Blood transport and genomic effects of olive oil components

By **Yolanda M. Pacheco, Beatriz Bermúdez, Sergio López, Rocío Abia**
and **Francisco J.G. Muriana***

Cellular and Molecular Nutrition, Instituto de la Grasa,
Consejo Superior de Investigaciones Científicas, Seville, Spain

*Corresponding author: Dr. Francisco J.G. Muriana. Tel: +34 954611550
Email: muriana@ig.csic.es

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RESUMEN

Transporte en sangre y efectos genómicos de los componentes del aceite de oliva.

Estudios epidemiológicos asocian el consumo de una dieta rica en aceite de oliva (virgen extra) con una baja incidencia de enfermedades crónicas, como las enfermedades cardiovasculares y el cáncer. Una concentración elevada de triglicéridos en plasma, tanto en ayunas como durante el metabolismo postprandial, está implicada en la patogénesis cardiovascular. Se ha demostrado que el aceite de oliva contribuye a modular los procesos metabólicos relativos a la secreción y al transporte de triglicéridos. Con respecto a otros aceites comestibles, el aceite de oliva genera lipoproteínas intestinales ricas en triglicéridos que se metabolizan con rapidez. En este contexto, se produce un aflujo masivo de lipoproteínas nacientes y remanentes, así como de metabolitos lipídicos y componentes liposolubles, que interaccionan con células hepáticas y de tejidos extra-hepáticos. La respuesta celular a la dieta implica multitud de productos génicos, incluyendo proteínas asociadas a la síntesis de lípidos, oxidación y diferenciación celular. Se conoce el efecto beneficioso de los ácidos grasos y otros componentes menores en la regulación transcripcional de una gran variedad de genes. En esta revisión se reafirma la importancia de una dieta rica en aceite de oliva virgen extra en la prevención de las enfermedades cardiovasculares y otras.

PALABRAS-CLAVE: Aceite de oliva; Ácidos grasos monoinsaturados; Compuestos menores; Secreción intestinal; Transporte de lipoproteínas; Respuesta celular; Genes; Dieta Mediterránea.

SUMMARY

Blood transport and genomic effects of olive oil components.

Epidemiological studies suggest that consuming diets rich in (extra virgin) olive oil is associated with a low incidence of chronic diseases, including cardiovascular disease and cancer. Recent evidence has emerged which implicates raised concentrations of plasma triglycerides in the pathogenesis of coronary artery disease (CAD). It has been demonstrated that olive oil contributes to modulate metabolic processes related to secretion and transport of triglycerides. Intestinal triglyceride-rich lipoproteins from olive oil are very efficiently cleared during postprandial metabolism compared to other oils. Then, there is a massive interaction of nascent and remnant triglyceride-rich lipoproteins, as well as lipid metabolites and fat-soluble components, with hepatic and non-hepatic tissues. A diet-related response involves a multitude of gene products, including proteins implicated in lipid synthesis, oxidation and cell differentiation. Particularly, it has been reported a health beneficial effect of several components from (extra virgin) olive oil (fatty acids and minor compounds), which are functioning as regulators of gene transcription. This review reaffirm that a diet rich in extra virgin olive oil is of vital importance in the prevention of cardiovascular and other diseases.

KEY-WORDS: Olive oil; Monounsaturated fatty acids; Minor compounds; Intestinal secretion; Lipoproteins transport; Cellular response; Genes; Mediterranean diet.

1. INTRODUCTION

Recent evidence has emerged which implicates raised concentrations of plasma triglycerides in the pathogenesis of coronary artery disease (CAD). Exaggerated triglyceride responses to standard fat-containing meals have been shown in subjects with CAD and in offspring with a parental history of premature CAD. The lower rate of CVD in Southern Europe suggests a potential role of the traditional Mediterranean diet in the prevention of CVD. Zampelas *et al.* (1998) have previously shown no

difference in postprandial triglyceride response to meals of varying monounsaturated (MUFA) and saturated (SFA) fatty acid content in Northern European men. Later studies they set out to determine whether the same response was observed in Southern European men whose habitual background diet was high in MUFA. Both populations showed similar lipemic responses when meals of varying MUFA and SFA content were fed, but they unexpectedly observed very different postprandial patterns of triglyceride response in the two European populations.

The Mediterranean diet has become a cultural model for dietary improvement. It is characterised by the use of olive oil as the major culinary fat, which is rich in MUFA. In addition, extra virgin olive oil contains a number of phenolic compounds, which via their antioxidant and other potent biological activities could partially account for the observed healthful effects of the traditional Mediterranean Diet.

Traditional Mediterranean Diet is constituted, mainly, plenty of fresh vegetables, fruit, grains and (extra virgin) olive oil with moderate consumption of wine (mainly red wine) and dairy products (mainly cheese). However, there is considerable debate about the nutrients responsible for health effects of a diet. The low SFA/high MUFA content has received considerable attention. The epidemiological evidence of a lower incidence of CHD in the Mediterranean area also led to the hypothesis of a protective effect of some (extra virgin) olive oil phenolics with respect to chemically induced oxidation of human plasma low-density lipoproteins (LDL), one of steps in the initiation of atherosclerosis (Steinberg *et al.,* 1989). In both cases, fatty acids (as triglycerides, whose fatty acid composition partially depends on the olive oil fatty acid composition) and minor compounds (in free or conjugated forms) have to be transported in blood and to reach hepatic and extra-hepatic systems.

