil initiation groups exhibited a high pattern and nuclear grade, but few mitotic figures. They presented a prominent lymphoplasmacytic reaction. (e and f): adenocarcinomas from promotion high corn oil groups presented invasive features, showing indisputable infiltration of neighbour tissues, as skeletal muscle, and the most prominent degree of cytological malignancy with prominent nucleoli and high mitotic count. (g and h): adenocarcinomas of the promotion high olive oil group were well differentiated, with prevailing papillary areas and no or light stromal response, but with mitotic activity. (Hematoxylin and eosin staining; original magnification $\times$ 10 (a, c, e, g); $\times$ 20 (b, h); $\times$ 40 (d, f) (Costa *et al.*, 2004).

promotional phases of carcinogenesis, although saturated fat seems to exert an enhancing effect during the initiation phase too. As for the n-6 PUFA, while case-control and prospective cohort studies have failed to show a consistent association between intake of these fatty acids and colorectal cancer risk, in general experimental studies have shown an increase in the risk of chemically induced colon carcinogenesis and high n-6 PUFA diets. On the contrary, epidemiological, case-control, and animal studies have found a protective effect of n-3 PUFA over colorectal cancer development (Reddy, 1992; Woutersen *et al.*, 1999; Rao *et al.*, 2001; Roynette *et al.*, 2004). Despite the wide ranging evidence of the potential benefit of n-3 PUFA in colon cancer, some ambiguity remains over their potential adverse effects with respect to secondary tumour formation. Thus, while it appears that n-3 PUFA may be useful in colon cancer prevention, there remain some uncertainties about their application in colon cancer patients (Roynette *et al.*, 2004).

As regards the n-9 MUFA in olive oil, although they have been less studied, ecological and case-control studies suggest that olive oil may also have a protective effect. Laboratory animal model studies have generally shown that olive oil has either no effect or a protective effect on the prevention of chemically induced tumours (Lipworth *et al.*, 1997; Stoneham *et al.*, 2000; Stark and Madar, 2002). Thus, some animal studies have shown a decrease in the number of aberrant foci at the early stages of tumour development as well as inhibition of tumour formation. For example, in female F344 rat AOM-induced colon cancer model, high olive oil diets had no promoting effect on colon tumour incidence in comparison with diets containing high corn or safflower oils (Reddy and Maeura, 1984). Likewise, Takeshita *et al.* (1997) found that the incidence of colon adenocarcinomas in DMH-treated mice was significantly higher in animals fed a diet rich in LA than in animals fed a diet enriched with OA or a low-fat diet. It has also been shown that an olive oil based diet with a normal amount of fat (5% dietary fat for rats), compared with a isolipidic safflower oil diet, prevented the development of aberrant crypt foci and colon carcinomas induced in male Sprague-Dawley rats with AOM, suggesting that olive oil may have a chemopreventive activity against colon carcinogenesis. The anticarcinogenic effect of this olive oil diet was similar to that of an isolipidic fish oil based diet (Bartolí *et al.*, 2000). Results obtained *in vitro* supplementing colon cancer cell lines with olive oil support the hypothesis that the prevention of colorectal cancer development by olive oil, and also by fish oil, may be the result of an early downregulation of cyclooxygenase-2 (*COX2*) followed by a decrease in the expression of the apoptosis inhibitory Bcl-2 protein (Llor *et al.*, 2003). The possible colon tumour preventive effect of high 15% olive oil diets in the offspring of pregnant female rats fed this diet has also been tested. The results indicated that the significant effect observed in adults was lost in the DMH-treated male offspring (Kossoy *et al.*, 2000).

Other *in vitro* and animal studies have provided support for the potential of olive oil to lower colon cancer risk. Thus, when heterocyclic amine formation during cooking of meat and fish was measured in a model system, olive oil was able to inhibit formation by 30–50% compared with controls. Because these compounds are considered to be possible human carcinogens, these results suggest that the use of olive oil in food preparation may be advantageous (Monti *et al.*, 2001).

Recently, the possible effects of olive oil–pharmacological combination on colon cancer incidence have been tested in the male Sprague Dawley rat DMH-induced colon cancer model. Diets containing high levels (15%) of olive oil exerted a significant protective effect from colon tumour development that was additive with the inhibitory effect of sulindac, a non-steroidal anti-inflammatory drug used in the treatment of familial adenomatous polyposis. These data provide experimental evidence on the suitability of the nutritional–pharmacological combination to reduce colon cancer incidence (Schwartz *et al.*, 2004). Another study carried out using archival paraffin-embedded colon tumour samples corresponding to 106 patients and epidemiological data from a previous case-control study, showed that high consumption of monounsaturated fats, mostly derived from olive oil, was associated with a statistically significant decrease in the risk of cancer with wild-type Ki-ras genotype, but not of Ki-ras mutated cancers. These results point out the fact that nutrients can have a differential protective effect, depending on the genetic makeup of the cells upon which they act, and this should be taken into account in the possible nutrient chemoprevention regimens (Bautista *et al.*, 1997).

Taken together, there is now substantial evidence that n-6 PUFA enhance the risk for colon cancer and metastasis, whereas relatively high intakes of n-3 PUFA (fish oil) and n-9 MUFA (olive oil) reduce cancer risk by different mechanisms that will be reviewed in section 5 of this chapter.

4.3. Prostate cancer

Prostate cancer has become the second most commonly diagnosed male cancer in many Westernized populations and the fourth leading cause of male cancer-related death (Ferlay *et al.*, 2004; Jemal *et al.*, 2005). As with breast and colorectal cancer, there is a wide geographical variation in prostate cancer rates; much of this variation is likely the result of environmental or lifestyle factors that also vary geographically. Fat has been the focus of dietary studies of prostate cancer more than any other dietary component, although the literature is not as extensive as for the other mentioned cancers (World Cancer Research Fund and American Institute for Cancer Research, 1997). International comparisons have shown that the same positive correlation exists between prostate cancer mortality rates and estimates of *per capita* fat intake as that observed for breast and colorectal cancer. Ecological analyses have also found positive associations with animal and saturated fat, but association with monounsaturated and polyunsaturated fat is generally weak. On the other hand, results from case-control and cohort studies lack consistency especially for specific fatty acids, probably due partially to methodological limitations (Rose, 1997a; Kolonel *et al.*, 1999; Kushi and Giovannucci, 2002). The results with regard to LNA have been particularly interesting because, whereas some analytical epidemiological studies have found a positive association between this essential fatty acid and prostate cancer risk, marine long-chain n-3 PUFA have not shown a similar association (Willett, 1997; Kolonel *et al.*, 1999; Brouwer *et al.*, 2004; De Lorgeril and Salen, 2004; Leitzmann *et al.*, 2004). In conclusion, from epidemiological studies the role of

dietary fat in prostate cancer remains controversial since although the pooled estimates suggest a small, significant association between prostate cancer and total fat consumption, the heterogeneity between studies is large, and the association is not supported for specific fatty acids (Dennis *et al.*, 2004).

Experimental data on the potential relationship between dietary fat and prostate cancer are limited, especially in the case of olive oil. The experimental evaluation of the role of dietary fat in the occurrence of prostate tumours has been hampered by a lack of suitable animal models. Also, small laboratory animals seldom develop this tumour spontaneously and their prostate gland differs anatomically from that of humans. However, some experimental models of prostate cancer hormonally or chemically induced, in addition to the more recent transgenic mouse models, have been developed. The experimental evidence suggests that dietary fatty acids influence the biological behaviour of prostatic cancer cells once neoplastic transformation has taken place; an association between high dietary fat intake and risk of having advanced stage disease when initially diagnosed with prostate cancer has been described (Rose, 1997a). In 1986, Pollard and Luckert (1986) reported, for the first time, that a high-fat diet increased prostate cancer incidence and shortened the latent period in Lobund Wistar rats treated with exogenous testosterone. In general, the animal studies suggest that dietary fat increases both the incidence and the rate of growth of adenocarcinomas of the prostate. Fat type has also been shown to influence prostate tumour cell growth both *in vitro* and *in vivo*. Generally, fish oils containing high levels of long-chain n-3 PUFA suppress tumour growth, whereas oils high in PUFA such as LA and LNA promote tumour growth (Rose and Connolly, 1992; Zhou and Blackburn, 1997; Kolonel *et al.*, 1999; Shirai *et al.*, 2002).

The studies with human prostate cell lines have also suggested an influence of fat on tumour growth. In the androgen-independent PC-3 human prostate cancer cell line, LA exerted a stimulatory effect on the cell growth. It also enhanced the invasive capacity of PC-3M cells, obtained from a relatively uncommon PC-3 cell metastasis. In another androgen-independent prostate cancer cell line, DU145, this fatty acid also stimulated the growth of a high-passage variant. Arachidonic acid (AA) has also been shown to exert a mitogenic effect on PC-3 and DU145 cells. Moreover, solid tumours developed in male athymic nude mice by subcutaneous injection of the DU145 cell line were inhibited by feeding a diet containing a high concentration of long-chain n-3 PUFA, but the supplementation with LNA was ineffective. Using the androgen-responsive human prostate cancer cell line LNCaP in nude mice, growth rates of transplantable tumours were significantly greater in mice fed a 40.5% fat diet compared with those of other lower fat diets (Rose, 1997b; Zhou and Blackburn, 1997; Moretti *et al.*, 2004). However, the study of Mukherjee *et al.* (1999) carried out in two transplantable prostate tumour models exhibiting dissimilar histopathological and biological features showed that energy restriction reduced prostate tumour growth, but that dietary fat concentration did not influence it. More recently, it has been shown that the increase in the incidence of prostate cancer induced by a high-fat diet (40% of calories from fat) in the *Lady* transgenic mice model, which mimics progressive forms of human disease, is blocked by the addition of antioxidants (vitamin E, selenium, lycopene) at an achievable

dose for humans. Moreover, administration of antioxidants increased the disease-free survival of mice (Venkateswaran *et al.*, 2004). These results are interesting because all of these antioxidants are notably present in the Mediterranean diet.

5. Mechanisms of the Modulating Action of Dietary Lipids on Cancer

The mechanisms through which dietary lipids could act on the development of cancer have not been well elucidated, but from experimental data, the influence of lipids could be established at several levels: (i) the carcinogenesis stages; (ii) hormonal levels; (iii) the cell membranes; (iv) the signal transduction pathways; (v) gene expression; and (vi) the immunological system. Probably, *in vivo*, lipids act through all these mechanisms in an integrated, simultaneous and/or sequential way.

5.1. Influence on the carcinogenesis stages

The influence of the lipids on carcinogenesis seems to be exerted mainly during the promotion stage. The mechanisms involved are those mentioned in the following sections. However, there is also evidence of a possible role of the lipids during the initiation stage of the carcinogenesis, whereby they act as co-carcinogens, facilitating the genotoxic action of several agents (Kritchevski *et al.*, 1984). Thus, lipids could alter the structure of the chromatin and affect the accessibility of carcinogens, DNA repair and/or the access of the transcription machinery to specific genes (Ronai *et al.*, 1991). In this sense, data from *in vitro* studies suggest that the electrostatic interactions between histones and DNA could be affected by interactions with membranes that contain acidic phospholipids and sphingosine, supporting the hypothesis that specific lipids could be directly involved in the regulation of the chromatin structure and function (Kinnunen *et al.*, 1994). Moreover, the presence of phospholipids, mostly sphingomyelin and phosphatidylserine, plasmalogens and cholesterol as components of chromatin is now well documented, and there are studies suggesting that these nuclear lipids may play crucial roles in transcriptional regulation that reflect the metabolic or development state of the cell (Albi and Viola Magni, 2004). On the other hand, in the rat 2-amine-1-methyl-6-phenylimidazo[4,5-b] piridin (PhIP)-induced mammary cancer model, it has been observed that a high n-6 PUFA diet increases the density and proliferation of epithelial cells in terminal end buds (TEBs) of the mammary gland, without altering the removal rates of PhIP-DNA adducts. These results prompted the authors to hypothesize that the stimulating effect of the high-fat diet may in part involve a further fixation of adduct-induced mutations through an enhanced proliferative stimulus rather than by an inhibition of DNA adduct repair processes (Snyderwine and Davis, 1998). As well, several studies have demonstrated that the exposition to CLA during the maturation period of the rat mammary gland provides a protective effect in the

initiation of the carcinogenesis. CLA could modify the potential development of a subgroup of target cells, which are susceptible to the carcinogen-induced transformation (Thompson *et al.*, 1997).

Moreover, the possible carcinogenic initiating action of substances that accompany the dietary fats (food pollutants, additives, hormones) should be taken into account, as well as the fact that the lipids could activate particular carcinogenic exogenous substances, or modulate the endogenous production of substances able to damage DNA (Armstrong *et al.*, 1982; King *et al.*, 1983; Nemoto, 1986).

Likewise, it also should be considered that PUFA metabolites resulting from peroxidation (epoxides and peroxides) could have a stimulatory effect on the development of human malignancies, since several antioxidant substances are protective from cancer (London *et al.*, 1985; Cohen *et al.*, 1986b; Fleshner *et al.*, 1999; Venkateswaran *et al.*, 2004). The free radicals that these compounds have can interact covalently with cell macromolecules and could affect tumour proliferation. A mechanism of DNA-adduct formation associated with a high intake of n-6 PUFA has been proposed. This would be based on the generation of reactive α,β-unsaturated aldehydes, such as *trans*-4-hidroxy-2-nonenal and malondialdehyde, which can form promutagenic exocyclic DNA adducts in human cells and may thus contribute to diet-related cancers. *trans*-4-hidroxy-2-nonenal, one of the major lipid peroxidation products, is formed by oxidation of LA or AA and is readily oxidized by fatty acid peroxides to form 2,3-epoxy-4-hydroxynonenal. This alkylating agent can react with DNA to yield exocyclic adducts with deoxyguanosine, which are highly miscoding lesions in mammalian cells and are thought to initiate the carcinogenic process through specific point mutations (Goldstein and Witz, 1990; Bartsch *et al.*, 1999). In general, these assumptions are based on the results of investigations of the *in vitro* oxidation of unsaturated fatty acids in homogeneous systems. However, some data suggest that lipid peroxidation *in vivo* may not correspond with that *in vitro* and, therefore, the role of lipid peroxidation is controversial. For instance, compared with the n-6 fatty acids, the n-3 fatty acids seem to exert a protective effect on cell growth that may, at least partly, be explained by the formation of oxidation products, which leads to cell growth arrest and, as they are cytotoxic, to apoptosis (Cave, 1997; Wynder *et al.*, 1997; Stoll, 2002; Larsson *et al.*, 2004). On the other hand, CLA is suggested to suppress peroxidation of unsaturated fatty acids, which would reduce oxidative damage (Ip *et al.*, 1996; Moya-Camarena *et al.*, 1999; Watson *et al.*, 2000).

Olive oil protects from oxidative damage or generates lower intracellular levels of such damage. On the one hand, the high content of OA is important because it is far less susceptible to oxidation than LA; on the other, some of the minor components of the virgin olive oil (α-tocopherol – vitamin E -, and the phenolic compounds, such as oleuropein, hydroxytyrosol and lignans) would have the ability to reduce the lipid peroxidation, reducing oxidative stress. In relation to their possible role in the cancer initiation processes, these hydrosoluble phenolic compounds seem to have a protective role which could be more important than that of vitamin E. Moreover, the antioxidant richness of its minor components contributes importantly to the high stability (shelf life) of

this oil, in addition to resulting in a lower level of potentially carcinogenic oxidized compounds (Gerber, 1997; Visioli and Galli, 1998; Owen *et al.*, 2000, 2004; Bartsch *et al.*, 2002; Visioli *et al.*, 2004). The effects of olive oil on oxidative stress have been previously mentioned and described extensively in Chapter 6 by Quiles *et al.*

Hydroxyl radical-induced DNA damage has also been linked to the progression of human cancers to the metastatic stage, notably because it results in loss of cell adhesion, which is a prerequisite for cellular detachment and invasion of host tissues (Malins *et al.*, 1996).

Finally, in colon tumorigenesis the effects of bile acids must be considered. Dietary fat induces excretion of bile acids, which may be converted to secondary bile acids by colonic bacteria. Metabolic epidemiological studies have demonstrated that populations at high risk for colon cancer excrete high levels of secondary bile acids. Laboratory animal model studies have shown that these compounds induce cell proliferation in the colonic mucosa and act as tumour promoters. The colonic luminal concentration of these secondary bile acids increases in response to diets high in lard, beef tallow or corn oil, whereas dietary fish oil at high concentrations does not have this enhancing effect (Woutersen *et al.*, 1999; Rao *et al.*, 2001). The secretion of these acids does not increase due to high olive oil diets (Stoneham *et al.*, 2000). Experimentally, the non-promoting effect of the high olive oil diets on the rat F344 AOM-induced colon cancer model has been also associated to a lack of increase in the level of colonic secondary bile acids (Reddy and Maeura, 1984). Squalene, a constituent of olive oil, itself has a similar effect due to its modulation of bile acid biosynthesis (Rao *et al.*, 1998).

5.2. Effects on the hormonal levels

In the hormone-dependent breast and prostate cancers, it has been proposed that dietary lipids may influence cancer development through modifications in the concentrations of circulating sex hormones, such as oestrogens and testosterone respectively. The oestrogens are the hormones that could be more directly involved in the action of lipids on breast cancer since they participate in all the processes, normal and pathological, of the mammary gland. The metabolism of these hormones could be altered by excessive lipid intake, and thus influence mammary carcinogenesis. Oestrone is the major oestrogen of postmenopausal women. This hormone can be synthesized by the adipose tissue, and other tissues, from the suprarenal and ovarian androgens. This peripheral conversion is exerted through the aromatase enzymes. An excessive intake of fat could result in an increase in adipocyte number and, as a consequence, an increase in oestrogen synthesis. This is supported by the observation that the higher breast cancer rates occur in postmenopausal and obese women (Deslypere *et al.*, 1985; Woutersen *et al.*, 1999). n-6 PUFA could increase oestrogenicity at three levels: (i) by the displacement of the oestrogens from their carrier protein (Bartsch *et al.*, 1999; Wynder *et al.*, 1997); (ii) by increasing the affinity for their receptor; or (iii) by inhibiting 17β-dehydrogenase enzyme activity and avoiding, in this way, the conversion of oestradiol (the most active oestrogen) into estrone (Martin *et*

al., 1986; Pansini *et al.*, 1990). Moreover, it has been observed that prostaglandin E_2 (PGE_2), an AA metabolite, stimulates the expression of aromatase P450 activity, which converts 19-carbons steroids into oestrogens. In contrast, PGE_3, a metabolic product of EPA, does not increase the expression of this aromatase. Hence, an increased intake of EPA, through fish consumption, which leads to increased production of PGE_3 and decreased production of PGE_2, is expected to decrease the local production of oestrogens and thus the growth stimulated by these hormones (Larsson *et al.*, 2004). Likewise, as discussed in the previous section, the increased intake of certain fats causes an increment in bile acid secretion. The bile acids and cholesterol derivatives can be transformed into oestrogens by certain bacteria of the intestinal flora (Hill *et al.*, 1971). The modulating activity of the bile acid biosynthesis characteristic of squalene could account at least partially for the preventive effect of this oil on carcinogenesis (Rao *et al.*, 1998).

