Mediterranean Diet and Health: Biological Importance of Olive Oil

C. Alarcón de la Lastra*, M.D. Barranco[a], V. Motilva and J.M. Herrerías

Departamento de Farmacología, Facultad de Farmacia, C/Profesor García González s/n.41012 Sevilla, Spain

Abstract: Olive oil, the main fatty component of the Mediterranean diet, is characterized by consisting of monounsaturated fatty acids as well as by its elevated content in antioxidant agents. This oil exhibits numerous biological functions which are beneficial for the state of health. A diet rich in monounsaturated fatty acids provides an adequate fluidity to the biological membranes, diminishing the hazard of lipid peroxidation which affects polyunsaturated fatty acids. Moreover, the antioxi dants present in olive oil are able to scavenge free radicals and afford an adequate protection against peroxidation. Regarding the heart, olive oil decreases the plasmatic levels of LDL-cholesterol and increases those of HDL-cholesterol, hence diminishing the risk of suffering from heart complaints. In this context, it has been suggested that increased consumption of monounsaturated fatty acids in place of polyunsaturated fatty acids will render circulating lipoproteins less sensitive to peroxidation and thereby diminish the development of atherosclerosis. Olive oil has also been proven to contribute to a better control of the hypertriglyceridemia accompanying diabetes and may reduce the risk of breast cancer and colorectum. On the other hand, several investigations have suggested that olive oil can be beneficial in inflammatory and autoimmune diseases, such as rheumatoid arthritis. In this sense, some reports have indicated that olive oil modifies inflammatory cytokines production. As for the digestive system, olive oil enhances gallbladder emptying consequently reducing cholelithiasis risk, decreases the pancreatic exocrine secretion and gastric secretory function in response to food. Finally, it has been demonstrated that a diet rich in olive oil is associated with a high percentage of gastric ulcer healing and affords a higher resistance against non steroidal antiinflammatory drugs-induced gastric ulcerogenesis.

I. INTRODUCTION

In the course of history, important changes in lifestyle and nutrition have taken place [1]. Nowadays, in the industrialized countries the alimentary error characterized by an excessive intake of calories (due to the higher food disponibility or to the less physical activity) is very extended. This energy-rich diet is often dysbalanced in the amount of nutrients: the intake of lipids increases, with a marked rise in saturated and polyunsaturated fatty acids (PUFA) versus monounsaturated fatty acids (MUFA); the consumption of hydrocarbons decreases, but among those, the oligosaccharides increase; the consumption of foods from animal source increases, with a higher supply of proteins of high biological value. At the same time, the intake of invisible saturated fats increases, fresh vegetables are canned and preserved by treatments not always respectful with their biological content. Furthermore, the subsequent transformation of foods reduces their vitamin, mineral, antioxidant as well as vegetable fiber content.

All these modifications which are due to the technological progress, to the much extended fast-food consumption or to the changes in taste and habits, can only negatively affect the state of health of the population and increase the incidence of degenerative metabolic diseases. Therefore, it is necessary to change alimentary habits and to reconduct people towards a more moderate and balanced diet as is the case of the Mediterranean diet [2, 3].

*Address correspondence to this author at the Departamento de Farmacología. Facultad de Farmacia, C/Profesor García González s/n.41012 Sevilla (Spain), Telephone number:+34 954556722. Fax: + 34 954233765

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II. THE MEDITERRANEAN DIET

The Mediterranean diet is, in its widest definition, not only the intake of certain foods; it also encompasses a lifestyle peculiar to the inhabitants of the Mediterranean basin. For these peoples, the act of eating is certainly not the rapid ingestion of healthy food products obtained from their habitat but rather the pleasure of eating at a table while sharing food with others, spending some time savouring the dishes and resting after the meal to aid digestion. These factors are fundamental to the lifestyle which is termed the "Mediterranean diet"[4].

This diet is compound mainly of [5]:

- *Cereals and grains.* Cereals and grains constitute the base of majority of the meals of the Mediterranean peoples. They range from wheat, pasta, polenta, couscous and rice, and provide the intake of complex carbohydrates.