2. SECRETION OF LIPOPROTEINS BY THE ENTEROCYTE

The function of chylomicron (CM) and very-low density lipoproteins (VLDL) is to pack the absorbed dietary lipids and fat-soluble components, stabilising

Table 1
Characteristics of intestinal lipoproteins: chylomicrons and very-low density lipoproteins (VLDL) (adapted from Mu and Hoy, 2004)

	Chylomicrons	VLDL
Sources	Intestine only	Both intestine and liver
Density	<0.95 g/mL	0.95-1.006 g/mL
Sdverdberg floation (Sf)	>400	20-400
Size	80-500 nm	30-80 nm
Approximate weight (KD)	50-1000x10^3	10-80x10^3
Total lipids	97-98% of particle mass	90% of particle mass
Triglycerides	≈95%	≈40%
Cholesterol	≈1%	≈15%
Phospholipids	≈4%	≈15%
Total protein	2% of particle mass	10% of particle mass
Major apolipoprotein components	B-48, A-I, A-IV, A-II	Probably similar to chylomicrons (B-100 instead B-48)
Minor apolipoprotein components	C and E (both acquired through interaction with other plasma lipoproteins)	Same as chylomicrons

them for transport in the aqueous plasma environment. Although plasma VLDL are mainly synthesized in the liver, there is incoming evidence that fasting intestine also produces these particles, which do not mix readily in individual Golgi vesicles (Mu and Hoy, 2004). Characteristics of these lipoproteins are detailed in Table 1.

Briefly, triglyceride-rich lipoproteins are spherical particles synthesised by intestinal epithelial cells (Luchoomun and Hussain, 1999), being CM higher (100 nm) than VLDL (25-80 nm). Another classification attends to the Svedberg flotation (S_f) rate, which defines CM as those lipoproteins that exceed 400 S_f and VLDL as those with S_f rate of 20-400. The major lipid components are triglycerides, cholesterol esters, free cholesterol and phospholipids. Attending to their distribution in the CM particle, amphipathic molecules form a monolayer that stabilises the lipid core that also contains dietary fat-soluble components. VLDL differ from CM in their density, size, lipid and protein content and composition.

Studies by Luchoomun and Hussain (1999) have shown that in the distribution of lipids in the intestine-assembled lipoproteins, triglyceride-rich lipoproteins contain higher amounts of preformed phospholipids compared with nascent phospholipids. However, VLDL contain similar amounts of preformed and nascent triglycerides, whereas CM rather contain higher amounts of nascent triglycerides. This is why the fatty acid composition of triglycerides, but not of phospholipids, reflects the composition of dietary triglycerides in CM.

Apolipoproteins constitute the protein fraction in lipoproteins and play a major role in their metabolism. Triglyceride-rich lipoproteins contain several different apolipoproteins, including apo B-48, apo B-100, apo AI, apo AIV, apo AII, apo C and apo E (the two last ones are acquired later) (Table 1). There are differences in the relative content of apolipoproteins between CM and intestinal VLDL. For instance, apo A-IV comprises about 40% of intestinal VLDL, but only 10% of apo A-IV is present in CM. Human enterocytes have the potential to secrete both apo B-48 and apo B-100, which differ in apoB mRNA post-transcriptional processes. Two approaches have been described to explain the production of apoB and apoB-containing lipoproteins. The first one involves the introduction of a unique RNA editing enzyme, the apoB mRNA editing enzyme catalytic polypeptide-1 (apobec-1) that gives a stop codon at codon 2153, resulting in synthesis of a protein which is 48% of the apo B-100 molecule. The second one relates the assembly and intracellular sorting of apoB-containing lipoproteins and the inhibition of lipid addition to apo B-100 (Innerarity *et al.*, 1996). Apolipoproteins B have also a critical role in the assembly of triglyceride-rich lipoproteins, because

apo B-48 and apo B-100 bind differently to LDL receptor (van Greevenbroek and de Bruin, 1998a). A general belief is that apo B-100 is exclusive of VLDL, as liver VLDL can only content apo B-100, but the truth is that large CM can contain both apo B-48 and apo B-100 (Luchoomun and Hussain, 1999).

It has been described that pathways for triglyceride synthesis influence the amount of apo B in the secreted triglyceride-rich lipoproteins (Mu and Hoy, 2004). The "triglyceride synthetase" pathway (predominant in the feeding state) produces larger particles with lesser apolipoprotein content, whereas the phosphatidic acid pathway (predominant in the fasting state) produces smaller particles with twice as much apolipoprotein per gram of fat than the former pathway.

3. INFLUENCE OF OLIVE OIL ON POSTPRANDIAL LIPOPROTEINS TRANSPORT

Intestinal chylomicrons (CM) and VLDL transport dietary fat but also triglycerides from endogenous sources (Shiau *et al.*, 1985). Endogenous triglycerides come in part from circulating fatty acids and remnant CM taken up by enterocytes, and do not readily enter in the CM secretory pathway. That is why the fatty acid composition of CM triglycerides closely reflects that of dietary triglycerides (Mu and Hoy, 2004).

The composition and structures of dietary triglycerides determine intestinal absorption and lipoprotein secretion (Aoe *et al.*, 1997). After stomach infusion of 1,3-dioleoyl-2-palmitoyl glycerol (OPO) and 1,2-dioleoyl-3-palmitoyl glycerol (OOP), the mean diameter of CM containing OPO are larger than those carrying OOP. OPO is better absorbed and transported than OOP. Other parameters related to composition of dietary triglycerides are the size and number of postprandial lipoproteins. The unsaturation degree of fatty acids tends to increase the size of CM (Sakr *et al.*, 1997). Even, the configuration of double bonds can condition the size of postprandial lipoproteins (van Greevenbroek *et al.*, 1998b).