The minor components of olive oil lignans seem to have antioestrogenic effects, because of their structural similarities with oestradiol and the synthetic antioestrogen tamoxifen. Moreover, they have been shown to inhibit oestradiol-induced proliferation of MCF-7 human breast carcinoma cells and to stimulate sex-hormone-binding globulin synthesis, with a subsequent decrease in free oestradiol (Owen *et al.*, 2000).

In relation to the oestrogen receptors, LA has recently been shown to increase expression of the oestrogen receptor α (ER_α) mRNA and decrease that of the androgen receptor (AR) in human mammary tumour cells T47D. This increase is particularly significant because the oestrogens are potent stimulators of the mammary cell growth, both normal and tumoral. The fact that AR is decreased would signify that this would have antagonist effects on ER_α-mediated signalling (Reyes *et al.*, 2004).

Despite the relationhips previously suggested, clear differences have not been found between high-fat diets and levels of either the gonadal steroid levels or their receptors (Ip and Ip, 1981; Welsch *et al.*, 1983; Sylvester *et al.*, 1986; Mizukami *et al.*, 1992).

Another aspect to take into account in the possible relationship between dietary lipids and oestrogens in cancer is the fact that the metabolic redox cycling of the metabolite catechol oestrogen 4-hydroxyoestradiol catalysed by CYP P450 enzymes leads to reactive oxygen species. These can damage DNA and could trigger lipid peroxidation of n-6 PUFAs to yield the DNA-reactive enals mentioned above (Bartsch *et al.*, 1999).

Prolactin has been also related to lipids and breast cancer, although the results are controversial (Wynder and Hill, 1977; Carroll, 1981; Cohen, 1981; Wetsel *et al.*, 1983; Sylvester *et al.*, 1986; Clinton *et al.*, 1995). Notable differences exist between human and experimental breast cancer regarding its prolactin-dependence (European Breast Cancer Group, 1972; Meites, 1972), and the fact that prolactin is an extremely labile hormone must be considered. Thus, it could be modified by multiple factors such as circadian rhythm, sleep, stress due to the manipulation or the moment of blood extraction and the type of anaesthetic used in the experiments (Neill, 1970). These factors on their own could explain the disparity of results. Insulin is another of the hormones studied.

It is suggested that high-fat diets enhance its cellular action, although the studies are not conclusive (Lomeo *et al.*, 1986; Cave, 1996; Woutersen *et al.*, 1999). In the same way, inconsistent results preclude any firm conclusion about the participation of other hormones – tiroxine, growth hormone and corticosterone – in relation to dietary lipids and breast cancer.

The possible effect of dietary lipids on hormone levels was studied by our group, although there were no modifications in the plasma levels of the main regulating hormones (17β-oestradiol, progesterone, insulin, prolactin and corticosterone) of mammary development, or in the tumour content of steroidal receptors (oestrogen and progesterone receptors) (Escrich, 1990, 1998). Likewise, various plasmatic biochemical parameters of the animals from the different experimental groups were studied, showing only a hypocholesterolemic effect of the diets rich in corn oil (Moral *et al.*, 2004).

In the case of prostate cancer, little is known about the possible influence of dietary fats on male sex hormone levels. For example, in athymic nude mice bearing human prostate cancer xenografts and fed a low-fat diet, an absence of any change in serum testosterone was reported. On the contrary, in a human study a low fat dietary intervention caused a reduction in serum total and unbound testosterone (Rose and Connolly, 1992; Rose, 1997b). In two other human feeding studies, a high-fat diet with a high ratio of saturated to polyunsaturated fat was shown to increase total urinary androgens and total plasma testosterone respectively. Moreover, in a third trial, a reduction in intake of dietary fat led to a decrease in serum testosterone and androstenedione levels. A strong association between increasing plasma testosterone levels and risk of prostate cancer was found in a recent prospective cohort study after adjustment for sex hormone-binding globulin levels (Kolonel *et al.*, 1999).

Finally, leptin has also been related to the effects of a high-fat diet on carcinogenesis. In particular, in the rat DMH-induced colon cancer model, the elevation in dietary fat (corn oil) caused, in a dose-dependent manner, high incidence of colonic aberrant crypt foci and aberrant crypt, high serum leptin levels and elevation in colon cell proliferation and expression of c-fos protein. These results have suggested that high-fat diets enhance colon carcinogenesis by elevating cell proliferation through higher serum leptin (Liu *et al.*, 2001). Recently, leptin has been shown to be able to control the proliferation of both normal and malignant breast epithelial cells, suggesting a novel association of diet-induced obesity, mammary gland development and the risk for breast cancer (Hu *et al.*, 2002).

5.3. Modification of cell membranes

Lipids modulate the biological activity of cell membranes since they are essential components of their structure. The composition of polar lipids (phospholipids, sphingomyelin and cardiolipin in mitochondria) and the cholesterol content of cell membranes is tightly regulated by cells, but can vary depending on the lipids habitually ingested (Spector and Burns, 1987; Clandinin *et al.*, 1991; Cave, 1997). Cell membranes seem to remain relatively constant in their saturated and

monounsaturated fatty acid levels over a wide range of dietary variation for these fatty acids. Membrane composition has been found to be more responsive to n-6 and n-3 polyunsaturated fatty acid levels in the diet, and specially to n-3 PUFA and the n-3:n-6 ratio. These differential responses are probably due to the fact that both n-6 and n-3 PUFA classes cannot be synthesized *de novo* by higher animals (Hulbert *et al.*, 2005). The changes in the lipid profile of the cell membranes due to the intake of a particular type of dietary fat can, in turn, modify cell behaviour by influencing membrane fluidity, lipid mediated cell signalling transduction pathways and the degree of lipid peroxidation in the cell membranes (Jump, 2004).

The changes in membrane fluidity, for example an increase due to a higher content of PUFA, may affect the lateral mobility of specific integral and membrane-bound proteins (transporters, receptors, enzymes), its conformation and its interaction with other membrane components, which could produce functional changes (Clandinin *et al.*, 1991; Merrill and Schroeder, 1993). In this way, polyunsaturated fatty acid enrichment has been described to reduce the number of sodium channels, whereas saturated and *trans*-unsaturated fatty acids have the opposite effect and OA does not produce any change (Spector and Burns, 1987). An increase in the unsaturated fatty acid content of the membrane phospholipids has also been reported to change adenylate cyclase (Gidwitz *et al.*, 1980) and Na^+-K^+-ATPase (Solomonson *et al.*, 1976) activities, as well as the binding of ligands to receptors as in the case of the insulin receptor (Lomeo *et al.*, 1986; Clandinin *et al.*, 1991). In all these cases, PUFA have been reported to have a greater effect than MUFA (Hulberg and Else, 2000; Hulbert *et al.*, 2005). Furthermore, specific membrane lipids regulate the function of amphitropic proteins, which are proteins localized to both the cytosol and membrane, which bind weakly and reversibly by means of covalent or non-covalent interactions (Burn, 1988). The interactions with membrane lipids can affect their assembly, folding, or topological organization, and therefore their function. Proteins functioning in transduction of signals generated in cell membranes, such as small GTPases Ras, Src-family protein tyrosine kinase (SFK), Ras-guanine nucleotide exchange factor, CTP:phosphocholine cytidylyltransferase, PKC and phospholipase C (PLC) are commonly regulated by amphitropism (Johnson and Cornell, 1999; Dowhan *et al.*, 2004). Several extracellular matrix and cytoskeletal-related proteins have been also demonstrated to interact with specific membrane phospholipids (Niggli and Burger, 1987; Grimard *et al.*, 1993).

Recently, in a mouse colon model it has been demonstrated that dietary n-3 PUFA, compared with n-6 PUFA, can profoundly alter the caveolar microenvironment, thereby influencing cellular signalling in the colon (Ma *et al.*, 2004a,b). Caveolae are specialized rafts enriched with caveolin-1. These cholesterol-rich microdomains attract key signalling proteins including: glycosylphosphatidylinositol-anchored proteins such as Thy-1; acylated proteins such as Src family kinases, heterotrimeric G proteins and endothelial nitric oxide synthase (eNOS); palmitoylated transmembrane proteins such as β-secretase; and lipid-modified proteins with saturated fatty acyl anchors, including H-Ras (Anderson, 1998; Sternberg and Schmid, 1999; Simons and Toomre, 2000; Prior and Hancock, 2001; Edidin, 2003; Parton and Hancock, 2004).

An increase in the membrane relative content of n-6 PUFA has been associated with a greater cell proliferation rate (Welsch, 1987; Stoll, 2002). Our studies have produced data on this. Thus, when analysing the composition of 14 fatty acids in six lipid fractions of experimental breast tumours from animals fed low and/or high corn oil diets, it was found that high-fat diet groups' tumours, with a more aggressive clinical behaviour and a higher histopathological degree, were characterized by a significant increase of LA relative content and a decrease of that of OA, in comparison with control low-fat diet tumours (Table 15.1, Fig. 15.6) (Escrich *et al.*, 2001). Within the phospholipids, this situation was strongly significant in the phosphatidylcholine (PC), whose hydrolysis products have been linked to tumour cell proliferation (Exton, 1990; Dennis *et al.*, 1991; Billah, 1993; Graber *et al.*, 1994). These results are in agreement with the experimental and epidemiological studies previously described, showing a stimulating role of LA and likely protective role of OA on mammary carcinogenesis. Given that OA is the major component of olive oil, these results would suggest the benefit of the regular intake of olive oil in the diet. Besides the beneficial effects of the antioxidant minor components of the olive oil, OA could also exert its protective effect in breast cancer by competing with LA for metabolic enzymes, and by its differing capacity for influencing membrane fluidity, both directly or through derivatives (Rose, 1997a). In this way, the results of our work show interesting changes: (i) a trend to find more AA in the fractions in which there was less LA, above all in phospholipids and particularly in the phosphatidylethanolamine (PE), indicating a different efficiency of conversion among the lipid fractions; and (ii) an increase in the unsaturated fatty acid content of the PC in tumours from the animals fed high-fat diets (Escrich *et al.*, 2001). The results of this study suggest that the metabolism of the fatty acids in tumour cells depends on the dietary lipid content and on specific factors of tumour tissue. Thus, the diet had less influence on the total fatty acid composition of different phospholipids than on that of the triglycerides, which reflected exactly the supplied dietary fatty acids. However, LA was significantly higher in almost all lipid fractions, irrespective of whether the total esterified quantity was high or low (Table 15.1), which suggests that this fatty acid would be involved in different functions. Thus, the low total quantity of this fatty acid in some fractions (mainly in phospholipids) could be due to its use by the tumour cells, both for its conversion into arachidonic acid or for the signal transduction pathways, as the results obtained in the PC and the free fatty acids fractions suggest. By contrast, in the structural lipids, as in the triacylglycerides, where the LA total content was important, this fatty acid could have a role as an energy reserve, as it would be characteristic of this fraction. However, OA decreased significantly in almost all the fractions of the more malignant tumours, despite being the second major component of the experimental diet.

On the other hand, dietary lipid composition can alter the phospholipid fatty acid profile of the nuclear membrane, resulting in altered membrane function. Thus, *in vitro* it has been described that there are DNA binding proteins which interact with phospholipid membranes and that their activities in DNA replica-

Table 15.1. Fatty acid content of lipid fractions of rat DMBA-induced mammary tumours.

Fatty acid	C	I	P	D
		Phosphatidylcholine		
16:0	42.1(1)	42.9(1)	42.5(1)	40.1(1)
18:1	25.0(2)	20.9(2)	19.5(2)	21.7(2)
20:4	16.4(3)	15.5(3)	17.7(3)	15.1(3)
18:0	8.8(4)	8.8(4)	9.6(4)	8.5(4)
18:2	5.2(5)	7.3(5)	6.6(5)	6.3(5)
		Phosphatidylinositol		
18:0	44.5(1)	52.6(1)	51.6(1)	46.5(1)
18:1	30.0(2)	27.0(2)	25.4(2)	28.0(2)
16:0	14.3(3)	10.7(3)	10.9(3)	13.2(3)
20:4	9.2(4)	6.7(4)	8.0(4)	6.7(4)
		Phosphatidylethanolamine		
20:4	45.7(1)	45.2(1)	45.5(1)	47.2(1)
18:0	20.4(2)	21.4(2)	18.8(2)	18.8(2)
18:1	15.9(3)	15.4(3)	14.1(3)	14.8(3)
16:0	9.5(4)	10.1(4)	10.4(4)	12.1(4)
		Free fatty acids		
16:0	34.0(1)	32.1(1)	37.8(1)	32.2(1)
18:1	27.3(2)	22.2(2)	18.6(2)	26.5(2)
18:0	14.4(3)	15.9(3)	12.1(5)	12.9(3)
16:1	12.9(4)	9.3(6)	12.6(4)	7.7(6)
20:4	9.9(5)	9.8(5)	14.5(3)	12.0(5)
18:2	5.3(6)	12.8(4)	11.1(6)	12.3(4)
		Triacylglycerides		
18:1	39.5(1)	27.5(2)	26.7(2)	36.5(1)
16:0	27.6(2)	22.5(3)	18.1(3)	24.0(3)
18:2	13.3(3)	34.4(1)	30.3(1)	24.6(2)
16:1	7.3(4)	–	–	–
18:0	5.0(3)	–	7.4(4)	5.0(4)
		Cholesteryl esters		
20:4	28.4(1)	18.4(3)	19.6(3)	22.9(2)
18:1	24.8(2)	20.4(2)	23.9(1)	21.0(3)
16:0	21.9(3)	24.7(1)	23.7(2)	23.5(1)
18:0	10.6(4)	10.1(5)	10.3(5)	10.7(5)
16:1	8.7(5)	7.3(6)	7.5(6)	8.0(6)
18:2	5.6(6)	10.4(4)	17.2(4)	9.7(5)

Values are area per cent (mean ± standard deviation). The order of magnitude of each fatty acid in the different fractions is indicated in parentheses. C: control group (low, 3%, corn oil diet all through the assay); I: initiation group (high, 20%, corn oil diet all through the assay); P: promotion group (low fat diet from weaning until carcinogen induction and high corn oil diet from that time onwards); and D: development group (low-fat diet until day 157 and then high corn oil diet, once tumours had appeared). The number of tumours analysed in each group was: C, 17; I, 15; P, 17; and D, 18.

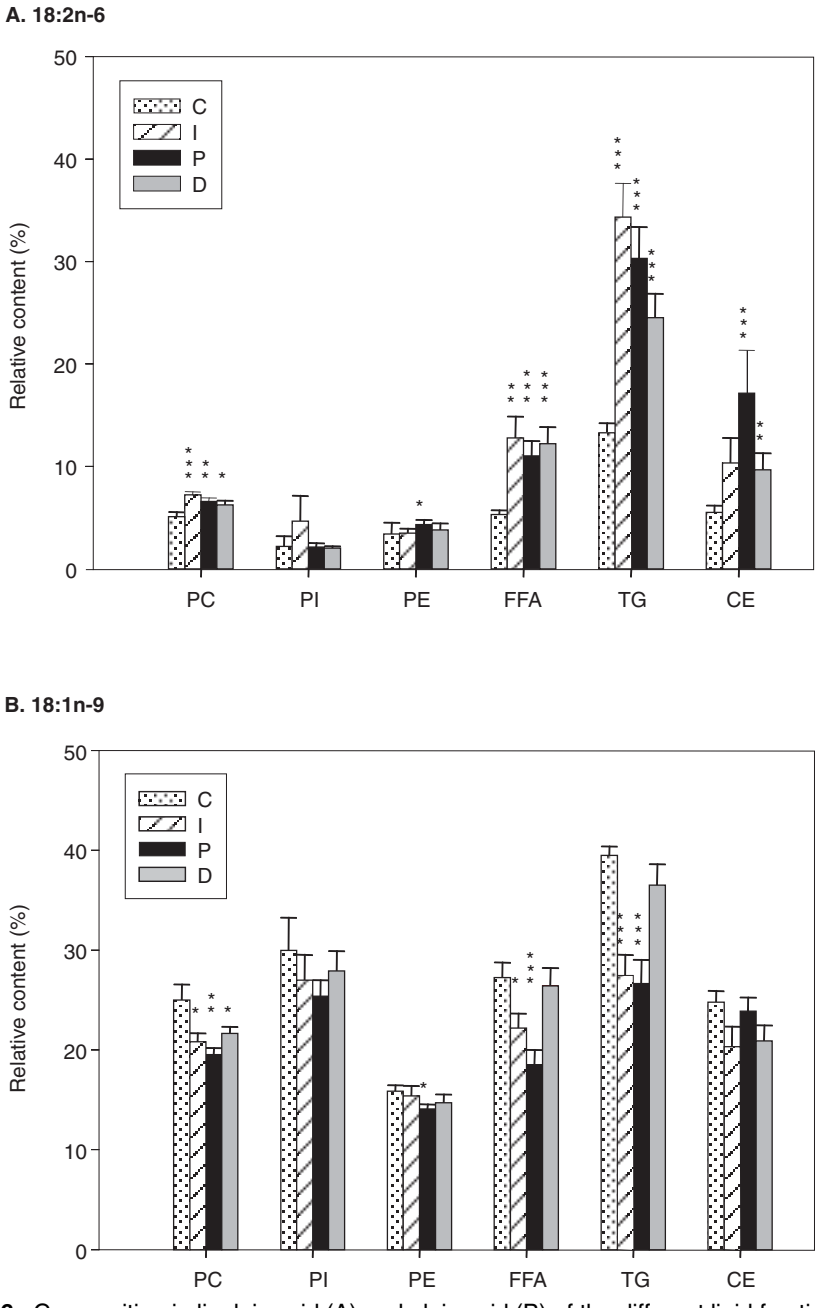


Fig. 15.6. Composition in linoleic acid (A) and oleic acid (B) of the different lipid fractions of rat DMBA-induced mammary tumours. PC: phosphatidylcholine; PI: phosphatidylinositol; PE: phosphatidylethanolamine; FFA: non-esterified fatty acids; TG: triacylglycerides; CE: cholesterol esters. The experimental groups are described in Table 15.1: C, control group; I, initiation group; P, promotion group; and D, development group. The values are area percent (mean ± standard deviation). The statistically significant differences from the control values are indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p << 0.01$ (Mann-Whitney's U test) (Escrich *et al.*, 2001).

tion, transcription and recombination are likely to be regulated by phospholipids, specially if they contain unsaturated fatty acids (Sekimizu, 1994). Likewise, high dietary LA levels, in comparison with low LA diet, has been demonstrated to increase the nucleosidetriphosphatase (NTPase) activity and consequently the RNA nucleocytoplasmic export in rat liver (Clandinin *et al.*, 1991). It has been suggested that the nuclear membrane fluidity changes caused by the increase of unsaturated fatty acids could affect the phosphorylation and consequently the activities and function of the nuclear membrane-associated enzymes involved in this RNA transport (Tomasoni *et al.*, 1999). These works are in favour of the possibility that dietary lipids may regulate gene expression at the posttranscriptional nuclear level.