- *Fruits and vegetables.* The taste of the dishes is obtained by carefully choosing seasonal products and cooking them simply. Throughout the Mediterranean area, dessert tends to be fruit (providing fiber, mineral salts, vitamins, carotenes and phenolic compounds).

- *Pulses and nuts.* In Mediterranean cooking a great variety of pulses and nuts are used, such as chickpeas, lentils, white beans, pine nuts, hazelnuts, walnuts, and almonds (providing fiber, antioxidants, phytoestrogens).

- *Dairy products.* These are taken in the form of cheese, yogurt and other dairy products (proteins rich in essential amino acids).

- *Fish.* Fish appears in the mediterranean diet as a first order protein, ranked before eggs, poultry and red meat. It is not only the variety of fish that is important but also traditional forms of intake and the way in which it is cooked. It is also high in PUFA and especially in omega-3 fatty acids.

- *Meat.* This diet is characterized by a moderate but sufficient meat intake.

- *Pastry products.* The lowest recommended allowance of these products is another feature of this diet model.

- *Seasonings.* Although seasonings may vary slightly in different places, garlic and aromatic herbs such as oregano, rosemary, thyme, sage, mint, coriander, cumin, parsley, fennel and laurel tend to be abundantly used. They are high in antioxidant phenolics.

- *Wine and exercise.* The importance of moderate wine consumption during meals (a glass of wine) and regular physical exercise should be also pointed out.

Finally, throughout the Mediterranean area, both *olive oil* and *extra-virgin olive oil* are used as dietary fats. The first is more extensively used for cooking, while the second type is used directly as a salad dressing or as a dip for bread.

III. CHEMICAL COMPOSITION OF OLIVE OIL

Edible oil constituents can be divided into the saponifiable and the unsaponifiable fractions [6, 7, 8].

1. The saponifiable fraction represents between 98,5 and 99,5 percent of the oil.

2. The unsaponifiable constituents of virgin olive oil account for 1,5-0,5 percent of the oil. Some of them contribute to its flavour quality and also increase its oxidative stability.

Composition of the Saponifiable Fraction

Triglycerides constitute the most important part of the saponifiable fraction. The rest is principally composed of free fatty acids together with other minor components of olive oil derived from fatty acids, such as mono- and diacylglycerols, phosphatides, waxes, and esters of sterols [7, 8].

Fatty Acids

Edible vegetable oils seldom contain branched-chain or odd-numbered fatty acids or unsaturated fatty acids with fewer than sixteen or more than twenty carbon atoms.

Olive oil is rich in oleic acid (monounsaturated), it contains a moderate amount

of palmitic and stearic acids (saturated) and a low quantity of linoleic and α-linolenic acids (polyunsaturated). The percentage of fatty acids distribution in olive oil is shown in Table1 [7].

Table 1. Percent of Fatty Acids of Olive Oil

Trivial name	Symbol	Percentage
Myristic	14:0	0.0 - 0.05
Palmitic	16:0	7.5 - 20.0
Palmitoleic	16:1*n*7	0.3 - 3.5
Margaric	17:0	0.0 - 0.3
Heptadecanoic	17:1	0.0 - 0.3
Stearic	18:0	0.5 - 5.0
Oleic	18:1*n*9	55.0 - 83.0
Linoleic	18:2*n*6	3.5 - 21.0
α-Linolenic	18:3*n*3	0.0 - 0.9
Arachidic	20:0	0.0 - 0.6
Eicosenoic	20:1*n*9	0.0 - 0.4
Behenic	22:0	0.0 - 0.2
Lignoceric	24:0	0.0 - 0.2

The composition in fatty acids differs from one sample to another, depending on the geographic origin of the olive oil. Among the principal factors affecting the fatty acids composition the following are to be mentioned: latitude, clime, genetic factors (variety) and grade of ripeness of the harvested olives.

For example, different authors maintain that the further south a variety is cultivated, the concentration of saturated fatty acids and linoleic acid of the oil increases, whereas the content in oleic diminishes [6]. The ratio unsaturated/saturated fatty acids rises with the latitude and the ratio oleic/linoleic increases similarly. This behaviour is due to a prolongation of the maturity cycle, hence states of higher ripeness grade are reached at low latitude.