Particularly, olive oil increases the number of CM compared with other oils, including saturated, *n*-6 and *n*-3 polyunsaturated-enriched oils (Jackson *et al.*, 2002). Possible mechanisms could imply the modulation in the enterocytes of the activity or expression of microsomal triglyceride transfer protein (MTP), or mRNA editing enzyme that involves the co-translational enrichment in lipids of apo B and further lipidation of the primordial particle along the secretory pathway (van Greevenbroek *et al.*, 1998c). Therefore, olive oil could be enabling the stabilization of the nascent CM particles with smaller lipid droplets by affecting the enzymes involved in the lipoprotein assembly. Indeed, unsaturated fatty acids, especially oleic acid, are more efficiently incorporated into lipoproteins by MTP (van Greevenbroek *et al.*, 1996).

The size and number of CM influence their fate and rate of clearance. Large CM are preferentially hydrolyzed by lipoprotein lipase (LPL), which produces remaining cholesterol ester-rich particles (remnant CM) after the removal of 70-90% of triglycerides. Remnant particles interact with hepatic lipase (HL) that further hydrolyses triglycerides and phospholipids, preparing remnant to be up taken by liver receptor-mediated process (Sultan *et al.*, 1990). It has been suggested that CM from olive oil are poorly hydrolysed by LPL due to the greater particle number, but rapidly removed by HL (Jackson *et al.*, 2002). Certainly, olive oil contributes to modulate metabolic processes related to secretion and transport of triglycerides.

4. INFLUENCE OF OLIVE OIL ON POSTPRANDIAL LIPOPROTEINS CLEARANCE

Endothelial cells by the heparan sulphate proteoglycan-dependent LPL and the endothelial lipase (EL) initially participate in the clearance of postprandial triglyceride-rich lipoproteins. LPL (with mainly acylglycerol hydrolase activity) and EL (with mainly phospholipase activity) co-ordinately remodel the surface of CM. This step is essential for accessing the CM triglyceride core. The largest CM have the highest affinity for LPL (Xiang *et al.*, 1999). The action of the LPL produces free fatty acids (FFA) and monoglycerides (MG), turning CM into remnant lipoproteins. Moreover, the amount of soluble LPL is proportional to the amount of triglycerides in plasma (Heeren *et al.*, 2002). It is interesting to note that CM with PUFA have faster lipolysis than CM with SFA (Chung *et al.*, 1998). The activity of pre-heparin LPL is sensitive to dietary fatty acids, enhancing in the presence of *n*-3 PUFA (Park *et al.*, 2003). In addition, EL bound to postprandial lipoproteins facilitates the hepatic clearance independently of its catalytic function (Heeren *et al.*, 2002).

Olive oil increases the amount of postprandially nascent and remnant triglyceride-rich lipoproteins compared with other fats, including safflower oil and milk fat (Higashi *et al.*, 1997). Similar findings have been described by Mekki *et al.* (2002), who found that a meal rich in butter has lower postprandial lipemic response than sunflower and olive oils. The time to reach the triglyceride postprandial peak is also higher with olive oil (Jackson *et al.*, 2002). CM-triglycerides from olive oil are very efficiently cleared during postprandial metabolism compared to other MUFA-containing oils, which depends on the distribution of fatty acids into triglyceride and phospholipids molecules (Abia *et al.*, 2003). Oleic acid (18:1*n*-9) is the main fatty acid found in the phospholipids of postprandial triglyceride-rich lipoproteins after a meal rich in olive oil, whereas stearic (18:0) and linoleic (18:2*n*-6) acids are those found with a meal rich in high-oleic sunflower oil and

exhibit higher time of clearance. This metabolic process is also regulated by the nature of carried triglycerides, being more quickly removed OOO (triolein) followed by POO (palmitoyl-dioleoyl glycerol) and OOL (dioleoyl-linoleoyl glycerol) (Abia *et al.*, 1999).

5. ROLE OF APOLIPOPROTEINS AND RECEPTORS ON MECHANISMS INVOLVED IN POSTPRANDIAL LIPOPROTEINS CLEARANCE

The mechanisms related to postprandial lipoproteins clearance are not well understood nowadays. The nascent CM are loaded with apo B-48. The nature of dietary fats modulates the expression of apo B-48. By using olive oil in a human model of postprandial metabolism, Abia *et al.* (2003) suggested that CM-phospholipids might interact with distinct cellular receptors to facilitate their incorporation. This agrees with the discovery of a novel and a uniquely receptor for apo B-48 in the human monocyte/macrophage line THP-1 and endothelial cells (Gianturco *et al.*, 1999; Brown *et al.*, 2000). It is of major importance for the knowledge of atherosclerosis development because macrophages loaded with triglyceride-rich lipoproteins, as oxidised-LDL, can turn on foam cells in the arterial intima (Gianturco *et al.*, 1999). Apo B-48 receptor is a non-heparan sulphate-dependent dimmer (200 and 235 KDa) (Bradley *et al.*, 1999). When heterologous expression of apo B-48 receptor is promoted in CHO cells, they become apparent as foam cells after incubation with triglyceride-rich lipoproteins (Brown *et al.*, 2000; Brown *et al.*, 2002). Unfortunately, we have no data on the effects of olive oil in the expression and activity of the receptor.