Moreover, as dealt with in Chapter 6 and in section 5.1 of this chapter, dietary fat can modify the degree of lipid peroxidation in the cell membranes. Despite antioxidant defence mechanisms, persistent cellular oxidative stress leads to macromolecular damage and disruption of signalling pathways, stimulating the development of cancer (Dreher and Junod, 1996). Fatty acid unsaturation degree of the membrane phospholipids determines susceptibility to peroxidation in such a way that the greater the unsaturation level, the more oxidative stress there is. Thus, it has been shown that a polyunsaturated fat source will lead to membranes more prone to oxidation than a saturated or a monounsaturated source. The modification of the degree of lipid peroxidation in the cell membranes by dietary fat is due to both the influence of their lipid profile and the structural and functional changes that occur in the membranes, including cholesterol mobilization, increased coenzyme Q synthesis and modulation of enzyme activities and levels (Huertas *et al.*, 1992; Mataix *et al.*, 1997; Ochoa-Herrera, 2001).

Finally, dietary lipids could also influence carcinogenesis by modulating gap junction-mediated intercellular communication (GJIC) or metabolic cooperation. Specifically, n-6 PUFA have been demonstrated to inhibit it in the rat DMBA-induced mammary cancer model, resulting in a blockade of the transfer of growth inhibitory signals among tumour cells via gap junctions (Aylsworth *et al.*, 1984, 1987). It has been shown that reduced GJIC is associated with increased cell motility, invasion and metastasis (Holder *et al.*, 1993). On the other hand, GLA reduces *in vitro* adhesion of human breast and colon cancer cells to the endothelium, partly by improved GJIC of this endothelium (Jiang *et al.*, 1997a).

5.4. Actions on the signal transduction pathways

The action of several phospholipases (PLA_2, PLC, PLD), activated by agonist–receptor complexes, generates a great quantity of bioactive molecules from the membrane lipids. The generated molecules can act as second messengers or as modulators within the intracellular signal cascade (Exton, 1994; Jump, 2004).

As a result of the action of the PLC on the membrane phospholipids (phosphatidylinositol 4,5-diphosphate – PIP_2 -), inositol triphosphate (IP_3) and diacilglycerol (DAG), which activates PKC, are generated. DAG can also be formed by the action of the PLC on the PC. PLA_2 produces free fatty acids (FFA) that enhance PKC activation. PLD generates phosphatidic acid (PA), a molecule with possible functions as second messenger and with an important role in cell proliferation and tumour promotion (Exton, 1990; Haeffner, 1993; Graber *et al.*, 1994; Uchida *et al.*, 1997). In this sense, PA would act as a coactivator of the Ras-mitogen-activated protein kinase (MAPK) signal transduction pathway by facilitating and stabilizing Raf-1 translocation into the membrane for the further activation of this pathway. PA, moreover, is metabolized into DAG, which is related to sustained PKC activation. This kinase phosphorylates serine and threonine residues of a great number of regulating cell proteins which are those that will produce the response to the membrane receptor ligands (Daniel *et al.*, 1999).

Furthermore, it has already been pointed out that changes in the physicochemical properties of the membrane which can be produced as a result of modifications in the quantity or type of dietary lipids will affect the production and the composition of the second messengers (see section 5.3). It has been shown that high-fat diets increase DAG levels and the quantity of membrane-associated PKC (Birt *et al.*, 1992; Choe *et al.*, 1992). It is also known that FFA modify the activity of phospholipases A_2, C and D, PKC, PKA (AMPc-dependent protein kinase), CaM-K II (Ca^{2+}/Calmoduline-dependent protein kinase II), G proteins, adenilate and guanylate cyclases, as well as ionic channels (of calcium, potassium, chloride and the Na^+,K^+-ATPase) and calcium mobilization (Casabiell *et al.*, 1991; Clandinin *et al.*, 1991; Sumida *et al.*, 1993; Graber *et al.*, 1994; Divecha and Irvine, 1995). Some authors have described the contrary effect of FFA on the activity of PLA_2, PLC and PLD that would be exerted through competitive inhibition (Sumida *et al.*, 1993; Graber *et al.*, 1994). However, the effect of FFA on the other factors is well established; for example it has been clearly determined that particular PUFA can activate directly some PKC isoforms, irrespective of DAG and calcium, enhance calcium release from the endoplasmic reticulum and thus inhibit PKC. The activation of PKC by non-esterified fatty acids would increase the range of possible substrate proteins and modify the intracellular localization of this enzyme (Lester, 1990). In colon, it has been described that n-3 PUFA (DHA and EPA) inhibit PKCβII activity, attenuating the hyperproliferation of the epithelium. This effect may be due to the direct inhibition of PKCβ catalytic unit by these fatty acids (Jump, 2004). On the other hand, it has been shown that DAG esterified with long-chain unsaturated fatty acids are the most effective in the activation of the PKC (Merrill and Schroeder, 1993). Moreover, FFA would act synergistically with DAG, on some PKC isoforms, allowing a sustained activation when calcium or DAG levels diminish. Regarding CaM-K II and PKA, it has been shown that they are inhibited by AA (Sumida *et al.*, 1993). Also, it is considered that a transitory increase of FFA concentration can be enough to activate G proteins (Graber *et al.*, 1994).

In relation to the modification of ion fluxes, it has already been mentioned that FFA can diminish the number of sodium and chloride channels, and increase those of potassium (Spector and Burns, 1987; Sumida *et al.*, 1993).

Moreover, they alter the conformation of the Na^+,K^+-ATPase, decreasing its activity (Solomonson *et al.*, 1976), and inhibit the mobilization of calcium, usually induced by the activation of several receptors (Casabiell *et al.*, 1991).

There are few data regarding direct effects of fatty acids on membrane receptors, which are mitogenic signal cascade effectors. However, some results point out that activation of the epidermal growth factor receptor (EGFR) by lipids could occur. In human endothelial cell lines, the activation of this receptor by OA has been observed, suggesting a direct interaction or the generation of changes in the membrane fluidity that would activate the receptor. It must also be taken into account that oxidized PUFA inhibit tyrosine phosphatases (PTPases), and so they would increase in general the phosphorylation of tyrosines of the cell proteins. In other studies *in vitro* it has been shown that particular LA metabolites can inhibit EGFR dephosphorylation, thus increasing the intracellular signal cascade (Glasgow *et al.*, 1997; Vacaresse *et al.*, 1999). However, our work in an *in vivo* model contradicts these results since they do not show a phospho-EGFR activity due to either dietary PUFA (corn oil) or MUFA (virgin olive oil) (see section 5.5.2 and Fig. 15.7).

Moreover, from the membrane phospholipids, essential fatty acids, which are precursor of a number of important, very active, short-lived, hormone-like compounds referred to as 'eicosanoids' (prostaglandins – PGE -, prostacyclines – PGI -, thromboxanes – TX -, and leukotrienes – LT -) are released. Two families of eicosanoids exist: 'series 2', derived from the LA (18:2n-6), via AA (20:4n-6), and 'series 3', derived from the LNA (18:3n-3), via EPA (20:5n-3) (Cave, 1997). Although both types are synthesized using the same enzymes, they have different and, in some cases, contrary effects (Weber, 1990). These molecules act via G protein-coupled receptors and have been demonstrated to have an important role in the modulation of potassium channels, adenylate cyclase, PLA_2 and PLC, and several kinases (Wood, 1990). Among the different eicosanoids synthesized, PGE_2, which is the product of the action of COX, particularly of COX2, on AA, promotes cell survival. It is increased in tumour cells, where it inhibits apoptosis and stimulates cell proliferation. It also enhances tumour progression, promoting tumour angiogenesis and the adhesion of tumour cells to endothelial cells. The products of the action of the 5-lipooxygenase (5-LOX) on AA, LTB_4 and 5-hydroxyeicosatetraenoic acid (5-HETE), also play a role in the adhesion of tumour cells and could increase their metastatic potential. LTB_4 increases the generation of reactive oxygen species that could attack DNA and lead to cancer initiation. Furthermore, cytochrome P450 monooxygenase-mediated oxidation of PUFA generates hydroxy fatty acids, dihydroxy fatty acids and epoxy fatty acids, which can influence cell proliferation, apoptosis and inflammation (Stoll, 2002; Larsson *et al.*, 2004).

The tumour promoting effect of high-fat diets on breast cancer has been correlated with a greater production of prostaglandins (Cohen *et al.*, 1986c). The inhibitors of prostaglandin synthesis partially block the promoting effect of high n-6 PUFA diets on rat DMBA-induced breast cancer (Mizukami *et al.*, 1992; Nakayama *et al.*, 1993). In the AOM-induced colon cancer model, the promoting effect of a high-fat diet containing mixed lipids, in particular high levels of saturated fatty acids, has been related to an increase in eicosanoid formation from AA through an increase in COX enzyme activity, leading to a suppression of apoptosis (Rao *et al.*,

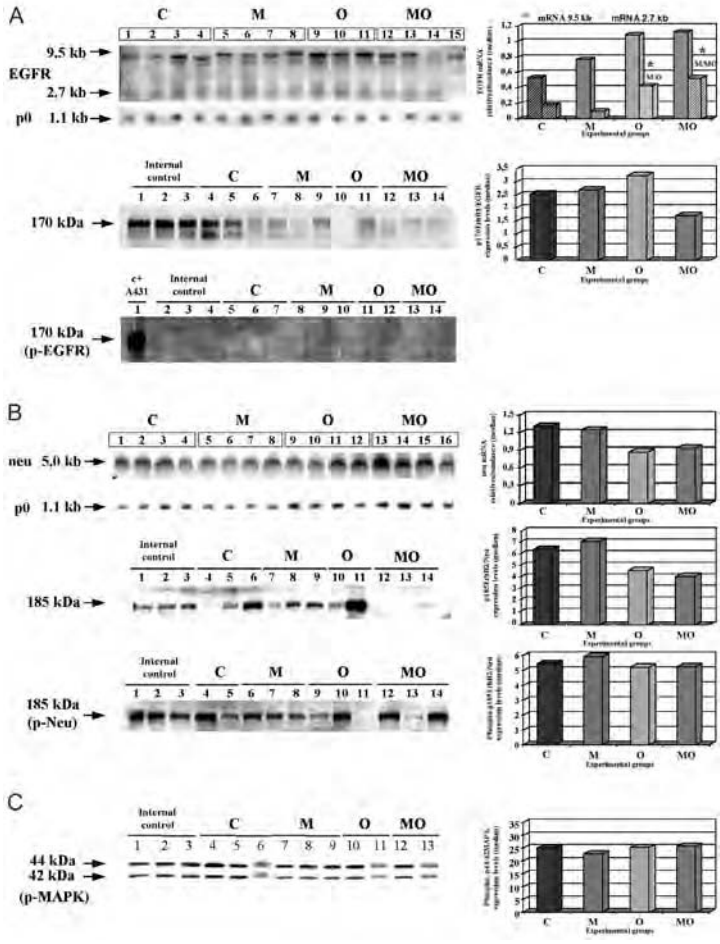


Fig. 15.7. Influence of a high virgin olive oil diet in comparison with a high corn oil diet both on the c-erbB1 and c-erbB2 mRNA and protein expression and on the phospho-MAPK protein levels in the promotion stage of rat DMBA-induced breast carcinogenesis. A. *Top*, representative Northern blot of c-erbB1/*EGFR* in the experimental mammary adenocarcinomas in the distinct experimental groups. Two c-erbB1/EGFR transcripts of 9.5 kb and 2.7 kb were detected. The p0 mRNA was used as a control transcript. *Middle*, representative Western blot of p170ErbB1/EGFR corresponding to the protein of the 9.5 kb transcript. *Bottom*, representative Western blot of the activated phosphorylated p170ErbB1/EGFR. A protein extract from EGF-stimulated A431 cells was used as a positive control. B. *Top*, representative Northern blot of c-erbB2/*neu* in the experimental mammary adenocarcinomas in the distinct experimental groups using the p0 mRNA as a control transcript. *Middle*, representative Western blot of p185ErbB2/Neu. *Bottom*, representative Western blot of the activated phosphorylated p185ErbB2/Neu. C. Representative Western blot of the activated phosphorylated p44/42 MAPK in the experimental mammary adenocarcinomas in the distinct experimental groups. C, control diet group; M, high corn oil diet group; O, high virgin olive oil diet group; MO, high corn and olive oils diet group, whose animals were fed sequentially the high corn oil diet and then the high virgin olive oil diet. The histograms show median values of mRNA or protein levels in the different experimental groups. *Differences statistically significant, $p < 0.05$ (Mann-Whitney's U and χ^2 tests).

2001). In prostate cancer cells, the significant proliferative effects of both LA and AA have been shown to be exerted through the 5-lipooxygenase pathway (Moretti *et al.*, 2004). In relation to the n-3 lipids, their protective effect would be based on the use of the same enzymes of the prostaglandin synthesis from n-6 lipids (desaturases and elongases), and on the inhibition of the COX activity. All this would induce a change in the proportions of the type of eicosanoids synthesized (Nakayama *et al.*, 1993; Cave, 1997; Larsson *et al.*, 2004; Roynette *et al.*, 2004). Likewise, it is also thought that CLA could act in the same way, decreasing LA desaturation products needed for eicosanoid synthesis (Banni *et al.*, 1999). In the same sense, the possible tumour protective effect of olive oil has been partly attributed to the competition between OA and LA for the desaturation process. Thus, although OA has lower affinitity for the Δ6 desaturase (which is the first step leading from LA to AA) than LA, at relatively high concentrations OA will inhibit the conversion of LA to GLA and, hence, its entry into the eicosanoid biosynthetic pathways (Brenner, 1981; Rose and Connolly, 1990; Bartsch *et al.*, 1999). Moreover, hydroxytyrosol, a minor component of olive oil, is capable of inhibiting the lipooxygenase enzyme, responsible for leukotriene synthesis (Visioli and Galli, 1998). In this sense, AA mobilization and the subsequent production of PGE_2 through the COX2 pathway are decreased in PMA (phorbol 12-myristate 13-acetate)-stimulated macrophages from rats fed olive oil and fish oil diets in comparison with a corn oil diet (Moreno *et al.*, 2001). Furthermore, the preventive effect of an olive oil based diet with a normal amount of fat (5% dietary fat for rats) on colon carcinogenesis induced with AOM has been attributed, at least partially, to the modulation of AA metabolism and local PGE2 synthesis (Bartolí *et al.*, 2000). *In vitro* this protective effect of olive oil has also been shown to be the result of an early downregulation of COX2 followed by a decrease in the expression of the inhibitory apoptosis Bcl-2 protein (Llor *et al.*, 2003). It has also been reported that a diet containing 10% high OA oil suppresses murine experimental lung tumorigenesis in comparison with a high LA oil diet, and that this suppression is correlated with inhibition of PGE2 production and inactivation of the MAPK cascade (Yamaki *et al.*, 2002).

Dietary lipids have also been shown to affect the activation of Ras proteins in several experimental studies. Thus, in the AOM-induced colon cancer model a high fish oil diet resulted in a decrease of activated membrane-bound Ras by interfering with posttranslational modification of the protein. By contrast, a high corn oil diet appeared to exert its promoting activity on colon carcinogenesis by increasing membrane localization of Ras (Singh *et al.*, 1997). *In vitro*, DHA has also been shown to reduce GTP-bound Ras in the plasma membrane in young adult mouse colon cells overexpressing H-ras compared with LA-treated and untreated cells (Collet *et al.*, 2001). This n-3 PUFA effect on Ras activation has been associated to a reduction of activity and concentration of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Likewise, the tumour-inhibitory effect of the constituent of olive oil squalene has been based on its strong inhibitory activity of this enzyme (Newmark, 1999). Thus, dietary administration of squalene inhibited rat AOM-induced colonic aberrant crypt foci, supporting the hypothesis that some of the observed protective effects of olive oil are due to squalene (Rao *et al.*, 1998). The inhibition of HMG-CoA reductase has also been proposed to explain the inhibitory effects of dietary olive oil and squalene on

chemically-induced lung tumorigenesis (Smith *et al.*, 1999). Our group has also associated this effect on HMG-CoA reductase activity with the protective effect of high virgin olive oil on experimental breast cancer (see section 5.5.2). Inhibition of HMG-CoA reductase activity, the rate-limiting control step in the normal biosynthetic pathway to cholesterol, presumably reduces the levels of the series of intermediates of this pathway, including mevalonate, geranyl pyrophosphate and farnesyl pyrophosphate (FPP). FPP is a source for, among others, the prenylation (farnesylation) of certain proteins, such as Ras. This posttranslational modification process enables Ras to acquire its full cell activities (Newmark, 1999).

5.5. Effects on gene expression and protein activity

A number of studies have shown that different dietary components (PUFA, cholesterol, glucose/fructose, specific minerals and liposolubles vitamins) can modulate specifically the gene transcription, the processing and the stability of the transcripts of genes involved in glycolysis, lipogenesis, etc. (Clarke and Abraham, 1992). It has been postulated that lipids could exert an influence on the gene expression through two ways: a direct, fast and acute control of the expression levels, and an adaptative long-term modulation of the cell membrane composition that would modify intracellular signalling (Clarke and Jump, 1993; Kaput and Rodríguez, 2004).

Whereas the regulation by dietary lipids of the expression of genes involved in cancer is just beginning to be known, their effect, at a physiological level, on metabolic genes is well documented (Clarke and Abraham, 1992; Clarke, 2001).

Almost all the mechanisms that could explain the modulating action of dietary lipids on the carcinogenesis could involve the modification of the expression of specific genes. Probably, the modulation of gene expression does not constitute an alternative mechanism of action, but contributes to the general effects of dietary lipids on cancer (Galli and Butrum, 1991; Glauert, 1993). In this sense, dietary lipids could affect the expression of genes potentially involved in cell transformation and the tumorigenesis processes through a long-term mechanism, modifying again the lipid composition of the cell membranes and, thus, the signal transduction pathways to the nucleus (Clarke *et al.*, 1990; Clandinin *et al.*, 1991; Sumida *et al.*, 1993). A priori, all the genes involved in cell proliferation and differentiation could be subject to study. However, the changes in the expression of these genes and/or in the activity of their proteins as a result of dietary lipids are relatively little known in cancer in comparison with those of the normal metabolism genes, even though some of them have been studied in several experimental systems.

Some experimental data suggest that dietary lipids could affect the expression of genes of the signal transduction pathway ErbB-Ras-MAPK. Thus, some authors have described changes in the steady-state cytoplasmic mRNA levels of c-erbB2 by n-6 and n-3 fatty acids in *ras*-transfected human breast cancer cells (Tiwari *et al.*, 1991). Another study showed that c-Ha-ras protein levels were reduced in MCF-7 breast tumours in fish oil fed mice (Fernandes and Venkatraman, 1991). Our group studied the effect of n-6 (corn oil) and n-9

(olive oil) lipids on genes of that signal transduction pathway in the DMBA-induced mammary tumours (Solanas *et al.*, 2001; Moral *et al.*, 2003). Although some of the results of the study will be the object of another independent publication, the main results can be advanced. These have been obtained in two experimental series developed in our laboratory and they are shown in Figs 15.7 and 15.8. The results show that the promoting effect of the high corn oil diet does not seem to be mediated by changes in the p21Ras expression or activation, or by alterations in the c-erbB1/EGFR and c-erbB2/Neu receptors, or by modification of the activated MAPK levels. On the contrary, the protective effect of the high virgin olive oil diet could be exerted, at least in part, through the diminution observed in p21Ras-GTP levels and through the changes found in the expression of *erbB* family genes. Thus, olive oil also tended to decrease the relative abundance of neu mRNA and of the p185ErbB2/Neu protein, suggesting the implication of this oncogene in this inhibitory mechanism. This decrease of c-erbB2/Neu was later detected by other authors in breast cancer cells with *neu* amplification, once treated with OA (Menéndez *et al.*, 2005). Our results also point out, primarily, that dietary lipids do not modify the mRNA and protein expression of the functional EGF receptor (9.5 kb mRNA; 170 kDa protein) and, secondly, that the activated form of this receptor does not seem to be involved either in this experimental breast cancer model or in the modulating effects of these dietary lipids on it. On the contrary, when we investigated the truncated form of the EGF receptor (2.7 kb mRNA; 95 kDa protein), we observed that the high corn oil diet slightly decreased its mRNA, and the high olive oil clearly increased it. This increase could also partly account for the inhibitory effect of the high olive oil diet, since this truncated form lacks enzymatic activity and, once secreted, forms inactive heterodimers with the different members of the c-erbB family, and binds and blocks ligands of these receptors. Thus, it exerts an inhibitory effect on this mitogenic signal transduction pathway (Figs 15.7 and 15.8). In the previously mentioned decrease of p21Ras activation due to olive oil, besides the described factors, the known strong inhibitory activity of HMG-CoA reductase of squalene could participate (see section 5.4). Our results showed that these changes, however, do not seem to be accompanied by alterations in the activated MAPK levels.

Another group of genes that have been described as modulated by dietary lipids are transcription factors. Some of them are, moreover, suppressor genes. Thus, diets with a high content of fish oil have been shown to reduce *c-myc* expression in murine MCF7 breast tumours (Fernandes and Venkatraman, 1991), whereas diets rich in LA have been shown to increase the expression of this oncogene in normal mammary gland and in rat PhIP-induced benign breast tumours (Davis and Snyderwine, 1995). Furthermore, certain studies carried out in other tissues point out that the effect of dietary lipids on gene expression could be exerted through their metabolites. In this sense, the COX product of LA, hydroperoxy-octadecadienoic acid, induces c-fos, c-jun and c-myc mRNA levels in aortic smooth muscle cells. In Swiss3T3 cells, the conversion of AA into prostaglandins stimulates DNA synthesis and mitosis by activating expression of *c-fos* and *Egf-1* through a PKC regulatory pathway (Jump and Clarke, 1999). The mechanism through which dietary lipids could modulate the expression of these

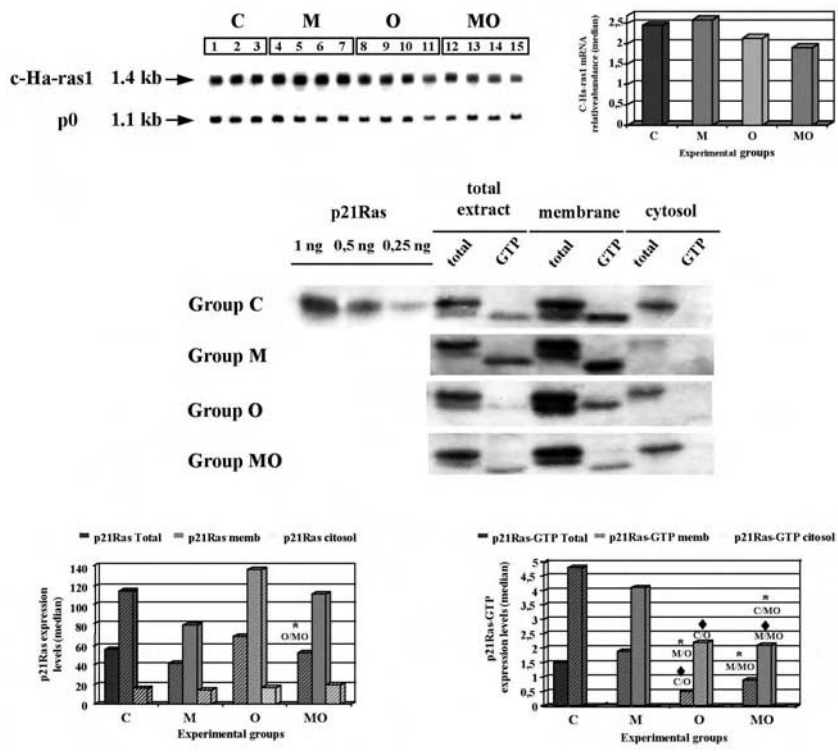


Fig. 15.8. Influence of a high virgin olive oil diet in comparison with a high corn oil diet on both the c-Ha-ras1 mRNA expression and the p21Ras protein expression and activity in the promotion stage of rat DMBA-induced breast carcinogenesis. *Top*, representative Northern blot of c-Ha-ras1 in the experimental mammary adenocarcinomas in the distinct experimental groups. The p0 mRNA was used as a control transcript. *Middle*, representative Western blots of the whole study of p21Ras. A p21Ras standard was used both as a positive control and to quantify p21Ras levels in the experimental samples. Total p21Ras protein levels as well as those of activated GTP bound p21Ras in total protein extract, membrane fraction and cytosol fraction were studied for each tumour analysed. *Bottom*, the histograms on the left show the median values of total, membrane and cytosol p21Ras expression. The histograms on the right show the median values of total, membrane and cytosol activated p21Ras-GTP expression. C, control diet group; M, high corn oil diet group; O, high virgin olive oil diet group; MO, high corn and olive oils diet group, whose animals were fed sequentially the high corn oil diet and then the high virgin olive oil diet. The statistically significant differences are indicated as follows: *, $p < 0.05$; ♦, $0.1 < p < 0.05$ (Mann-Whitney's U test).

genes is complex. Recently, a study has identified genes with recognition sequences to PPARα, among which *c-myc* has been described in murine liver (Mandard *et al.*, 2004). Moreover, as previously discussed, fatty acids can regulate a number of transcription factors, such as NFκB and SREBP (Jump, 2004; Larsson *et al.*, 2004).

Regarding the tumour suppressor genes, in *in vitro* studies using mammary tumour cells, the addition of LA increased DNA synthesis and decreased *p53* protein levels, whereas DHA induced further suppression of cell proliferation

and upregulated expression of *p53*. These data suggest that growth stimulation of tumour cells by LA is mediated in part by modulating *p53* expression through a mechanism still unknown (Tillotson *et al.*, 1993; Ronai *et al.*, 1995). In the AOM-induced colon cancer model, dietary corn oil (10%) did not decrease the wild type (*wt*) *p53* mRNA and protein levels. However, it decreased mitochondrial localization of *wt p53* and increased inactive cytosolic *wt p53*, leading to a reduced activity of *wt p53* in colon cancer. This effect resulted in the upregulation of Bcl-2 and Bcl-xL, and the downregulation of Bak in the mitochondria, suggesting that dietary corn oil promoted this cancer partly by inhibiting the tumour suppressor gene *p53*-mediated mitochondria-dependent apoptosis (Wu *et al.*, 2004). On the other hand, dietary lipids have also been described to affect the expression of other suppressor genes as BRCA1 and BRCA2, which are involved both in normal mammary gland development and in spontaneous and, mainly, inherited mammary cancer. Thus, in the MCF-7 tumour cell line both LA and oestradiol showed an appreciably decreased expression of BRCA1 mRNA compared with controls or cells treated with LA or oestradiol alone. This synergistic effect suggests that n-6 PUFA have the ability to modulate this tumour suppressor expression in mammary cancer cells in presence of oestradiol (Kachhap *et al.*, 2000). Increases in the BRCA1 and BRCA2 mRNA expression in MCF-7 and MDA-MB231 tumour cell lines have been demonstrated after treatment with n-3 PUFA (EPA and DHA), but no effects were noted with n-6 PUFA (AA). Moreover, no variation of the expression of these two suppressor genes was detected in the MCF-10A normal breast cell line treated by n-3 and n-6 PUFA (Bernard-Gallon *et al.*, 2002).

As discussed in section 5.4, data exist on the possible modulating effect of high-fat diets on tumorigenesis through changes in prostaglandin production by acting on the activity and/or the expression of the involved enzymes. Thus, some results suggest that the promoting effect of n-6 PUFA on mammary and colon tumorigenesis could be mediated by overexpression of COX2 and, to some extent, COX1 genes, whereas the n-3 PUFA could exert their antitumoral effect through the inhibition of the COX2 expression (Hamid and Sing, 1999; Rao *et al.*, 2001; Lu *et al.*, 2002; Stoll, 2002; Larsson *et al.*, 2004). COX2 has been shown to play a key role in the early stages of carcinogenesis by promoting the proliferation of tumour cells and their resistance to apoptosis, as well as angiogenesis, tumour cell invasion and setting up of the metastatic process (Gasparini *et al.*, 2003). In CaCo-2 colon cancer cells, besides inhibiting COX2 expression, DHA has also been shown to inhibit inducible nitric oxide synthase (iNOS) and expression of related proinflammatory genes (Narayanan *et al.*, 2003). However, in a recent study developed in the *in vivo* rat AOM-induced colon cancer model, neither *iNOS* nor COX2 expression was affected by DHA (Davidson *et al.*, 2004). Some products of lipid processing as the hydroperoxides aroused from LA have also been shown to upregulate the COX2 expression as well as that of the vascular endothelial growth factor (VEGF) in human colon adenoma and carcinoma cells (Jurek *et al.*, 2005).

The anticancer properties of GLA have been associated with the upregulation of the expression of the cell to cell adhesion molecule E-cadherin. In lung, colon, breast, melanoma and liver cancer cells the increased expression of E-cadherin protein induced by GLA has been correlated with reduced *in vitro* invasion

and increased aggregation. LA and AA failed to induce these changes (Jiang *et al.*, 1995). Moreover, GLA has also been found to upregulate the expression of maspin mRNA and protein, which is associated with a reduction in the motility of cancer cells. LA had an inhibitory effect and LNA and AA had no significant effect (Jiang *et al.*, 1997b).

The unspecific effects of the dietary lipids related to the energetic contribution that they represent on experimental breast cancer have been associated by our group with their influence on the regulation of the hepatic expression of genes involved in lipid metabolism. The hepatic expression of CPT I, mitochondrial HMG-CoA synthase and PPARα in the DMBA-induced breast cancer model, as well as the influence of this cancer on the regulation of the expression of these genes by dietary n-6 lipids, were analysed. The expression results, together with observed changes in the lipid profile and in the body weight and mass, suggested that the cancerous state modified the normal regulation of the expression of these genes by dietary lipids and that changes in the expression of *PPARα* mRNA could contribute to such alterations. The fat mobilization and the non-induced hepatic fatty acid oxidative capacity in mammary tumour-bearing animals fed a high corn oil diet suggested a preferential redirection of long-chain fatty acids into energetic and specific pathways of the extra-hepatic cancer cells. This effect was more remarkable with increasing tumour aggressiveness (Moral *et al.*, 2004).

Using cDNA microarray technology in experimental breast cancer, and after verifying the results obtained through other more conventional expression analysis techniques, and increasing the number of studied tumours, we have, finally, identified four novel differentially expressed genes by the effect of a high n-6 fat diet: submaxillary gland α-2u globulin, VDUP1 (vitamin D3-upregulated protein 1), the paternally imprinted gene *H19* and the unknown function gene that codifies an expressed sequence tag (EST) Rn.32385. The downregulation of these genes in the high-fat diet tumours associated with their clinical and anatomopathological malignancy (Escrich *et al.*, 2004) (Fig. 15.9). We have also determined that the expression of these genes is not modified by a virgin olive oil diet. These results, together with the fact that VDUP1, H19 and this globulin have been associated with cell proliferation and differentiation, open a new line of research about how the differential expression of these genes contributes to the modulating effects of high corn oil and virgin olive oil diets on experimental mammary carcinogenesis.

As far as we know we have demonstrated for the first time the influence of dietary lipids in the cell differentiation alterations in *in vivo* experimental breast cancer. We have contributed to this field demonstrating, primarily, that the different modulating effects of the dietary lipids correspond to different histopathological features of the tumours, which consist in a higher degree of morphological malignancy of tumours from corn oil-fed animals compared with those fed control and olive oil diets (Costa *et al.*, 2004) (see in section 4.1 and Fig. 15.5). Moreover, some of the genes differentially expressed in response to dietary lipids appear to be involved in cell differentiation and we have been able to confirm this relationship. This is the case for submaxillary gland α-2u globulin, *VDUP1* and *H19* genes (Escrich *et al.*, 2004) and for the *PCPH* gene, a proto-oncogene that acts synergistically with *ras*, presents homology with GDP/GTP exchange factors and participates in the response to cell stress. We characterized

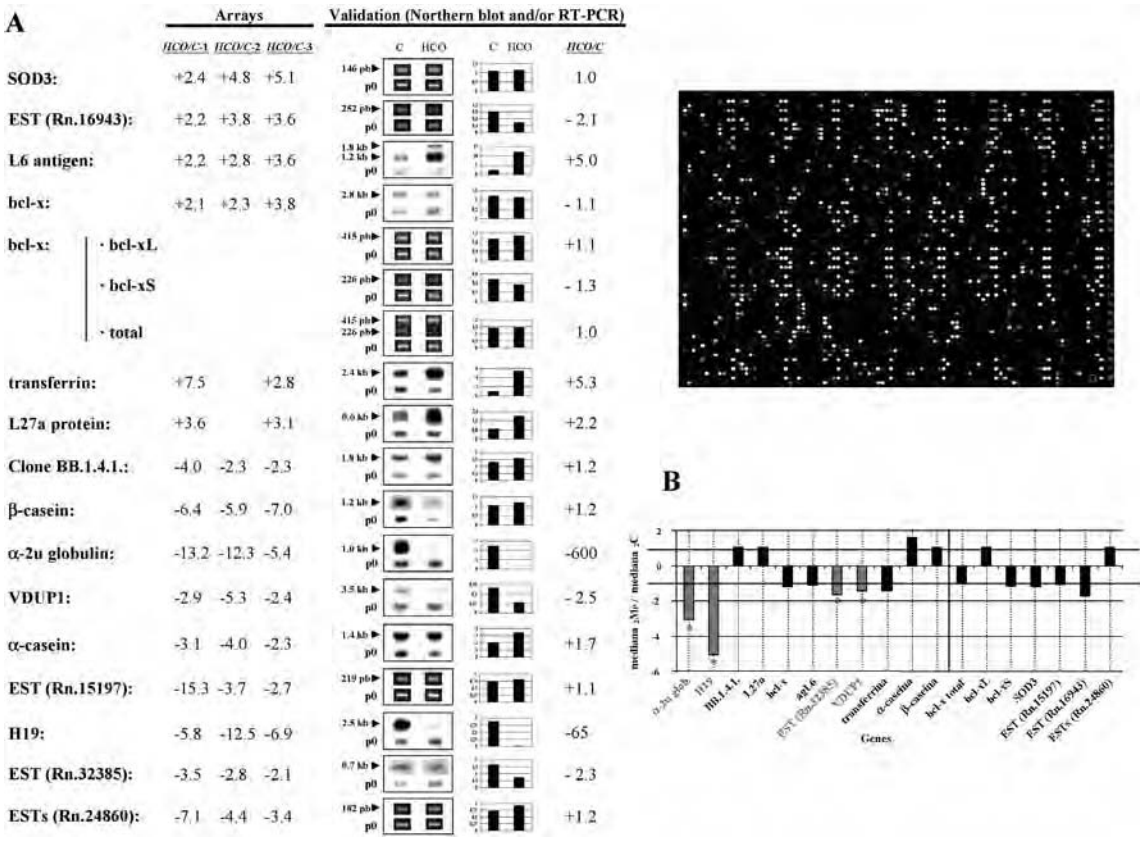


Fig 15.9. Screening of genes differentially expressed in rat DMBA-induced mammary adenocarcinomas by the effect of high n-6 PUFA diet using cDNA microarrays technology. A. The analysis was performed in triplicate using Rat GENEFILTERS® I, GF300 Microarrays filters containing 5184 rat cDNA corresponding to genes potentially related to cell proliferation and/or differentiation (Research Genetics). Sixteen genes with reproducible results as differentially expressed in the high corn oil diet tumour compared with the control (6 upregulated and 9 downregulated) were selected for verifying expression changes by Northern blot and/or RT-PCR. The size of the detected sequences is indicated. Graphic bars represent densitometric values normalized with control p0 mRNA. C: control diet group, HCO: high corn oil diet group, HCO/C: mRNA normalized level in group HCO relative to mRNA normalized level in group C. Positive values indicate that the transcript is more abundant in group HCO and negative values, the opposite. B. Further investigation in a higher number of mammary tumours from animals fed high corn oil or control diet, showed that this high n-6 fat diet significantly decreased the expression levels of *submaxillary gland α-2u globulin*, *VDUP1*, *H19* and the unknown function gene that codifies the EST Rn.32385. The x-fold change in the median of tumour mRNA levels from high corn oil group (HCO) relative to median from control group for each selected gene is indicated. *: significant mRNA levels changes ($p < 0.05$) (Escrich *et al.*, 2004).

the *PCPH* gene in breast cancer and related it to cell differentiation and, moreover, we showed that its expression decreases in response to a high n-6 lipid diet (Solanas *et al.*, 2002a). Despite the relationship found between dietary lipids and differentiation, it is interesting to note that in our studies these dietary factors did not modify the expression of *α-* and *β-casein*, indicating that in the experimental mammary tumours, unlike the normal mammary gland, the expression of these genes would not be a good indicator of the cell differentiation stage.