Apolipoproteins E are also involved in the clearance of lipoproteins. There are three main polymorphisms of apo E: E2, E3 and E4. E3 allele is present in 77% of the population and previous studies shown that correlates with plasma lipid profile. Over-expression of apo E3 in transgenic animals increases the level in plasma of total cholesterol and triglycerides. It appears that apo E3 leads to normolipemia, hypercholesterolemia or combined hyperlipidemia (hypercholesterolemia and hypertriglyceridemia) when expressed at low, medium or high levels, respectively (Tomkin *et al.*, 2001). E4 allele is associated with high levels in plasma of LDL-cholesterol, whereas E2 allele prevents the postprandial increase of plasma triglycerides (Minihane *et al.*, 2000) and the fasting levels of plasma cholesterol (Larson *et al.*, 2000).

The importance of lipids supply for extra-hepatic tissues by CM during postprandial processes determines the existence of a protector mechanism from too rapid uptake by the liver. Apolipoprotein C-III interferes with apo E binding sites at the receptors

and inhibits the activity of apolipoprotein C-II, an activator of LPL. These apolipoproteins are not formed in the enterocytes, but are constituent of the high-density lipoproteins (HDL). MUFA-rich diets increase the content of apo E in postprandial triglyceride-rich lipoproteins (Madigan *et al.*, 2000). It is not known whether olive oil can modify the apo C-III/apo C-II ratio.

6. SECRETION OF LIPOPROTEINS BY THE LIVER

The liver quickly metabolizes the postprandial remnant lipoproteins. Those of size smaller than 100 nm are up taken by hepatocytes in the space of Disse (Fraser *et al*, 1995). Larger triglyceride-rich lipoproteins re-circulate over the peripheral tissues. In the liver, triglycerides can be used for energy and cholesterol for the biosynthesis of membranes, conferring the optimal fluidity to their physiological functions. Throughout the postprandial metabolism, the liver secretes new triglyceride-rich lipoproteins (VLDL) to the blood circulation. This process occurs usually after 5-6 hours post-meal period (Abia *et al.*, 1999).

MTP mediates the formation of VLDL in hepatocytes, by regulating triglyceride accumulation in the microsomal lumen and triglyceride assembly with apo B-100 (Kulinski *et al.*, 2002). A deficiency in MTP causes abetalipoproteinemia, but up-regulation of MTP has been shown to increase the amount of triglycerides in VLDL (Liao *et al.*, 1999). Nascent VLDL touch with small lipoproteins charged with apo E and apo C-II (HDL), activating LPL and HL. The size of VLDL diminishes and lipoproteins turn on intermediary-density lipoproteins (IDL) and finally on LDL, the remnant particle of VLDL. LDL contains large amounts of cholesterol and almost none of triglycerides (Chapell *et al.*, 1998). Independently of its lipolytic activity, HL promotes the hepatic uptake of remnants and IDL particles (Perret *et al.*, 2002; Zambon *et al.*, 2003).

The expression and secretion of apo B-100 correlate with the pool of fatty acids in the hepatocytes. Particularly, oleic acid increases (29 %) the amount of apo B-100 in the medium of hepatoma cells, with no change in triglyceride synthesis (Arrol *et al.*, 2000). VLDL particles secreted had lower density compared to other unsaturated fatty acids, such as linoleic, arachidonic (20:4n-6) and eicosapentaenoic (20:5n-3) acids, and SFA, such as lauric (12:0), myristic (14:0), palmitic (16:0) and arachidic (20:0) acids. All fatty acids, except stearic and a-linolenic (18:3n-3) acids, were stimulators of apo B and VLDL secretion. In addition, MUFA and PUFA were more potent than SFA.

The composition of VLDL may be modulated, at least partially, by the nature of triglycerides coming from the diet. In fact, it is well accepted that dietary fatty acids have influence at long-term on the fatty acid composition of cholesterol esters and phospholipids in LDL (Sarkkinen *et al.*, 1994).

7. METABOLIC EFFECTS OF OLIVE OIL

Epidemiological studies suggest that consuming diets rich in (extra virgin) olive oil is associated with a low incidence of chronic disease, including cardiovascular disease and cancer. However, the effects of olive oil on health and disease cannot be understood without a profound understanding of how (extra virgin) olive oil components act at the cellular level.

The development of a diet-related disease involves a multitude of gene products, including proteins involved in lipid synthesis and oxidation, thermogenesis and cell differentiation. Nutrients we consume are among the most influential environmental factors that govern the expression of genes encoding these proteins. Nutrients govern the tissue content and activity of different proteins by functioning as regulators of gene transcription, nuclear RNA processing, mRNA degradation and mRNA translation, as well as functioning as post-translational modifiers of proteins.

The regulation of cell functions by fatty acids and minor compounds from (extra virgin) olive oil can occur on two general levels, modulation of signal transduction via manipulation of membrane fatty acid composition (non-genomic effect) and rapid and direct modification of gene transcription (genomic effect).

The initiation of gene transcription is a complex and tightly regulated process in which transcription factors, upon the appropriate stimuli, recognize and bind to specific DNA sequence motifs in the promoter region of the target gene. Upon this binding, mRNA for that particular gene is transcribed, and accumulation of its gene product begins. Transcription factors can be activated by phosphorylation, translocation of inactive complexes or synthesis *de novo* by other transcription factors.