In the human breast cancer cell line T47D, it has been shown that the treatment with 100 ng/ml of LA increases the proportion of cells in the S phase of cell cycle, and that this effect is exerted by modulation of the gene expression. Thus, using a microarray analysis, changes in oestrogen receptor α, the G13α G protein and p38 MAP kinase gene expression, as well as genes involved in RNA transcription and cell cycle regulation, have been detected (Reyes *et al.*, 2004).

As for prostate cancer, a microarray analysis of diet-induced alterations in gene expression has been developed in the ACI rat model for spontaneous, age-onset prostate cancer. A number of genes were found to be upregulated or downregulated in the prostate of animals fed a high beef-fat diet in comparison with a control diet. Data analysis indicated that the high-fat diet affected the expression of genes involved in inflammation, glucose and fatty acid metabolism, androgen metabolism, potential tumour suppression and protein kinase activity, as well as intracellular and extracellular matrix molecules, growth factors and androgen responsive genes (Reyes *et al.*, 2002).

Finally, in the above-mentioned study from Davidson *et al.* (2004), developed in the AOM-induced colon cancer model, DNA microarray analysis has linked the protective effect of dietary n-3 PUFA at both the initiation and promotion stages of colon carcinogenesis with specific alterations of the gene expression profile in comparison with n-6 PUFA and n-9 MUFA. Interestingly, in this study, n-9 MUFA did not have chemopreventive activity against colon carcinogenesis, probably attributable to the presence in the diet of a highly fermentable fibre source, and generated a similar pattern of expression to n-6 PUFA, but different from n-3 PUFA.

5.6. Immunosuppressor effect

Dietary lipids are able to modulate the immune response and modify inflammatory cytokine production (Calder *et al.*, 2002; Stark and Madar, 2002). The immunosuppressive effect of dietary PUFA has been observed in several studies (Utermohlen and Tucker, 1986; Calder, 1999). Thus, in a human breast cancer cell line it has been described that the LA has an inhibitory effect on the lymphocytotoxicity (Samlaska, 1978). Regarding the n-3 fatty acids, a suppressive effect of the immune system has also been observed (Calder, 1999; Hwang, 2000). The eicosanoids modulate the inflammatory and immune response, besides playing a critical role in platelet aggregation, and cell growth and differentiation. The n-6 PUFA generate eicosanoids with pro-inflammatory effects, whereas the n-3 PUFA generates eicosanoids with anti-inflammatory effects (Stulnig, 2003; Larsson *et al.*, 2004). Diets with a high proportion of n-6:n-3 PUFA would likely increase the generation of inflammatory eicosanoids, whereas diets with a low proportion of

n-6:n-3 would have an inhibitory effect on the production of these eicosanoids (Jump, 2004). Moreover, it has been demonstrated that the hyperlipidaemias have adverse consequences on the macrophage activity and reduce the lymphocytes' peripheral concentration (Vitale and Broitman, 1981; Wagner *et al.*, 1982). The prostaglandins could therefore be involved in the immunosuppressive effect of dietary PUFA (Hillyard and Abraham, 1979). Thus, although the mechanism is still unknown, PGE_2 inhibits the immune response: inhibits macrophages and T and B cells, and stimulates immunosuppressor cells (Devries and Vannoorden, 1992; Hwang, 2000; Stulnig, 2003). Furthermore, PPAR activation by fatty acids suppresses the expression of cytokines and other molecules involved in the inflammatory response (Hwang, 2000). Recently, it has been shown that the $PPAR_\gamma$ agonists regulate negatively the transcription of the inflammation response genes and that PUFA inhibit the LXR_α and LXR_β activation in macrophages. An inhibition of *in vitro* lymphocyte proliferation due to AA, EPA and DHA has been observed. A decrease in the TNF_α, IL1, IL2 and IL6 secretion and an inhibition of the NK cell and cytotoxic T lymphocyte activity have also been reported (Stulnig, 2003).

Research investigating the effects of olive oil on the immune system is sparse, but available data indicate that it may be a potent mediator of the immune response and modify inflammatory cytokine production. The attenuation of these processes that it elicits could explain the beneficial effects on cancer risk (Stark and Madar, 2002). Oleic acid has been demonstrated to have anti-inflammatory effects (Calder *et al.*, 2002). Also, some extra virgin olive oil phenolics have been shown to inhibit the production of inflammatory eicosanoids and cytokines by animal and human cells *in vitro* (Visioli *et al.*, 1998; Moreno, 2003; Miles *et al.*, 2005). In contrast, consumption by healthy human subjects of a diet rich in oleic acid did not appear to bring about general suppression of immune cell functions (Yaqoob *et al.*, 1998). Moreover, in rats it has been shown that feeding mothers a 15% olive oil diet had a cancer-inhibiting role in offspring, predominantly via changes at the cellular level such as increasing the total number of lymph cells and the number of CD8(+) lymphocytes (Kossoy *et al.*, 2001).

6. Final Considerations on Olive Oil in the Cancer Prevention

As has been reviewed in this chapter, a whole body of experimental evidence exists supporting the beneficial effects of olive oil, the main component of the Mediterranean diet, in relation to cancer. Habitual high intakes of olive oil (especially extra virgin) will provide a continuous supply of monounsaturated fatty acids, mainly oleic acid, and elevated levels of antioxidants. These two classes of components, by means of different specific mechanisms, are responsible for olive oil's observed preventive effects against this disease. From the experimental data, it can be stated that olive oil acts principally on the promotion of carcinogenesis, slowing the tumour growth rather than regressing already-established tumours. When extrapolating this situation to humans, it must not be forgotten that human feeding involves interaction between its multiple components and other factors, for instance, environmental. In this sense, the beneficial effect of olive oil intake should be contextualized within the wide concept of the Mediterranean diet and lifestyle.

When the possibility of the prevention of cancer through feeding, and in particular through the high habitual intake of virgin olive oil, is considered, it should be taken into account that tumour induction is a process that could take a long time, 20, 30 and even 40 years. Between the beginning of exposure to initiating and/or promoting factors and the appearance of the tumour, a very long period can pass. Moreover, certain dietary factors during puberty can be relevant. This fact is of great importance because it emphasizes that the possible benefits of healthy changes in feeding patterns cannot be expected immediately, but after many years. On the other hand, in the case of potentially harmful dietary factors, such as the high intake of particular dietary lipids, this fact means that their effects could be exerted over a very long period in the life of a person, and therefore, would represent an added risk. Such a risk is, moreover, imperceptible, but foreseeable from the available experimental data, such as are described in this chapter. Furthermore, taking into account what is possible with the current methods of cancer screening (mammography for breast cancer, colonoscopy for colorectal cancer and the prostatic specific antigen, PSA, determination and other methods for prostate cancer), this dietary factor would be acting before the stated screening techniques would permit detection of a tumour existence.

From the point of view of public health, prevention strategies against cancer should consider potentially beneficial dietary factors, such as extra virgin olive oil, for both secondary prevention and possibly also primary prevention of cancer.

Acknowledgements

The work of the authors cited in this chapter was supported in part by grants from 'Comision Interministerial de Ciencia y Tecnología' (CICYT SAF92-0537), 'Pla de Recerca de Catalunya, Generalitat de Catalunya' (GRQ93-2094, 1995SGR-495), 'Comision Interministerial de Ciencia y Tecnología' (CICYT OLI96-2114), 'Acadèmia de Ciències Mèdiques de Catalunya i Balears' (ACMCB-2002 163.985), 'Plan Nacional de I+D+I 2000–2003' and FEDER (BFI 2003-07341). The authors thank Irmgard Costa, MD, for the histological analyses described in this chapter and Mrs Laura Grau for her technical assistance.

7. References

Alberts, B., Jonson, A., Lewis, J., Raff, M., Roberts, K. and Walter, P. (2002) *Molecular Biology of the Cell*. 4th edn. Garland, New York, 1616 pp.

Albi, E. and Viola Magni, M.P. (2004) The role of intranuclear lipids. *Biology of the Cell* 96, 657–667.

Anderson, R.G.W. (1998) The caveolae membrane system. *Annual Review of Biochemistry* 67, 199–225.

Armstrong, B. and Doll, R. (1975) Environmental factors and cancer incidence and mortality in different countries with special reference to dietary practices. *International Journal of Cancer* 15, 617–631.

Armstrong, B.K., McMichael, A.J. and Maclennan, R. (1982) Diet. In: Schottenfeld, D. and Fraumeni, Jr, J.F. (eds) *Cancer Epidemiology and Prevention*. W.B. Saunders Company, Philadelphia, pp. 419–432.

Assmann, G., De Backer, G., Bagnara, S., Betteridge, J., Crepaldi, G., Fernandez-Cruz, A., Godtfredsen, J., Jacotot, B., Paoletti, R., Renaud, S., Ricci, G., Rocha, E., Trautwein, E., Urbinati, G.C., Varela, G. and Williams, C. (1997) Olive oil and the Mediterranean diet: implications for health in Europe. *British Journal of Nursing* 6, 675–677.

Aylsworth, C.F., Jone, C., Trosko, J.E., Meites, J. and Welsch, C.W. (1984) Promotion of 7,12-dimethylbenz[a]anthracene-induced mammary tumorigenesis by high dietary fat in the rat: possible role of intercellular communication. *Journal of the National Cancer Institute* 72, 637–645.

Aylsworth, C.F., Welsch, C.W., Kabara, J.J. and Trosko, J.E. (1987) Effects of fatty acids on gap junctional communication: possible role in tumor promotion by dietary fat. *Lipids* 22, 445–454.

Balmain, A., Gray, J. and Ponder, B. (2003) The genetics and genomics of cancer. *Nature Genetics* 33, 238–244.

Banni, S., Angioni, E., Casu, V., Melis, M.P., Carta, G., Corongiu, F.P., Thompson, H. and Ip, C. (1999) Decrease in linoleic acid metabolites as a potential mechanism in cancer risk reduction by conjugated linoleic acid. *Carcinogenesis* 6, 1019–1024.

Barnes, D.E. and Lindahl, T. (2004) Repair and genetic consequences of endogenous DNA base damage in mammalian cells. *Annual Reviews of Genetics* 38, 445–476.

Bartolí, R., Fernández-Bañares, F., Navarro, E., Castellà, E., Mañé, J., Alvarez, M., Pastor, C., Cabré, E. and Gassull, M.A. (2000) Effect of olive oil on early and late events of colon carcinogenesis in rats: modulation of arachidonic acid metabolism and local prostaglandin E(2) synthesis. *Gut* 46, 191–199.

Bartsch, H., Nair, J. and Owen, R.W. (1999) Dietary polyunsaturated fatty acids and cancers of the breast and colorectum: emerging evidence for their role as risk modifiers. *Carcinogenesis* 26, 2209–2218.

Bartsch, H., Nair, J. and Owen, R.W. (2002) Exocyclic DNA adducts as oxidative stress markers in colon carcinogenesis: potential role of lipid peroxidation, dietary fat and antioxidants. *Biological Chemistry* 383, 915–921.

Bautista, D., Obrador, A., Moreno, V., Cabeza, E., Canet, R., Benito, E., Bosch, X. and Costa, J. (1997) Ki-ras mutation modifies the protective effect of dietary monounsaturated fat and calcium on sporadic colorectal cancer. *Cancer Epidemiology, Biomarkers and Prevention* 6, 57–61.

Bégin, M.E., Ells, G. and Horrobin, D.F. (1988) Polyunsaturated fatty acid-induced cytotoxicity against tumor cells and its relationship to lipid peroxidation. *Journal of the National Cancer Institute* 80, 188–194.

Beremblum, I. and Shubik, P. (1947) The role of croton oil applications associated with a single painting of a carcinogen in tumor induction of the mouse's skin. *British Journal of Cancer* 1, 379.

Bernard-Gallon, D.J., Vissac-Sabatier, C., Antoine-Vincent, D., Rio, P.G., Maurizis, J.C., Fstier, P. and Bignon, Y.J. (2002) Differential effects of n-3 and n-6 polyunsaturated fatty acids on *BRCA1* and *BRCA2* gene expression in breast cell lines. *British Journal of Nutrition* 87, 281–289.

Billah, M.M. (1993) Phospholipase D and cell signaling. *Current Opinion in Immunology* 5, 114–123.

Bingham, S. and Riboli, E. (2004) Diet and cancer – the European prospective investigation into cancer and nutrition. *Nature Reviews* 4, 206–215.

Birt, D.F., Kris, E.S., Choe, M. and Pelling, J.C. (1992) Dietary energy and fat effects on tumor promotion. *Cancer Research* 52, 2035s–2039s.

Bishop, J.M. (1987) The molecular genetics of cancer. *Science* 235, 305–311.

Bishop, J.M. (1991) Molecular themes in oncogenesis. *Cell* 64, 235–248.

Bohnsack, B.L. and Hirschi, K.K. (2004) Nutrient regulation of cell cycle progression. *Annual Reviews of Nutrition* 24, 433–453.

Borek, C. (2004) Dietary antioxidants and human cancer. *Integrative Cancer Therapies* 3, 333–341.

Bray, F., McCarron, P. and Parkin, D.M. (2004) The changing global patterns of female breast cancer incidence and mortality. *Breast Cancer Research* 6, 229–239.

Brenner, R.R. (1981) Nutritional and hormonal factors influencing desaturation of essential

fatty acids. *Progress in Lipid Research* 20, 41–47.

Brouwer, I.A., Katan, M.B. and Zock, P.L. (2004) Dietary alpha-linolenic acid is associated with reduced risk of fatal coronary heart disease, but increased prostate cancer risk: a meta-analysis. *Journal of Nutrition* 134, 919–922.

Burn, P. (1988) Amphitropic proteins: a new class of membrane proteins. *Trends in Biochemical Sciences* 13, 79–83.

Calder, P.C. (1999) Dietary fatty acids and the immune system. *Lipids* 34, S137–S140.

Calder, P.C., Yaqoob, P., Thies, F., Wallace, F.A., Miles, E.A. (2002) Fatty acids and lymphocyte functions. *British Journal of Nutrition* 87, Suppl. 1, S31–S48.

Campillo, J.E. (1997) *Alimentación: ciencia, tradición y salud*. Arán Ediciones SA, Madrid, 398 pp.

Carroll, K.K. and Khor, H.T. (1975) Dietary fat in relation to tumorigenesis. *Progress in Biochemical Pharmacology* 10, 308–353.

Carroll, K.K. (1981) Neutral fats and cancer. *Cancer Research* 41, 3695–3699.

Carroll, K.K. (1992) Dietary fat and breast cancer. *Lipids* 27, 793–797.

Casabiell, X., Pandiella, A. and Casanueva, F.F. (1991) Regulation of epidermal-growth-factor-receptor signal transduction by cis-unsaturated fatty acids. *The Biochemical Journal* 278, 679–687.

Cave, W.T. (1996) Dietary ω-3 polyunsaturated fats and breast cancer. *Nutrition* 12, S39–S42.

Cave, W.T. Jr. (1997) Omega-3 polyunsaturated fatty acids in rodent models of breast cancer. *Breast Cancer Research and Treatment* 46, 239–246.

Choe, M., Kris, E.S., Luthra, R., Copenhaver, J., Pelling, J.C., Donnelly, T.E. and Birt, D.F. (1992) Protein kinase C is activated and diacylglicerol is elevated in epidermal cells from Sencar mice fed high fat diets. *The Journal of Nutrition* 122, 2322–2329.

Christman, J.K., Chen, M.L., Sheiknejad, G., Dizik, M., Abileah, S. and Wainfan, E. (1993) Methyl deficiency, DNA methylation, and cancer: studies on the reversibility of the effects of the lipotrope-deficient diet. *The Journal of Nutritional Biochemistry* 4, 672–680.

Clandinin, M.T., Cheema, S., Field, C.J., Garg, M.L., Venkatraman, J. and Clandinin, T.R. (1991) Dietary fat: exogenous determination of membrane structure and cell function. *The FASEB Journal* 5, 2761–2768.

Clarke, S.D. (2001) Polyunsaturated fatty acid regulation of gene transcription: a molecular mechanism to improve the metabolic syndrome. *The Journal of Nutrition* 131, 1129–1132.

Clarke, S.D. and Abraham, S. (1992) Gene expression: nutrient control of pre- and post-transcriptional events. *The FASEB Journal* 6, 3146–3152.

Clarke, S.D. and Jump, D.B. (1993) Regulation of gene transcription by polyunsaturated fatty acids. *Progress in Lipid Research* 32, 139–149.

Clarke, S.D., Armstrong, M.K. and Jump, D.B. (1990) Nutritional control of rat liver fatty acid synthase and S14 mRNA abundance. *The Journal of Nutrition* 120, 218–224.

Clinton, S.K., Li, P.S., Mulloy, A.L., Imrey, P.B., Nandkumar, S. and Visek, W.J. (1995) The combined effects of dietary fat and estrogen on survival, 7,12-dimethylbenz(a)anthracene-induced breast cancer and prolactin metabolism in rats. *The Journal of Nutrition* 125, 1192–1204.

Cohen, L.A. (1981) Mechanisms by which dietary fat may stimulate mammary carcinogenesis in experimental animals. *Cancer Research* 41, 3808–3810.

Cohen, L.A. (1992) Lipids in cancer: an introduction. *Lipids* 27, 791–792.

Cohen, L.A., Thompson, D.O., Maeura, Y., Choi, K., Blank, M.E. and Rose, D.P. (1986a) Dietary fat and mammary cancer. I – Promoting effects of different dietary fats on N-Nitrosomethylurea-induced rat mammary tumorigenesis. *Journal of the National Cancer Institute* 77, 33–42.

Cohen, L.A., Choi, K., Numoto, S., Reddy, S., Berke, B. and Weisburger, J.H. (1986b) Inhibition of chemically induced mammary carcinogenesis in rats by long-term exposure to butylated hydroxytoluene (BHT): interrelations among BHT concentration, carcinogen dose and diet. *Journal of the National Cancer Institute* 76, 721–730.

Cohen, L.A., Thompson, D.O., Choi, K., Karmali, R.A. and Rose, D.P. (1986c) Dietary fat and

mammary cancer: II – Modulation of serum and tumor lipid composition and tumor prostaglandins by different dietary fats: association with tumor incidence patterns. *Journal of the National Cancer Institute* 77, 43–51.

Cohen, L.A., Epstein, M., Pittman, B. and Rivenson, A. (2000) The influence of different varieties of olive oil on N-methylnitrosourea(NMU)-induced mammary tumorigenesis. *Anticancer Research* 20, 2307–2312.

Colditz, G.A., Samplin-Salgado, M., Ryan, C.T., Dart, H., Fisher, L., Tokuda, A., Rockhill, B. and Harvard Center for Cancer Prevention (2002) Harvard report on cancer prevention, volume 5: fulfilling the potential for cancer prevention: policy approaches. *Cancer Causes and Control* 13, 199–212.

Collett, E.D., Davidson, L.A., Fan, Y.Y., Lupton, J.R. and Chapkin, R.S. (2001) n-6 and n-3 polyunsaturated fatty acids differentially modulate oncogenic Ras activation in colonocytes. *American Journal of Physiology. Cell Physiology* 280, C1066–C1075.

Costa, I., Esquius, J., Solanas, M., Moral, R. and Escrich, E. (2001) Histopathologic characteristics of chemically-induced mammary adenocarcinomas in rats fed diets high in (n-6) polyunsaturated lipids. *Virchows Archive: an International Journal of Pathology* 439, 310–311.

Costa, I., Solanas, M. and Escrich, E. (2002) Histopathologic characterization of mammary neoplastic lesions induced with 7,12-dimethylbenz(α)antracene in the rat. A comparative analysis with human breast tumours. *Archives of Pathology and Laboratory Medicine* 126, 915–927.

Costa, I., Moral, R., Solanas, M. and Escrich, E. (2004) High-fat corn oil diet promotes the development of high histologic grade rat DMBA-induced mammary adenocarcinomas, while high olive oil diet does not. *Breast Cancer Research and Treatment* 86, 225–235.

Daniel, L.W., Sciorra, V.A. and Ghosh, S. (1999) Phospholipase D, tumor promoters, proliferation and prostaglandins. *Biochimica et Biophysica Acta* 1439, 265–276.

Darnell, J., Lodish, H. and Baltimore, D. (1990) *Molecular Cell Biology.* 2nd edn. Scientific American books Inc., New York, 1105 pp.

Davidson, L.A., Nguyen, D.V., Hokanson, R.M., Callaway, E.S., Isett, R.B., Turner, N.D., Dougherty, E.R., Wang, N., Lupton, J.R., Carroll, R.J. and Chapkin, R.S. (2004) Chemopreventive n-3 polyunsaturated fatty acids reprogram genetic signatures during colon cancer initiation and progression in the rat. *Cancer Research* 64, 6797–6804.

Davis, C.D. and Snyderwine, E.G. (1995) Analysis of EGFR, TGF-α, neu and c-myc in 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced mammary tumors using RT-PCR. *Carcinogenesis* 16, 3087–3092.

Davis, L.A., McNeill, G.P. and Caswell, D.C. (1999) Analysis of conjugated linoleic acid isomers by ^{13}C NMR spectroscopy. *Chemistry and Physics of Lipids* 97, 155–165.

De Lorgeril, M. and Salen, P. (2004) alpha-linolenic acid, coronary heart disease, and prostate cancer. *Journal of Nutrition* 134, 3385.

Dennis, E.A., Rhee, S.G., Billah, M.M. and Hannun, Y.A. (1991) Role of phospholipases in generating lipid second messengers in signal transduction. *The FASEB Journal* 5, 2068–2077.

Dennis, L.K., Snetselaar, L.G., Smith, B.J., Stewart, R.E. and Robbins, M.E. (2004) Problems with the assessment of dietary fat in prostate cancer studies. *American Journal of Epidemiology* 160, 436–444.

Deslypere, J.P., Verdonck, L. and Vermeulen, A. (1985) Fat tissue: a steroid reservoir and site of steroid metabolism. *The Journal of Clinical Endocrinology and Metabolism* 61, 564–570.

Devries, C.E.E. and Vannoorden, C.J.F. (1992) Effects of dietary fatty acid composition on tumor growth and metastasis. *Anticancer Research* 12, 1513–1522.

Diplock, A.T., Aggett, P.J., Ashwell, M., Bornet, F., Fern, E.B. and Roberfroid, M. (1999) Scientific concepts of functional foods in Europe: consensus document. *The British Journal of Nutrition* 81, S1.

Divecha, N. and Irvine, R.F. (1995) Phospholipid signaling. *Cell* 80, 269–278.

Doll, R. and Peto, R. (1981) The causes of cancer: quantitative estimates of avoidable risk in the United States today. *Journal of the National Cancer Institute* 66, 1191–1308.

Dowhan, W., Mileykovskaya, E. and Bogdanov, M. (2004) Diversity and versatility of lipid-protein interactions revealed by molecular genetic approaches. *Biochimica et Biophysica Acta* 1666, 19–39.

Dreher, D. and Junod, A.F. (1996) Role of oxygen free radicals in cancer development. *European Journal of Cancer* 32, 30–38.

Edidin, M. (2003) The state of lipid rafts: from model membranes to cells. *Annual Review of Biophysics and Biomolecular Structure* 32, 257–83.

Escrich, E. (1990) Hormone-dependence of experimental mammary tumours. *Revista Española de Fisiología* 46, 89–94.

Escrich, E. (1998) Endocrine Aspects of Breast Cancer. In: Cardoso, J. (ed.) *Senology*. Monduzzi Editore, Bologna, pp. 599–603.

Escrich, E., Ribalta, T., Muntané, J., Ruiz de Villa, M.C., Murillo, J. and Saez, S. (1991) Effects of an androgenic derivative on pre-established mammary tumours chemically induced in the rat. *Journal of Cancer Research and Clinical Oncology* 117, 575–582.

Escrich, E., Muntane, J., Ribalta, T., Colom, J., Solanas, M. and Segura, R. (1992) Efectos de una dieta hiperlipídica sobre la carcinogénesis mamaria experimental: contenido y tipo de tumores. *Neoplasia* 9, 54–56.

Escrich, E., Solanas, M., Soler, M., Ruiz De Villa, M.C., Sánchez, J.A. and Segura, R. (2001) Dietary polyunsaturated n-6 lipids effects on the growth and fatty acid composition of rat mammary tumors. *The Journal of Nutritional Biochemistry* 12, 536–549.

Escrich, E., Moral, R., García, G., Costa, I., Sánchez, J.A. and Solanas, M. (2004) Identification of novel differentially expressed genes by the effect of a high-fat n-6 diet in experimental breast cancer. *Molecular Carcinogenesis* 40, 73–78.

European Breast Cancer Group (1972) Clinical trial of 2-Br-α-ergocryptine (CB154) in advanced breast cancer. *European Journal of Cancer* 8, 155–156.

Evan, G.I. and Vousden, K.H. (2001) Proliferation, cell cycle and apoptosis in cancer. *Nature* 411, 342–348.

Exton, J.H. (1990) Signaling through phosphatidylcholine breakdown. *The Journal of Biological Chemistry* 265, 1–4.

Exton, J.H. (1994) Phosphoinositide phospholipases and G proteins in hormone action. *Annual Review of Physiology* 56, 349–369.

Fay, M.P., Freedman, L.S., Clifford, C.K. and Midthune, D.N. (1997) Effect of different types and amounts of fat on the development of mammary tumors in rodents: a review. *Cancer Research* 57, 3979–3988.

Ferlay, J., Bray, F., Pisani, P. and Parkin, D.M. (2004) *GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide*, Version 2.0. IARC CancerBase no. 5. IARCPress, Lyon. *http://www-dep.iarc.fr*

Fernandes, G. and Venkatraman, J.T. (1991) Modulation of breast cancer growth in nude mice by n-3 lipids. *World Review of Nutrition and Dietetics* 66, 488–503.

Fleshner, N., Fair, W.R., Huryk, R. and Heston, W.D. (1999) Vitamin E inhibits the high-fat diet promoted growth of established human prostate LNCaP tumors in nude mice. *The Journal of Urology* 161, 1651–1654.

Floid, R.A. (1990) Role of oxygen free radicals in carcinogenesis and brain ischemia. *The FASEB Journal* 4, 2587–2597.

Food and Agriculture Organisation and World Health Organization (1991) *Protein Quality Evaluation: Report of the Joint FAO/WHO*. FAO Food and Nutrition Paper 51, Rome, 66 pp.

Foulds, L. (1958) The natural history of cancer. *Journal of Chronic Diseases* 8, 2–37.

Freedman, L.S., Clifford, C. and Messina, M. (1990) Analysis of dietary fat, calories, body weight, and the development of mammary tumors in rats and mice: a review. *Cancer Research* 50, 5710–5719.

Futreal, P.A., Coin, L., Marshall, M., Down, T., Hubbard, T., Wooster, R., Rahman, N. and Stratton, M.R. (2004) A census of human cancer genes. *Nature Reviews Cancer* 4, 177–183.

Galli, C. and Butrum, R. (1991) Dietary ω-3 fatty acids and cancer: an overview. In: Simopoulos, A.P., Kifer, R.R., Martin, R.E. and Barlow, S.M. (eds) Health effects of ω-3 polyunsaturated fatty acids in seafoods. *World Review of Nutrition and Dietetics* 66, Karger, Basel, pp. 446–461.

Galli, C. and Visioli, F. (1999) Antioxidant and other activities of phenolics in olives/olive oil, typical components of the Mediterranean diet. *Lipids* 34, S23–S26.

Gasparini, G., Longo, R., Sarmiento, R. and Morabito, A. (2003) Inhibitors of cyclooxygenase 2: a new class of anticancer agents? *Lancet Oncology* 4, 605–615.

Gerber, M. (1997) Olive oil, monounsaturated fatty acids and cancer. *Cancer Letters* 114, 91–92.

Gidwitz, S., Pessin, J.E., Weber, M.J., Glaser, M. and Storm, D.R. (1980) Effect of membrane phospholipid composition changes on adenylate cyclase activity in normal and rous-sarcoma-transformed chicken embryo fibroblasts. *Biochimica et Biophysica Acta* 628, 263–276.

Glasgow, W.C., Hui, R., Everhart, A.L., Jayawickreme, S.P., Angerman-Stewart, J., Han, B.B. and Eling, T.E. (1997) The linoleic acid metabolite, (13S)-hydroperoxyoctadecadienoic acid, augments the epidermal growth factor receptor signalling pathway by attenuation of receptor dephosphorylation. Differential response in Syrian hamster embryo tumor suppressor phenotypes. *The Journal of Biological Chemistry* 272, 19269–19276.

Glauert, H.P. (1993) Dietary fat, gene expression, and carcinogenesis. In: Berdanier, C.D. and Hargrove, J.L. (eds) *Nutrition and Gene Expression*. CRC Press, Inc., Boca Raton, pp. 247–268.

Goldstein, B.D. and Witz, G. (1990) Free radicals and carcinogenesis. *Free Radical Research Communications* 11, 1–3.

Graber, R., Sumida, C.H. and Nunez, E.A. (1994) Fatty acids and cell signal transduction. *Journal of Lipid Mediators and Cell Signalling* 9, 91–116.

Grande Covian, F. (1993) *Nutrición y Salud*. Ediciones Temas de Hoy, Madrid, 203 pp.

Grimard, R., Tancrede, P. and Gicquard, C. (1993) Interaction of actin with positively charged phospholipids: a monolayer study. *Biochemical and Biophysical Research Communications* 190, 1017–1022.

Haeffner, E.W. (1993) Diacylglycerol: formation and function in phospholipid-mediated signal transduction. *Comparative Biochemistry and Physiology* 105C, 337–345.

Hamid, R. and Sing, J. (1999) Inhibition by dietary menhaden oil of cycloxygenase-1 and 2 in NMU-induced rat mammary tumors. *International Journal of Oncology* 14, 523–528.

Hanahan, D. and Weinberg, R.A. (2000) The hallmarks of cancer. *Cell* 100, 57–70.

Harris, H. (1988) The analyses of malignancy by cell fusion: the position in 1988. *Cancer Research* 48, 3302–3306.

Hathway, D.E. (1986) *Mechanisms of chemical carcinogenesis*. Butterworths, London, 134 pp.

Hetts, S.W. (1998) To die or not to die: an overview of apoptosis and its role in disease. *JAMA: The Journal of the American Medical Association* 279, 300–307.

Hill, M.J., Goddard, P. and Williams, R.E.O. (1971) Gut bacteria and aetiology of cancer of the breast. *Lancet* 2, 472–473.

Hillyard, L.A. and Abraham, S. (1979) Effect of dietary polyunsaturated fatty acids on growth of mammary adenocarcinomas in mice and rats. *Cancer Research* 39, 4430–4437.

Hoeijmakers, J.H.J. (2001) Genome maintenance mechanisms for preventing cancer. *Nature* 411, 366–374.

Holder, J.W., Elmore, E. and Barret, J.C. (1993) Gap junction function and cancer. *Cancer Research* 53, 3475–3485.

Hu, X., Juneja, S.C., Maihle, N.J. and Cleary, M.P. (2002) Leptin-a growth factor in normal and malignant breast cells and for normal mammary gland development. *Journal of the National Cancer Institute* 94, 1704–1711.

Huertas, J.R., Battino, M., Barzanti, V., Parenti-Castelli, G., Littarru, G.P., Turchetto, E., Mataix, F.J. and Lenaz, G. (1992) Mitochondrial and microsomal cholesterol mobilization after oxidative stress induced by adriamicin in rats fed with dietary olive and corn oil. *Life Science* 50, 2111–2118.

Hulberg, A.J. and Else, P.L. (2000) Mechanisms underlying the cost of living in animals. *Annual Review of Physiology* 62, 207–35.

Hulbert, A.J., Turner, N., Storlien, L.H. and Else, P.L. (2005) Dietary fats and membrane function: implications for metabolism and disease. *Biological Reviews of the Cambridge Philosophical Society* 80, 155–169.

Hung, R.W. and Chow, A.W. (2004) Dissecting the 'end game': clinical relevance, molecular mechanisms and laboratory assessment of apoptosis. *Clinical and Investigative Medicine* 27, 324–344.

Hunter, D.J. and Willet, W.C. (1994) Diet, body build, and breast cancer. *Annual Review of Nutrition* 14, 393–418.

Hwang, D. (2000) Fatty acids and immune responses– A new perspective in searching for clues to mechanism. *Annual Review of Nutrition* 20, 431–456.

International Agency for Research on Cancer (2003) *World Cancer Report.* Stewart, B.W., Kleihues, P. (eds) IARC Press, Lyon, 351 pp.

Ip, C. (1987) Fat and essential fatty acid in mammary carcinogenesis. *The American Journal of Clinical Nutrition* 45, S218–S224.

Ip, C. (1997) Review of the effects of trans fatty acids, oleic acid, n-3 polyunsaturated fatty acid, and conjugated linoleic acid on mammary carcinogenesis in animals. *The American Journal of Clinical Nutrition* 66, S15235–S15295.

Ip, C. and Ip, M.M. (1981) Serum estrogens and estrogen responsiveness in 7,12-dimethylbenz[a]anthracene-induced mammary tumors as influenced by dietary fat. *Journal of the National Cancer Institute* 66, 291–295.

Ip, C., Ip, M.M. and Sylvester, P.W. (1986) Relevance of trans fatty acids and fish oil in animal tumorigenesis studies. In: Rogers, A., Birt, D., Mettlin, C. and Ip, C. (eds) *Dietary Fat and Cancer*, Alan R. Liss Inc., New York, pp. 283–294.

Ip, C., Briggs, S.P., Haegele, A.D., Thompson, H.J., Storkson, J. and Scimeca, J.A. (1996) The efficacy of conjugated linoleic acid in mammary cancer prevention is independent of the level or type of fat in the diet. *Carcinogenesis* 17, 1045–1050.

Ip, M.M., Masso-Welch, P.A. and Ip, C. (2003) Prevention of mammary cancer with conjugated linoleic acid: Role of the stroma and the epithelium. *Journal of Mammary Gland Biology and Neoplasia* 8, 103–118.

Jacobson, M.D., Weil, M. and Raff, M.C. (1997) Programmed cell death in animal development. *Cell* 88, 347–54.

James, W.P.T., Ferro-Luzzi, A. and Szostak, W.B. (1990) *Alimentation et santé. La prevention des maladies d'origine alimentaire en Europe.* Organisation Mondiale de la Santé. OMS publications régionales. Série européenne no. 24. Bureau régional de l'Europe, Copenhagen, 161 pp.