7.1. Non-genomic effects of olive oil

Olive oil phenols interfere with leukotriene generation by inhibiting 5-lipoxygenase and 12-lipoxygenase activities in macrophages (Kohyama *et al.*, 1997), but not cyclooxygenase (COX) activity. Therefore, their role in prostaglandin biosynthesis is due not only to their action on COX activity but also to their effects on COX levels. Reactive oxygen species (ROS) may be involved in the pathways that result in arachidonic acid mobilization from membranes and PGE$_2$ release by COX-2. The tyrosol and β-sitosterol treatments that inhibit ROS production can prevent COX-2 expression induced by phorbol ester (PMA).

However, tyrosol and β-sitosterol have no effect on COX-2 activity in a cell-free enzyme system from macrophages stimulated by PMA (Moreno, 2003). Taken together, these results are consistent with the hypothesis that tyrosol and β-sitosterol inhibit arachidonic acid cascade through ROS release modulation. This may result from the transcriptional or post-transcriptional regulation of mRNA stability, which remains to be established. The promoter region of the COX-2 gene contains several binding sites for transcriptional factors that may be regulated by the cellular redox state, like NF-κB (Zwacka *et al.*, 1998). Thus, antioxidants like tyrosol and β-sitosterol may interfere with NF-κB. It confirms that both olive oil minor components reduce the NF-κB activation induced by PMA, whose interaction amplifies COX-2 promoter activation.

7.2. Genomic effects of olive oil

The following section will review transcription factors that play a role in diet-related diseases regulated by (extra virgin) olive oil components (Table 2).

7.2.1. *NF-κB*

The transcription factor NF-κB complex is present in the cytoplasm as a heterodimer composed of NFκB1 (p50) and Rel a (p65) subunits bound to one of a number of inhibitory proteins called IκBs. In response to diverse stimuli, including inflammatory cytokines, mitogens and several viral proteins, sheer stress and oxidative processes, NF-κB dissociates from IκB, the p50/p65 complex translocates to the nucleus because of the complete proteolytic degradation of the IκB proteins. NF-κB activation has been linked to the onset of atherosclerosis. It activates a variety of target genes that regulate cell proliferation and mediate cell survival as well as genes relevant to the pathophysiology of the vessel wall, including genes that regulate the expression of cytokines (interferon γ and β, interleukin-3, interleukin-6, GM-CSF), chemokines, vaso-regulatory molecules (endothelial tissue factor, nitric oxide synthase), and leukocyte adhesion molecules (ICAM-1, ELAM-1, VCAM-1).

The effect of olive oil on proliferation, cytokine production and pleiotropic genes expression through NF-κB activation has been considered beneficial or neutral by several authors (Rusyn *et al.*, 1999; Verlengia *et al.*, 2003). Thus, oleic acid does not stimulate induction of NF-κB and therefore does not activate endothelial cells (Henning *et al.*, 2000; Massaro *et al.*, 2002). However, the effect of oleic acid on NF-κB activation remains controversial. Recent studies have suggested that elevated levels of oleic and linoleic acids in plasma result in endothelial dysfunction, through an increase in

Table 2
Transcription factor pathways mediating nutrient-gene interaction

TRANSCRIPTION FACTOR	COMPOUNDS	TARGET GENES TRANSACTIVATED
NF-κB	-Oleic acid (activation, inhibition) -Phenolics (oleuropein, hybroxytyrosol) (inhibition) -ß-carotene (inhibition) -Linoleic acid (activation)	Inflammatory cytokines, chemokines, vaso-regulatory molecules, leukocyte adhesion molecules, genes that regulate cell proliferation
C-myc	-Oleic and linoleic acids -ß-carotene -α-Tocopherol (inhibition)	Proliferation (cyclins A and B), apoptosis and differentiation
AP-1	-Oleic acid (inhibition) -Linoleic and α-linolenic acid (activation) -Phenolics, ß-carotene, α-tocopherol (inhibition)	Proliferation, p21, apoptosis, p53, p19, cytokines
LXR	Polyunsaturated>monounsaturated>saturated fatty acids (pattern of inhibition)	Genes for lipoprotein lipase, sterol regulatory-element-binding protein 1c, ATP-binding cassette transporter Al
PPARs	Polyunsaturated>monounsaturated>saturated fatty acids (pattern of affinity)	Genes involved in lipid and glucose metabolism

endothelin-1 (Park *et al.*, 2003) and vascular adhesion molecule-1 (VCAM-1) protein expression (Dichtl *et al.*, 2002) via activation of protein kinase C and NF-κB.

Phenolics present in extra virgin olive oil, transcriptionally inhibit endothelial adhesion molecules expression by inhibiting NF-κB activation, partially explaining the vascular athero-protective effect of extra virgin olive oil (Massaro *et al.*, 2002; Carluccio *et al.*, 2003). Analysis of the NF-κB activation pathways reveals that the antioxidants do not inhibit activation of the IκB kinase activity, degradation of IκBα, or translocation of activated NF-κB into the nucleus, but they do block the formation of p50/p65/DNA binding complexes (Ma *et al.*, 2003).

It has been recently reported that ß-carotene induces growth-inhibitory and pro-oxidant effects, and increases the DNA-binding activity of nuclear proteins at NF-κB site in leukemic and colon adenocarcinoma cells (Palozza *et al.*, 2003). In these cells, treatment with α-tocopherol supports the hypothesis that carotenoids regulate this transcription factor through a pro-oxidant mechanism. Other minor compounds from extra virgin olive oils have been shown to inhibit translocation of NF-κB and interrupt cardiac inflammatory cytokine secretion (Horton *et al.*, 2001).

7.2.2.   *c-Myc*

A growing body of evidence indicates that c-Myc can play a pivotal role in both neoplasia and cardiovascular diseases. This protein product of cellular proto-oncogen c-myc is widely known as one of the essential transcription factors that perform cellular growth, proliferation, apoptosis, cell cycle entry and differentiation.

C-Myc is a sequence specific DNA binding protein that has a helix-loop-helix (HLH) and a leucine zipper (Zip) for protein dimerization and a basic region (b) for DNA binding at its C-terminal end. Heterodimerization of c-Myc with another b/HLH/Zip protein Max and binding of Myc-Max heterodimer to an E-box DNA motif [5-CA(C/T)GTC] is a prerequisite for the transcriptional regulation and activity of the c-Myc protein. C-Myc-Max also controls other transcription factors by sequestering or interacting with them, including activator protein (AP)-2 and elongation-2 factor, which are involved in the basal machinery of cell.

Some olive oil components may have anti-tumoral and anti-atherogenic effects by inhibiting c-Myc activation. Some studies have demonstrated that c-Myc activation is an early event in lipid accumulation within coronary arterial wall, occurring prior to development of atherosclerosis. MUFA and PUFA have strong inhibitory effects on the binding of

the c-Myc-Max heterodimer to an E-box DNA site. The interaction with the protein dimmer, not with DNA, blocks the entire c-Myc-Max-DNA complex formation, causing anti-proliferative effects in human cancer cells (Chung *et al.*, 2002). Similar to NF-κB, c-Myc is a redox-sensitive transcription factor. C-Myc is a NF-κB target gene and is implicated in apoptosis induction. Accordingly, ß-carotene is able to increase the expression of c-Myc, which is directly related to apoptosis induction (Palozza *et al.*, 2003). Some studies have demonstrated that α-tocopherol is able to reduce the activation of c-Myc-dependent signalling pathways in coronary lesions of animals and humans (de Nigris *et al.*, 2000; 2001).

7.2.3.   *AP-1*

Activator protein-1 (AP-1) is a transcription factor composed of homodimeric or heterodimeric combinations of the transcription factor proteins Fos and Jun, which bind to a common DNA consensus site. The AP-1 consensus site is located in the promoters of many genes involved in inflammatory responses relevant to vascular pathophysiology, including basic fibroblast growth factor (bFGF), transforming growth factor ß (TGFß), endothelin-1 and plasminogen activator-1. AP-1 protein also affects cell growth and survival by their ability to transactivate the expression of genes that regulate the cell cycle.

The process of inflammation is now believed to be the etiological event that precedes the development and continual process of atherosclerosis. There is accumulating evidence that point out the beneficial influence of extra virgin olive oil in the immune function (Yaqoob *et al.*, 1998; Adam *et al.*, 2000; Miles *et al.*, 2001). PUFA, such as linoleic acid and to a lesser extent α-linolenic acid, can contribute to induction of inflammatory genes in vascular endothelial cells via AP-1 activation. However, oleic acid seems to have no effect or down-regulate the activity of AP-1, resulting in a decreased inflammatory gene mRNA (Toborek *et al.*, 2002; Viswanathat *et al.*, 2003). Thus, the high ratio of MUFA to PUFA found in olive oil may contribute to its substantial benefit in cardiovascular diseases.

Phenolics from extra virgin olive oil at nutritionally relevant concentrations transcriptionally inhibit endothelial adhesion molecular expression via inhibition of AP-1 (Carluccio *et al.*, 2003). Further studies have provided molecular evidence supporting the beneficial effect of α-tocopherol in the development of inflammation via suppression of IL-4 expression by blocking binding of transcription factors to IL-4 promoter binding sites for AP-1 (Li-Weber *et al.*, 2002). ß-Carotene is also an effective inhibitor of breast cancer, possibly mediated through down-regulation of cell cycle regulatory

proteins and/or inhibition of AP-1 transcriptional activity (Tibaduiza *et al.*, 2002).

7.2.4. *Nuclear receptors*

Nuclear receptors are one of the largest groups of transcription factors, with 49 distinct members presently identified in the human genome. Nuclear receptors are regulators integrating different homeostatic controls:

- Energy and glucose metabolism [peroxisome proliferators-activated receptor gamma (PPARλ)].
- Fatty acid, triglyceride and lipoprotein metabolism (PPARα, ß/δ and γ).
- Reverse cholesterol transport and cholesterol absortion [liver X receptors (LXR) and liver receptor homolog-1 (LRH-1)].
- Bile acid metabolism [farnesol X receptor (FXR), LXR, LRH-1].
- Defence against xeno- and endobiotics [pregnane X, receptor/steroid and xenobiotic receptor (PXR/SXR)].

Nuclear receptors have a modular structure characterized by a ligand-independent AF-1 transactivation domain in the N-terminal region, a highly conserved DNA-binding domain composed of two zinc fingers recognizing specific DNA sequences, and a ligand-binding and dimerization domain that contains a ligand-dependent AF-2 transactivation domain in its C-terminal portion. Most nuclear receptors are active as dimmers with the retinoid X receptor (RXR, NR2B1) and bind to specific nucleotide sequences (response element) in the promoter region of a large number of genes. During ligand binding, nuclear receptors undergo a conformational change that results in the coordinated dissociation of co-repressors and the recruitment of co-activator proteins to enable transcriptional activation (Francis *et al.*, 2003).