Jemal, A., Murray, T., Ward, E., Samuels, A., Tiwari, R.C., Chafoor, A., Feuer, E.J. and Thun, J. (2005) Cancer Statistics. *CA: a Cancer Journal for Clinicians* 55, 10–30.

Jiang, W.G., Hiscox, S., Hallett, M.B., Horrobin, D.F., Mansel, R.E. and Puntis, M.C. (1995) Regulation of the expression of E-cadherin on human cancer cells by gamma-linolenic acid (GLA). *Cancer Research* 55, 5043–5048.

Jiang, W.G., Bryce, R.P. and Mansel, R.E. (1997a) Gammalinolenic acid regulates gap junction communication in endothelial cells and their interaction with tumour cells. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 56, 307–316.

Jiang, W.G., Hiscox, S., Horrobin, D.F., Bryce, R.P. and Mansel, R.E. (1997b) Gamma linolenic acid regulates expression of maspin and the motility of cancer cells. *Biochemical and Biophysical Research Communications* 237, 639–644.

Johnson, J.E. and Cornell, R.B. (1999) Amphitropic proteins: regulation by reversible membrane interactions (review). *Molecular Membrane Biology* 16, 217–35.

Jump, D.B. (2004) Fatty acid regulation of gene transcription. *Critical Reviews in Clinical Laboratory Sciences* 41, 41–78.

Jump, D.B. and Clarke, S.D. (1999) Regulation of gene expression by dietary fat. *Annual Review of Nutrition* 19, 63–90.

Jurek, D., Udilova, N., Jozkowicz, A., Nohl, H., Marian, B. and Schulte-Hermann, R. (2005) Dietary lipid hydroperoxides induce expression of vascular endothelial growth factor (VEGF) in human colorectal tumor cells. *The FASEB Journal* 19, 97–99.

Kachhap, S.K., Dange, P. and Ghosh, S.N. (2000) Effect of ω-6 polyunsaturated fatty acid (linoleic acid) on BRCA1 gene expression in MCF-7 cell line. *Cancer Letters* 154, 115–120.

Kaput, J. and Rodríguez, R.L. (2004) Nutritional genomics: the next frontier in the postgenomic era. *Physiological genomics* 16, 166–177.

Kelekar, A. and Thompson, C.B. (1998) Bcl-2-family proteins: the role of the BH3 domain in apoptosis. *Trends in Cell Biology* 8, 324–330.

Kenny, F.S., Gee, J.M., Nicholson, R.I., Ellis, I.O., Morris, T.M., Watson, S.A., Bryce, R.P. and

Robertson, J.F. (2001) Effect of dietary GLA+/-tamoxifen on the growth, ER expression and fatty acid profile of ER positive human breast cancer xenografts. *International Journal of Cancer* 92, 342–347.

Khan, S.A. and Heuvel, J.P.V. (2003) Current topics role of nuclear receptors in the regulation of gene expression by dietary fatty acids. *The Journal of Nutritional Biochemistry* 14, 554–567.

King, M.M., McCay, P.B. and Russo, I.H. (1983) Dietary fat may influence DMBA-initiated mammary gland carcinogenesis by modification of mammary gland development. In: Roe, D.A. (ed.) *Current Topics in Nutrition and Disease: Diet, Nutrition, and Cancer, from Basic Research to Policy Implications*, Vol. 9. Alan R. Liss Inc., New York, pp. 61–90.

Kinnunen, P.K.J., Jukka, A.K., Lehtonen, J.Y.A., Rytomaa, M.A. and Mustonen, P. (1994) Lipid dynamics and peripheral interactions of proteins with membrane surface. *Chemistry and Physics of Lipids* 73, 181–207.

Klurfeld, D.M. and Bull, A.W. (1997) Fatty acids and colon cancer in experimental models. *American Journal of Clinical Nutrition* 66(6 Suppl), 1530S–1538S.

Knudson, A.G. (1971) Mutation and cancer: statistical study of retinoblastoma. *Proceedings of the National Academy of Sciences of the United States of America* 68, 820–823.

Kolonel, L.N., Nomura, A.M. and Cooney, R.V. (1999) Dietary fat and prostate cancer: current status. *Journal of the National Cancer Institute* 91, 414–428.

Kossoy, G., Yarden, G., Benhur, H., Sandler, B., Zusman, I.I., Stark, A. and Madar, Z. (2000) Transplacental effects of a 15% olive-oil diet on chemically-induced tumorigenesis in offspring. *Oncology Reports* 7, 1145–1148.

Kossoy, G., Madar, Z., Ben-Hur, H., Gal, R., Stark, A., Cohen, O. and Zusman, I. (2001) Transplacental effect of a 15% olive-oil diet on functional activity of immune components in the spleen and colon tumors of rat offspring. *Oncology Reports* 8, 1045–1049.

Kritchevsky, D. (1999) Caloric restriction and experimental carcinogenesis. *Toxicological Sciences* 52, 13–16.

Kritchevski, D., Weber, M.M. and Klurfeld, D.M. (1984) Dietary fat versus caloric content in initiation and promotion of 7,12-dimethylbenz(α)anthracene-induced mammary tumorigenesis. *Cancer Research* 44, 3174–3177.

Kritchevsky, D. and Klurfeld, D.M. (1987) Caloric effects in experimental mammary tumorigenesis. *The American Journal of Clinical Nutrition* 45, 236–242.

Kumar, V., Abbas, A.K. and Fausto, N. (2004) *Robbins and Cotran Pathologic Basis of Disease.* 7th edn. W.B. Saunders Company, Elsevier Health Science, Philadelphia, 1552 pp.

Kundu, J.K. and Surh, Y.J. (2004) Molecular basis of chemoprevention by resveratrol: NF-kappaB and AP-1 as potential targets. *Mutation Research* 555, 65–80.

Kushi, L. and Giovannucci, E. (2002) Dietary fat and cancer. *The American Journal of Medicine* 113(9B), 63–70S.

Larsson, S., Kumlin, M., Ingelman-Sundberg, M. and Wolk, A. (2004) Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. *The American Journal of Clinical Nutrition* 79, 935–945.

Lee, M.M. and Lin, S.S. (2000) Dietary fat and breast cancer. *Annual Review of Nutrition* 20, 221–248.

Leitzmann, M.F., Stampfer, M.J., Michaud, D.S., Augustsson, K., Colditz, G.C., Willett, W.C. and Giovannucci, E.L. (2004) Dietary intake of n-3 and n-6 fatty acids and the risk of prostate cancer. *American Journal of Clinical Nutrition* 80, 204–216.

Lester, D.S. (1990) In vitro linoleic acid activation of protein kinase C. *Biochimica et Biophysica Acta* 1054, 297–303.

Lipworth, L., Martínez, M.E., Angell, J., Hsieh, C.-C. and Trichopoulos, D. (1997) Olive oil and human cancer: an assessment of the evidence. *Preventive Medicine* 26, 181–190.

Liu, Z., Uesaka, T., Watanabe, H. and Kato, N. (2001) High fat diet enhances colonic cell proliferation and carcinogenesis in rats by elevating serum leptin. *International Journal of Oncology* 19, 1009–1014.

Llor, X., Pons, E., Roca, A., Alvarez, M., Mane, J., Fernández-Bañares, F. and Gassull, M.A. (2003) The effects of fish oil, olive oil, oleic

acid and linoleic acid on colorectal neoplastic processes. *Clinical Nutrition* 22, 71–79.

Loeb, K.R. and Loeb, L.A. (2000) Significance of multiple mutations in cancer. *Carcinogenesis* 21, 379–385.

Lomeo, F., Khokher, M.A. and Dandona, P. (1986) Unsaturated fatty acids potentiate insulin action on adypocites. *Progress in Lipid Research* 25, 511–513.

London, R.S., Murphy, L. and Kitlowski, K.E. (1985) Hypothesis: breast cancer prevention by supplemental vitamin *Journal of the American College of Nutrition* 4, 559–564.

Lowe, S.W., Cepero, E. and Evan, G. (2004) Intrinsic tumour suppression. *Nature* 432, 307–315.

Lu, S., Zhang, X., Badawi, A.F., El-Sohemy, A. and Archer, M.C. (2002) Cyclooxygenase-2 inhibitor celecoxib inhibits promotion of mammary tumorigenesis in rats fed a high fat diet rich in n-6 polyunsaturated fatty acids. *Cancer Letters* 184, 7–12.

Ma, D.W.L., Seo, J., Davidson, L.A., Callaway, E.S., Fan, Y.Y., Lupton, J.R. and Chapkin, R.S. (2004a) n-3 PUFA alter caveolae lipid composition and resident protein localization in mouse colon. *The FASEB Journal* 18, 1040–1042.

Ma, D.W.L., Seo, J., Switzer, C., Fan, Y.Y., McMurray, D.N., Lupton, J.R. and Chapkin, R.S. (2004b) n-3 PUFA and membrane microdomains: a new frontier in bioactive lipid research. *Journal of Nutritional Biochemistry* 15, 700–706.

Mahan, L.K. and Escott-Stump, S. (2000) *Food, nutrition, and diet therapy*, 10th edn. W.B. Saunders Company, Philadelphia, 1194 pp.

Malins, D.C., Polissar, N.L. and Gunselman, S.J. (1996) Progression of human breast cancers to the metastatic state is linked to hydroxyl radical-induced DNA damage. *Proceedings of the National Academy of Sciences of the United States of America* 93, 2557–2563.

Mandard, S., Müller, M. and Kersten, S. (2004) Peroxisome proliferator-activated receptor α target genes. *Cellular and Molecular Life Sciences* 61, 393–416.

Marañón, G. (1929) Breve ensayo sobre la cocina Española. Prólogo. In: García del Real, E. (ed.) *Cocina Española y Cocina Dietética.* Imp. Sobrinos de la Sucesora de M. Minuesa de los Ríos, Madrid, pp. v–xxii.

Martin, M.E., Vranckx, R., Benassayag, C. and Nunez, E.A. (1986) Modifications of the properties of human sex steroid-binding protein by nonesterified fatty acids. *The Journal of Biological Chemistry* 261, 2954–2959.

Martin-Moreno, J.M. (2000) The role of olive oil in lowering cancer risk: Is this real gold or simply pinchbeck? *Journal of Epidemiology and Community Health* 54, 726–727.

Mataix, J., Mañas, M., Quiles, J.L., Battino, M., Cassinello, M., López-Frias, M. and Huertas, J.R. (1997) Coenzyme Q content depends upon oxidative stress and dietary fat unsaturation. *Molecular Aspects of Medicine* 18, S129–S135.

McConkey, D.J. (1998) Biochemical determinants of apoptosis and necrosis. *Toxicology Letters* 99, 157–168.

Meites, J. (1972) Relation of prolactin and estrogen to mammary tumorigenesis in the rat. *Journal of the National Cancer Institute* 48, 1217–1224.

Menendez, J.A., Vellon, L., Colomer, R. and Lupu, R. (2005) Oleic acid, the main monounsaturated fatty acid of olive oil, suppresses Her-2/neu (erb B-2) expression and synergistically enhances the growth inhibitory effects of trastuzumab (Herceptin™) in breast cancer cells with Her-2/neu oncogene amplification. *Annals of Oncology* 16, 359–371.

Merrill, A.H. and Schroeder, J.J. (1993) Lipid modulation of cell function. *Annual Review of Nutrition* 13, 539–559.

Michalik, L., Desvergne, B. and Wahli, W. (2004) Peroxisome-proliferator-activated receptors and cancers: complex stories. *Nature Reviews* 4, 61–70.

Miles, E.A., Zoubouli, P. and Calder, P.C. (2005) Differential anti-inflammatory effects of phenolic compounds from extra virgin olive oil identified in human whole blood cultures. *Nutrition* 21, 389–394.

Miller, S.J. (2004) Cellular and physiological effects of short-chain fatty acids. *Mini Reviews in Medicinal Chemistry* 4, 839–845.

Mizukami, Y., Nonomura, A., Noguchi, M., Taniya, T., Thomas, M.A., Nakamura, S. and Miyazaki, I. (1992) Effects of high and low dietary fat and indomethacin on tumour

growth, hormone receptor status and growth factor expression in DMBA-induced rat breast cancer. *International Journal of Tissue Reactions* 14, 269–276.

Moller, P. and Loft, S. (2004) Interventions with antioxidants and nutrients in relation to oxidative DNA damage and repair. *Mutation Research* 551, 79–89.

Monti, S.M., Ritieni, A., Sacchi, R., Skog, K., Borgen, E. and Fogliano, V. (2001) Characterization of phenolic compounds in virgin olive oil and their effect on the formation of carcinogenic/mutagenic heterocyclic amines in a model system. *Journal of Agricultural and Food Chemistry* 49, 3969–3975.

Moolgavkar, S.H. and Knudson, A.G. (1981) Mutation and cancer: a model for human carcinogenesis. *Journal of the National Cancer Institute* 66, 1037–1052.

Moral, R., Solanas, M., García, G., Colomer, R. and Escrich, E. (2003) Modulation of EGFR and neu expression by n-6 and n-9 high fat diets in experimental mammary adenocarcinomas. *Oncology Reports* 10, 1417–1424.

Moral, R., Solanas, M., Manzanares, E.M., Haro, D. and Escrich, E. (2004) Influence of DMBA-induced mammary cancer on the liver CPT I, mit HMG-CoA synthase and PPARα mRNA expression in rats fed low or high corn oil diet. *International Journal of Molecular Medicine* 14, 283–287.

Moreno, J.J. (2003) Effect of olive oil minor components on oxidative stress and arachidonic acid mobilization and metabolism by macrophaes Raw 264.7. *Free Radical Biology & Medicine* 35, 1073–1081.

Moreno, J.J., Carbonell, T., Sanchez, T., Miret, S. and Mitjavila, M.T. (2001) Olive oil decreases both oxidative stress and the production of arachidonic acid metabolites by the prostaglandin G/H synthase pathway in rat macrophages. *The Journal of Nutrition* 131, 2145–2149.

Moretti, R.M., Marelli, M.M., Sala, A., Motta, M. and Limonta, P. (2004) Activation of the orphan nuclear receptor RORalpha counteracts the proliferative effect of fatty acids on prostate cancer cells: crucial role of 5-lipoxygenase. *International Journal of Cancer* 112, 87–93.

Moya-Camarena, S.Y., Vandel Heuvel, J.P. and Belury, M.A. (1999) Conjugated linoleic acid activates peroxisome proliferator-activated receptor α and β subtypes but does not induce hepatic peroxisome proliferator in Sprague-Dawley rats. *Biochimica et Biophysica Acta* 1436, 331–342.

Mukherjee, P., Sotnikov, A.V., Mangian, H.J., Zhou, J.R., Visek, W.J. and Clinton, S.K. (1999) Energy intake and prostate tumor growth, angiogenesis, and vascular endothelial growth factor expression. *Journal of the National Cancer Institute* 91, 512–523.

Nakayama, M., Ran, J.U.H., Sugano, M., Hirose, N., Ueki, T., Doi, F. and Eynard, A.R. (1993) Effect of dietary fat and cholesterol on dimethylbenz(a)-anthracene-induced mammary tumorigenesis in Sprague-Dawley rats. *Anticancer Research* 13, 691–698.

Narayanan, B.A., Narayanan, N.K., Simi, B. and Reddy, B.S. (2003) Modulation of inducible nitric oxide synthase and related proinflammatory genes by the omega-3 fatty acid docosahexaenoic acid in human colon cancer cells. *Cancer Research* 63, 972–979.

Narod, S.A. and Foulkes, W.D. (2004) BRCA1 and BRCA2: 1994 and beyond. *Nature Reviews Cancer* 4, 665–676.

Neill, J.D. (1970) Effect of 'stress' on serum prolactin and luteinizing hormone levels during the estrous cycle of the rat. *Endocrinology* 87, 1192–1197.

Nemoto, N. (1986) Marked activation of benzo(a)pyrene to protein-binding forms in the presence of unsaturated fatty acids and heme-compounds. *Carcinogenesis* 7, 267–271.

Newmark, H.L. (1999) Squalene, olive oil, and cancer risk. Review and hypothesis. *Annals of the New York Academy of Sciences* 889, 193–203.

Niggli, V. and Burger, M.M. (1987) Interaction of the cytoskeleton with the plasma membrane. *The Journal of Membrane Biology* 100, 97–121.

Nowell, P.C. (2002) Tumour progression: a brief historical perspective. *Seminars in Cancer Biology* 12, 261–266.

O'Carroll, P. (1999) The shape of functional foods. *The World of Ingredients*, 54–57. *https://www77.ssldomain.com/twoi/results_articles.asp?type=Title*

Ochoa-Herrera, J.J., Huertas, J.R., Quiles, J.L. and Mataix, J. (2001) Dietary oils high in oleic acid, but with different non-glyceride contents, have different effects on lipid profiles and peroxidation in rabbit hepatic mitochondria. *The Journal of Nutritional Biochemistry* 12, 357–364.

Oommen, A.M., Griffin, J.B., Sarath, G. and Zempleni, J. (2005) Roles for nutrients in epigenetic events. *The Journal of Nutritional Biochemistry* 16, 74–77.

Owen, R.W., Giacosa, A., Hull, W.E., Haubner, R., Wurtele, G., Spiegelhalder, B. and Bartsch, H. (2000) Olive-oil consumption and health: the possible role of antioxidants. *Lancet Oncology* 1, 107–112.

Owen, R.W., Haubner, R., Wurtele, G., Hull, E., Spiegelhalder, B. and Bartsch, H. (2004) Olives and olive oil in cancer prevention. *European Journal of Cancer Prevention* 13, 319–326.

Pansini, F., Bonaccorsi, G., Genovesi, F., Folegatti, M.R., Bagni, B., Bergamini, C.M. and Mollica, G. (1990) Influence of estrogens on serum free fatty acid levels in women. *The Journal of Clinical Endocrinology and Metabolism* 71, 1387–1389.

Pariza, M.W. (1987) Fat calories, and mammary carcinogenesis: net energy effects. *The American Journal of Clinical Nutrition* 45, 261–263.

Parkin, D.M., Whelan, S.L., Ferlay, J., Teppo, L. and Thomas, D.B. (2002) *Cancer incidence in five continents*, Vol. VIII. IARC Scientific Publication no. 155. Lyon, 781 pp.