In the past few years, it has become clear that fatty acids induce or inhibit the mRNA expression of a variety of different genes by acting both, as agonist and as antagonists for nuclear hormone receptors. The present section briefly reviews recent data on the mechanisms by which fatty acids influence DNA transcription and focus especially on the importance of two nuclear receptors, LXR and PPARs.

LXR is believed to act as a sterol sensor in cells up-regulating genes involved in cholesterol elimination when cells are faced with excess sterol. LXR forms obligate heterodimers with RXR, which then enhance the transcription of such genes as those for LPL, SREBP-1c (sterol regulatory-element-binding protein 1 c) and ABC sterol transporters, such as ABCA1 (ATP-binding cassette transporter A1, a sterol transporter critical for facilitating the cholesterol and phospholipids to lipid-poor apolipoproteins, resulting in the formation of nascent HDL particles). Two different LXR genes have been described, LXRα and LXRß. Fatty acids induce LXRα gene expression in the liver (Tobin *et al.*, 2000) and activate LXRα transcription factor in the intestine (Murthy *et al.*, 2004). Furthermore, these studies have postulated that SREBP-1c and ABCA1 are gene targets of LXR through fatty acid activation, suggesting an important cross-talk between fatty acids metabolism and cholesterol regulation. The pattern of inhibition clearly favours unsaturated fatty acids over SFA and, in most studies, PUFA over MUFA (Hannah *et al.*, 2001; Ou *et al.*, 2001). Thus, PUFA but not oleic acid decrease both LXR-mediated SREBP-1c mass and gene expression (Field *et al.*, 2002). Murthy *et al.* (2004) have recently shown that fatty acids decrease the efflux of cholesterol in Caco-2 cells in response to LXR activation, without changing (by linoleic and stearic acids) or modestly attenuating [by oleic, arachidonic and docosahexaenoic (22:6*n*-3) acids] ABCA1 gene expression. Except for oleic acid, all fatty acids substantially attenuated an increase in ABCA1 mass secondary to LXR activation. The authors suggested that oleic acid probably decreases cholesterol efflux by diverting cholesterol away from ABCA1 towards ACAT (acyl-CoA:cholesterol acyltransferase). This fact would indicate that diets enriched in oleic acid would preferentially promote intestinal cholesterol secretion in triglyceride-rich lipoproteins and less cholesterol secretion in nascent HDL.

In addition, it has become clear that induction of gene transcription by fatty acids is often mediated by PPARs. PPARα is mostly expressed in brown adipose tissue and liver; PPARß/δ is present at high concentrations in numerous tissues but it is especially abundant in the intestine; PPARγ is most abundant in white adipose tissue and to a lesser extent in colon and macrophages. Some authors have provided support for an anti-atherogenic role of PPARγ (Chawla *et al.*, 2001; Chinetti *et al.*, 2001). PUFA, as well as MUFA and SFA, induce the expression of reporter genes in a PPAR-dependent manner. Although most fatty acids including SFA and unsaturated fatty acids bind PPARs with low to moderate affinity, it is not entirely clear whether their relative affinities differ greatly. In general, all isoforms of PPARs are more responsive to 18-22 carbon *n*-6 and *n*-3 PUFA than to MUFA and SFA. According to Xu *et al.* (1999), SFA and MUFA bind to the PPARα subtype with approximately equal affinity. The highest binding affinity was found for linoleic acid.

8. METABOLISM AND EXCRETION OF OLIVE OIL MINOR COMPOUNDS

Studies on phenolics metabolism show differences between administered doses and excreted metabolic products in urine. It is unknown

whether the remaining fraction is evacuated with the faeces without be absorbed, destroyed in the gut, or accumulated in organs or circulating cells, such as erythrocytes. The quantities of phenolic compounds found intact in urine vary from one to another. The low recovery for some of them is not explained by chemical degradation in the gut, as they have shown a great stability in gastric or intestinal juices (Olthof *et al.*, 2001).

Intestinal absorption of phenolic compounds can be high; however, the plasma concentration of any individual molecule rarely exceeds 1 μM after the consumption of 10-100 mg of a single compound. The plasma antioxidant capacity suggests that more phenolics should be present, mainly in the form of metabolites generated by either tissues or colonic microflora (Scalbert *et al.*, 2000). It is assumed that dietary doses, contrary to drug doses, do not readily saturate the metabolic pathways and hence circulating species would be expected to be conjugated (Bell *et al.*, 2000). The dose will also determine the primary site of metabolism. Large doses are metabolised primarily in the liver, whereas small doses are rather metabolised by the intestinal mucosa, with the liver playing a secondary role to modify conjugates from the small intestine (Piskula *et al.*, 1998).

In the colon, the microflora can also hydrolyse flavonoid glucuronides excreted in the bile and the resulting aglycone reabsorbed, thus entering into an entero-hepatic cycle (Aura *et al.*, 2002). Aglycones can also further be metabolised to a wide variety of low molecular weight aromatic acids (phenylvaleric, phenylpropionic, phenylacetic and benzoic acids), which are well absorbed through the colonic barrier (Deprez *et al.*, 2000). The amount of substrates reaching the colon and the tissue exposure to their microbial metabolites could be highest than the amount of the absorbed phenolic compounds (Scalbert *et al.*, 2002).

The metabolism of phenolic compounds implies reactions of conjugation on aglycones, after the hydrolysis of phenol derivatives is produced. Major conjugation reactions include the formation of *o*-glucuronides (Spencer *et al.*, 1999; Donovan *et al.*, 2001), sulphate esters and *o*-methyl ethers (Manna et al., 2000). They occur firstly in the gut and then in plasma facilitating its urinary and biliary excretion (Scalbert *et al.*, 2002). The formation of phenol conjugates can dramatically alter the biological properties of the circulating metabolites.

Miro-Casas *et al.* (2001) quantified the amount of excreted tyrosol in urine, reporting a maximal concentration in the first 4 h after (extra virgin) olive oil ingestion. Total urine recoveries were about 25% of the ingested tyrosol, in which 6-11% consisted of free tyrosol and the remaining as glucuronide conjugates. Vissers *et al.* (2001) estimated the human absorption of a phenol-rich supplement obtained from (extra virgin) olive oil, finding the net absorption >55 mol/100 mol and the re-excretion of tyrosol and hydroxytyrosol 5-16 mol/100 mol.

Hydroxytyrosol and tyrosol are excreted in urine as glucuronide conjugates with a positive correlation between the amounts of phenolics ingested and glucuronide conjugation of urine metabolites (Visioli *et al.*, 2000). Other metabolites of hydroxytyrosol [homovanillic acid (4-hydroxy-3-methoxy phenylacetic acid) and homovanillic alcohol] in humans (Caruso *et al.*, 2001), and oxidised and methylated derivatives of hydroxytyrosol (3,4-dihydrophenilacetic acid; 3,4-dihydroxyphenylacetaldehyde; and sulfoconjugated derivatives) (D'Angelo *et al.*, 2001) in animals were later detected. Figure 1 shows different metabolites of hydroxytyrosol including enzymatic pathways of formation.

Recently, it has been determined hydroxytyrosol and 3-*o*-methyl-hydroxytyrosol in plasma after real-life doses of extra virgin olive oil (Miro-Casas *et al.*, 2003), finding that approximately 98% of hydroxytyrosol appears to be present in plasma and urine in glucuronide-conjugated forms, which suggests extensive first-pass intestinal/hepatic metabolism of the ingested hydroxytyrosol.

Into the enterocytes, ß-carotene can be converted to vitamin A. This process is highly regulated, and consequently toxic levels of vitamin A from ingested carotenoids are rare (Olson, 1994). Carotenoids distribute into lipoproteins (LDL) where they exert antioxidant activities, and in tissues, mainly adipose tissue (85%), liver (12%) and muscle (3%). Particularly, lutein has been shown to suffer oxidative modifications in human serum, forming metabolites with anti-tumoral activities (Khachik *et al.*, 1995). In further studies, Khachik *et al.* (1997) identified dehydrated metabolites of lutein.
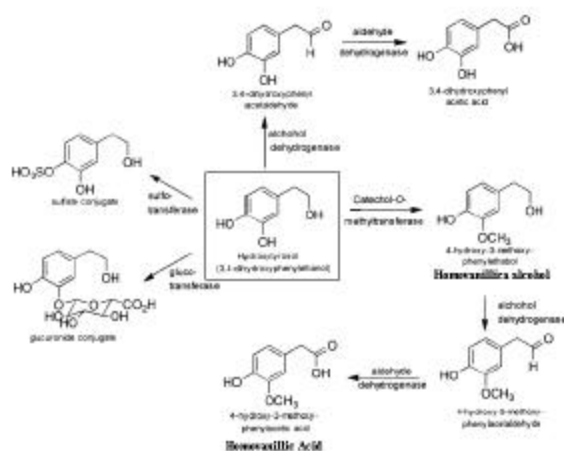

Figure 1
Metabolites from hydroxytyrosol (adapted from Tuck *et al.*, 2002).

Tocopherols are metabolised in the liver, by a pathway involving cytochrome P450-mediated ω-hydroxylation of the phytyl side chain followed by stepwise removal of two- or three-carbon moieties, ultimately yielding the 3-carboxychromanol metabolite that is excreted in urine (Sontang and Parker, 2002). The α-tocopherol transfer protein (α-TTP) specifically binds α-tocopherol and sorts it out for incorporation into plasma lipoproteins (Schuelke *et al.*, 2000), what suggests a high regulation of the plasma and tissue levels of α-tocopherol. That is in accordance to the fact that only a-tocopherol, among other tocopherols, has signalling functions in vascular smooth muscle cells (Brigelius-Flohe and Traber, 1999; Azzi *et al.*, 2000). The metabolic pathway of the hepatic degradation of α-tocopherol has not been fully established, but ω- and ß-oxidations probably occur after ω-hydroxylation, suggesting the participation of a CYP3A-type cytochrome (Birringer *et al.*, 2001). Furthermore, the same authors pointed out that a competition of microsomal ω-oxidation with specific binding by the α-TTP could determine the metabolic fate of tocopherols.

Finally, some of these metabolites are recently probed to have effects on cancer cell proliferation, by specific down-regulation of cyclin expression (Galli *et al.*, 2004). The presence of vitamin E metabolites in plasma is parallel to the ingested parent compounds, as have been assessed by high performance liquid chromatography (Lodge *et al.*, 2001; Radosavac *et al.*, 2002) and gas-chromatography/mass spectrometry (Galli *et al.*, 2002).

ACKNOWLEDGMENTS

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