Parton, R.G. and Hancock, J.F. (2004) Lipid rafts and plasma membrane microorganization: insights from Ras. *TRENDS in Cell Biology* 14, 141–147.

Pitot, H. (1993) The molecular biology of carcinogenesis. *Cancer* 72 (suppl.), 962–970.

Poirier, L.A. (1987) Stages in carcinogenesis: alteration by diet. *The American Journal of Clinical Nutrition* 45, 185.

Pollard, M. and Luckert, P.H. (1986) Promotional effects of testosterone and high fat diet on the development of autochthonous prostate cancer in rats. *Cancer Letters* 32, 223–227.

Ponder, B.A. (2001) Cancer genetics. *Nature* 411, 336–341.

Prior, I.A. and Hancock, J.F. (2001) Compartmentalization of Ras proteins. *Journal of Cell Science* 114, 1603–1608.

Rao, C., Newmark, H. and Reddy, B. (1998) Chemopreventive effect of squalene on colon cancer. *Carcinogenesis* 2, 287–290.

Rao, C.V., Hirose, Y., Indranie, C. and Reddy, B.S. (2001) Modulation of experimental colon tumorigenesis by types and amounts of dietary fatty acids. *Cancer Research* 61, 1927–1933.

Reddy, B.S. (1992) Dietary fat and colon cancer: animal model studies. *Lipids* 27, 807–813.

Reddy, B.S. and Maeura, Y. (1984) Tumor promotion by dietary fat in azoxymethane-induced colon carcinogenesis in female F344 rats: influence of amount and source of dietary fat. *Journal of the National Cancer Institute* 72, 745–750.

Reyes, N., Iatropoulos, M., Mittelman, A. and Geliebter, J. (2002) Microarray analysis of diet-induced alterations in gene expression in the ACI rat prostate. *European Journal of Cancer Prevention* 11 (suppl. 2), S37–S42.

Reyes, N., Reyes, I., Tiwari, R. and Geliebter, J. (2004) Effect on linoleic acid on proliferation and gene expression in the breast cancer cell line T47D. *Cancer Letters* 209, 25–35.

Rock, C.L. (2003) Diet and breast cancer: Can dietary factors influence survival? *Journal of Mammary Gland Biology and Neoplasia* 8, 119–132.

Rogers, A.E. and Longnecker, M.P. (1988) Biology of disease. Dietary and nutritional influences on cancer: a review of epidemiologic and experimental data. *Laboratory Investigation* 59, 729–759.

Ronai, Z., Lau, Y. and Cohen, L.A. (1991) Dietary n-3 fatty acids do not affect induction of Ha-ras mutations in mammary glands of NMU-treated rats. *Molecular Carcinogenesis* 4, 120–128.

Ronai, Z., Tillotson, J. and Cohen, L. (1995) Effect of dietary fatty acids on gene expression in breast cells. *Advances in Experimental Medicine and Biology* 375, 85–95.

Rose, D.P. (1997a) Dietary fatty acids and cancer. *The American Journal of Clinical Nutrition* 66 (suppl. 4), 998S–1003S.

Rose, D.P. (1997b) Effects of dietary fatty acids on breast and prostate cancers: evidence

from in vitro experiments and animal studies. *The American Journal of Clinical Nutrition* 66(6 suppl.), 1513S–1522S.

Rose, D.P. and Connolly, J.M. (1990) Effects of fatty acids and inhibitors of eicosanoid synthesis on the growth of a human breast cancer cell line in culture. *Cancer Research* 50, 7139–7144.

Rose, D.P. and Connolly, J.M. (1992) Dietary fat, fatty acids and prostate cancer. *Lipids* 27, 798–803.

Rose, D.P. and Connolly, J.M. (1997) Dietary fat and breast cancer metastasis by human tumor xenografts. *Breast Cancer Research and Treatment* 46, 225–237.

Rose, D.P., Hatala, M.A., Connolly, J.M. and Rayburn, J. (1993) Effect of diets containing different levels of linoleic acid on human breast cancer growth and lung metastasis in nude mice. *Cancer Research* 53, 4686–4690.

Rous, P. and Kidd, J.G. (1941) Conditional neoplasms and subthreshold neoplastic states. *The Journal of Experimental Medicine* 73, 365.

Roynette, C.E., Calder, P.C., Dupertuis, Y.M. and Pichard, C. (2004) n-3 polyunsaturated fatty acids and colon cancer prevention. *Clinical Nutrition* 23, 139–151.

Ruiz de Villa, M.C., Cabral, M.S.E., Escrich, E. and Solanas, M. (1999) A non-parametric regression approach to repeated measures analysis in cancer experiments. *Journal of Applied Statistics* 26, 601–611.

Samlaska, C. (1978) Linoleic acid inhibition of lymphocytotoxicity. *Federation Proceedings* 37, 1273.

Schi, C.Y., Chua, S., Ong, C.N. and Lee, H.P. (1994) Dietary selenium inhibits DNA binding of aflatoxin B^1 in rats. *Proceedings of the American Association for Cancer Research* 34, 133.

Schwartz, B., Birk, Y., Raz, A. and Madar, Z. (2004) Nutritional-pharmacological combinations – a novel approach to reducing colon cancer incidence. *European Journal of Nutrition* 43, 221–229.

Sekimizu, K. (1994) Interactions between DNA replication-related proteins and phospholipid vesicles in vitro. *Chemistry and Physics of Lipids* 73, 223–230.

Sherr, C.J. (2004) Principles of tumor suppression. *Cell* 116, 235–246.

Shirai, T., Asamoto, M., Takahashi, S. and Imaida, K. (2002) Diet and prostate cancer. *Toxicology* 181–182, 89–94.

Simons, K. and Toomre, D. (2000) Lipid rafts and signal transduction. *Nature reviews. Molecular Cell Biology* 1, 31–39.

Singh, J., Hamid, R. and Reddy, B.S. (1997) Dietary fat and colon cancer: modulation of cyclooxygenase-2 by types and amount of dietary fat during the postinitiation stage of colon carcinogenesis. *Cancer Research* 57, 3465–3470.

Smith, T., Yang, G., Seril, D., Liao, J. and Kim, S. (1999) Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis by dietary olive oil and squalene. *Carcinogenesis* 4, 703–706.

Snyderwine, E.G. and Davis, C.D. (1998) Proliferation, development and DNA adduct levels in the mammary gland of rats given 2-amino-1-methyl-phenylimidazo [4,5-b] pyridine and a high fat diet. *Carcinogenesis* 19, 1209–1215.

Solanas, M., Moral, R., Colomer, R. and Escrich, E. (2000) Effects of dietary (n-6) polyunsaturated lipids on experimental mammary carcinogenesis. *Journal of Women's Cancer* 2, 67–72.

Solanas, M., Moral, R. and Escrich, E. (2001) The stimulating effect of a high-fat n-6 polyunsaturated diet on rat DMBA-induced mammary tumors is not related to changes in c-*Ha-ras1* mRNA tumor expression. *Nutrition Research* 21, 1261–1273.

Solanas, M., Escrich, E., Rouzaut, A., Costa, I., Martínez, A. and Notario, V. (2002a) Deregulated Expression of the PCPH Proto-Oncogene in Rat Mammary Tumors Induced with 7,12-Dimethylbenz[a]antracene. *Molecular Carcinogenesis* 33, 219–227.

Solanas, M., Hurtado, A., Costa, I., Moral, R., Menendez, J.A., Colomer, R. and Escrich, E. (2002b) Effects of high olive oil diet on the clinical behavior and histopathological features of rat DMBA-induced mammary tumors compared with a high corn oil diet. *International Journal of Oncology* 21, 745–753.

Solomonson, L.P., Liepkalns, V.A. and Spector, A.A. (1976) Changes in (Na^+K^+)-ATPase activity of Ehrlich Ascites tumor cells produced by alteration of membrane fatty acid composition. *Biochemistry* 15, 892–897.

Spector, A.A. and Burns, C.P. (1987) Biological and therapeutic potential of membrane lipid modification in tumors. *Cancer Research* 47, 4529–4537.

Stark, A.H. and Madar, Z. (2002) Olive oil as a functional food: epidemiology and nutritional approaches. *Nutrition Reviews* 60, 170–176.

Steinmetz, K. and Potter, J.D. (1991) A review of vegetables, fruit, and cancer II: Mechanisms. *Cancer Causes and Control* 2, 427–442.

Steller, H. (1995) Mechanisms and genes of cellular suicide. *Science* 267, 1445–1449.

Sternberg, P.W. and Schmid, S.L. (1999) Caveolin, cholesterol and Ras signalling. *Nature cell Biology* 1, E35–E37.

Stevens, J.F. and Page, J.E. (2004) Xanthohumol and related prenylflavonoids from hops and beer: to your good health! *Phytochemistry* 65, 1317–1330.

Stoll, B.A. (2002) N-3 fatty acids and lipid peroxidation in breast cancer inhibition. *British Journal of Nutrition* 87, 193–198.

Stoneham, M., Goldacre, M., Seagroatt, V. and Gill, L. (2000) Olive oil, diet and colorectal cancer: an ecological study and a hypothesis. *Journal of Epidemiology and Community Health* 54, 756–760.

Stulnig, T.M. (2003) Immunomodulation by polyunsaturated fatty acids: mechanisms and effects. *International Archives of Allergy and Applied Immunology* 132, 310–321.

Sumida, C., Graber, R. and Nunez, E. (1993) Role of fatty acids in signal transduction: modulators and messengers. *Prostaglandins, Leukotrienes, and Essential Fatty Acids* 48, 117–122.

Sylvester, P.W., Rusell, M., Ip, M. and Ip, C. (1986) Comparative effects of different animal and vegetable fats fed before and during carcinogen administration on mammary tumorigenesis, sexual maturation, and endocrine function in rats. *Cancer Research* 46, 757–762.

Takeshita, M., Ueda, H., Shirabe, K., Higuchi, Y. and Yoshida, S. (1997) Lack of promotion of carcinogenesis by high-oleic safflower oil. *Cancer* 8, 1487–1493.

Tannenbaum, A. (1942) The genesis and growth of tumors. III Effects of a high fat diet. *Cancer Research* 2, 468–475.

Thompson, H., Zhu, Z., Banni, S., Darcy, K., Loftus, T. and Ip, C. (1997) Morphological and biochemical status of the mammary gland as influenced by conjugated limoleic acid: implications for a reduction in mammary cancer risk. *Cancer Research* 57, 5067–5072.

Tillotson, J.K., Darzynkiewicz, Z., Cohen, L.A. and Ronai, Z. (1993) Effects of linoleic acid on mammary tumor cell proliferation are associated with changes in p53 protein expression. *International Journal of Oncology* 3, 81–87.

Tiwari, R.K., Mukhopadhyay, B., Telang, N.T. and Osborne, M.P. (1991) Modulation of gene expression by selected fatty acids in human breast cancer cells. *Anticancer Research* 11, 1383–1388.

Tomasoni, M.L., Amori, D. and Magni, M.V. (1999) Changes of nuclear membrane lipid composition affect RNA nucleocytoplasmic transport. *Biochemical and Biophysical Research Communications* 258, 476–481.

Tsujimoto, Y. and Shimizu, S. (2000) Bcl-2 family: life-or-death switch. *FEBS Letters* 466, 6–10.

Uchida, N., Okamura, S.I., Nagamaghi, Y. and Yamashita, S. (1997) Increased phospholipase D activity in human breast cancer. *Journal of Cancer Research and Clinical Oncology* 123, 280–285.

Utermohlen, V. and Tucker, M.A.M. (1986) Possible effects of dietary n-6 series polyunsaturated fatty acids on the development of immune dysfunction and infection. *The Proceedings of the Nutrition Society* 45, 327–331.

Vacaresse, N., Lajoie-Mazenc, I., Auge, N., Suc, I., Frisach, M.F., Salvayre, R. and Negre-Salvayre, A. (1999) Activation of epithelial growth factor receptor pathway by unsaturated fatty acids. *Circulation Research* 85, 892–899.

Venkateswaran, V., Fleshner, N.E., Sugar, L.M. and Klotz, L.H. (2004) Antioxidants block prostate cancer in *Lady* transgenic mice. *Cancer Research* 64, 5891–5896.

Visioli, F. and Galli, C. (1998) Olive oil phenols and their potential effects on human health. *Journal of Agricultural and Food Chemistry* 46, 4292–4296.

Visioli, F., Bellosta, S. and Galli, C. (1998) Oleuropein, the bitter principle of olives, enhances nitric oxide production by mouse macrophages. *Life Sciences* 62, 541–546.

Visioli, F., Grande, S., Bogani, P. and Galli, C. (2004) The role of antioxidants in the Mediterranean diets: focus on cancer. *European Journal of Cancer Prevention* 13, 337–343.

Vitale, J.J. and Broitman, S.A. (1981) Lipids and immune function. *Cancer Research* 41, 3706–3710.

Volgestein, B. and Kinzler, K.W. (2002) *The Genetic Basis of Human Cancer.* 2nd edn. McGraw Hill, New York, 821 pp.

Wagner, D.A., Naylor, P.H., Kim, U., Shea, W., Ip, C. and Ip, M.M. (1982) Interaction of dietary fat and the thymus in the induction of mammary tumors by 7,12-dimethylbenz(a)anthracene. *Cancer Research* 42, 1266–1273.

Wainfan, E. and Poirier, L.A. (1992) Methyl groups in carcinogenesis: effects on DNAmethylation and gene expression. *Cancer Research* 52 (suppl.), 2071s–2077s.

Watson, W.H., Cai, J. and Jones, D.P. (2000) Diet and apoptosis. *Annual Review of Nutrition* 20, 485–505.

Weber, P.C. (1990) n-3 fatty acids and human disease. *Scandinavian Journal of Clinical and Laboratory Investigation* 50, 14–19.

Weinberg, R.A. (1994) Molecular mechanisms of carcinogenesis. In: Leder, P., Clayton, D.A. and Rubenstein, E. (eds) *Introduction to Molecular Medicine*. Scientific American Inc, New York, pp. 235–275.

Welsch, C. (1987) Enhancement of mammary tumorigenesis by dietary fat: review of potential mechanisms. *The American Journal of Clinical Nutrition* 45, 192–202.

Welsch, C.W. (1992) Relationship between dietary fat and experimental mammary tumorigenesis: a review and critique. *Cancer Research* 52 (suppl.), 2040S–2048S.

Welsch, C.W. and Aylsworth, C.F. (1983) Enhancement of murine mammary tumorigenesis by feeding high levels of dietary fat: a hormonal mechanism? *Journal of the National Cancer Institute* 70, 215– 221.

Wetsel, W.C., Rutledge, A. and Rogers, A.E. (1983) Dietary corn oil does not alter plasma prolactin in rats. *Federation Proceedings* 42, 526.

Willett, W.C. (1995) Diet, nutrition and avoidable cancer. *Environmental Health Perspectives* 103 (suppl. 8), 165–170.

Willett, W.C. (1997) Specific fatty acids and risks of breast and prostate cancer: dietary intake. *American Journal of Clinical Nutrition* 66 (6 suppl.), 1557S–1563S.

Willett, W.C. (1999) Dietary fat and breast cancer. *Toxicological Sciences* 52, S127–S146.

Willet, W.C. (2001a) Diet and cancer: One view at the start of the millennium. *Cancer Epidemiology, Biomarkers and Prevention* 10, 3–8.

Willet, W.C. (2001b) Diet and breast cancer. *Journal of Internal Medicine* 249, 395–411.

Wogan, G.N., Hecht, S.S., Felton, J.S., Conney, A.H. and Loeb, L.A. (2004) Environmental and chemical carcinogenesis. *Seminars in Cancer Biology* 14, 473–486.

Wood, J.N. (1990) Essential fatty acids and their metabolites in signal transduction. *Biochemical Society Transactions* 18, 785–786.

World Cancer Research Fund and American Institute for Cancer Research (1997) *Food, Nutrition and the Prevention of Cancer: a Global Perspective.* American Institute for Cancer Research (eds), Washington, 670 pp.

Woutersen, R.A., Appel, M.J., Garderen-Hoetmer, A. and Wijnands, M. (1999) Dietary fat and carcinogenesis. *Mutation Research* 443, 111–127.

Wu, B., Iwakiri, R., Ootani, A., Tsunada, S., Fujise, T., Sakata, Y., Sakata, H., Toda, S. and Fujimoto, K. (2004) Dietary corn oil promotes colon cancer by inhibiting mitochondria-dependent apoptosis in azoxymethane-treated rats. *Experimental Biology and Medicine* 229, 1017–1025.

Wynder, E.L. and Hill, P. (1977) Prolactin, oestrogen, and lipids in breast fluid. *Lancet* 2, 840–842.

Wynder, E.L., Cohen, L.A., Muscat, J.E., Winters, B., Dwyer, J.T. and Blackburn, G. (1997) Breast cancer: weighing the evidence for a promoting role of dietary fat. *Journal of the National Cancer Institute* 11, 766–775.

Yamaki, T., Yano, T., Satoh, H., Endo, T., Matsuyama, C., Kumagai, H., Miyahara, M., Sakurai, H., Pokorny, J., Shin, S.J. and Hagiwara, K. (2002) High oleic acid oil suppresses lung tumorigenesis in mice through the modulation of extracellular signal-regulated kinase cascade. *Lipids* 37, 783–788.

Yaqoob, P., Knapper, J.A., Webb, D.H., Williams, C.M., Newsholme, E.A. and Calder, P.C. (1998) *American Journal of Clinical Nutrition* 67, 129–135.

Yarnold, J.R. (1996) What are cancer genes and how do they upset cell behaviour? In: Yarnold, J.R., Stratton, M. and McMillan, T.J. (eds) *Molecular Biology for Oncologists*. Chapman & Hall, London, pp. 3–15.

Zhou, J.R. and Blackburn, G.L. (1997) Bridging animal and human studies: what are the missing segments in dietary fat and prostate cancer? *American Journal of Clinical Nutrition* 66(6 suppl.), 1572S–1580S.

Zusman, I., Gurevich, P., Madar, Z., Nyska, A., Korol, D., Timar, B. and Zuckerman, A. (1997) Tumor-promoting and tumor-protective effects of high-fat diets on chemically induced mammary cancer in rats. *Anticancer Research* 17, 349–356.

Index