On the other hand, oils of different varieties have been studied and characteristic proportions of fatty acids have been found for each variety, regardless of the environment where they were cultivated. Table 2 shows the percentages of oleic and linoleic of some of the principal Spanish varieties [6].

Table 2. Percentage of Oleic and Linoleic Fatty Acids in Several Spanish Varieties

Varieties	Oleic acid (%)	Linoleic acid (%)
Picual	78.3	5.1
Hojiblanca	75.7	9.2
Lechín	69.7	13.3
Picudo	66.6	14.7
Cornicabra	80.3	5.6
Arbequina	70.2	11.4
Empeltre	74.6	9.4

Triacylglycerols

Most of the fatty acids of edible oil are present as triacylglycerols. Olive oil triacylglycerol compositions follow a pattern in which the fatty acids at the central or 2-position of the glycerol molecule are unsaturated, generally oleic acid. Table 3 depicts the different types of triglycerides found in significant amounts in olive oil [9, 10].

Table 3. Olive Oil Triacylglycerols

Triglycerides	Percentage
OOO	40-59
POO	15-22
OOL	12-20
POL	5.5-7
SOO	3-7
PLO	4-5
POP	2-4

O, oleic acid; P, palmitic acid; L, linoleic acid; S, stearic acid.

Composition of the Unsaponifiable Fraction

The unsaponifiable fraction is constituted by non volatile compounds which are obtained after saponification of the oil with an alkaline hydroxide and subsequent extraction with a certain solvent. It contains lipids of natural origin such as sterols, superior aliphatic alcohols, pigments and hydrocarbons, as well as strange organic particles which are not volatile at 103ºC [11].

Classically, in this group other minor nonglycerol (unsaponifiable) constituents have

been included although they are not extracted by the former method, for example, polar phenolic compounds.

Hydrocarbons

The unsaponifiable fraction or olive oil comprises an important part of the hydrocarbons, which ranges from 30 to 50 percent of the total amount. This group includes:

Squalene, a biochemical precursor of sterols, is an important hydrocarbon of both virgin and refined olive oils. Olive oil contains the largest amount of squalene among vegetable oils (2500-9250 μg/g) compared with other edible oils (16-370 μg/g) [12].

Carotenoids. The carotenoid fraction (0.5 to 1 mg/100 g of oil) is formed by *lutein* (30-60 percent), β-*carotene* (5-15 percent), and several xanthenes [7, 12, 13]. These components are pigments.

Chlorophylls

The chlorophyll fraction is made up of *chlorophylls a* and *b* and their magnesium-free derivatives, *pheophytins a* and *b* [7, 8]. In natural olive oil, this fraction is found in concentrations ranging from 1 to 20 ppm, and its major components are *pheophythin a* (20-40 percent) and *chlorophyll a* (4-7 percent) [12]. In oils that have undergone refining, the pigment content decreases remarkably.

Tocopherols

The total content of tocopherols in olive oil depends on the variety of olive and varies from 5 to 300 ppm [14], α-*tocopherol* being the main one (52-87 percent). β-*tocopherol* (15-20 percent) and γ-*tocopherol* (7-23 percent) are found in smaller amount [7]. In high quality virgin olive oils, the content is usually between 100 and 300 ppm [15, 16]. In oils that have undergone refining, the tocopherols content decreases notably [17]. Because they oxidize easily, tocopherols are excellent natural antioxidant agents and give stability to virgin olive oil.

Aliphatic Alcohols

Saturated straight-chain aliphatic alcohols with even-numbered carbon atoms (C18 to C28) are found in olive oil. The main linear aliphatic alcohols present in olive oil are *hexacosanol*, *octacosanol*, and *tetracosanol*. Also, *tricosanol*, *pentacosanol*, and *heptacosanol* might be present in traces [18, 19].

Tacchino and Borgoni [20] determined the aliphatic alcohols in pressed olive oil and found that it varies from 10 to 70 mg/100g.

Sterols

Olive oil contains sterols in a concentration ranging from 100 to 220 mg/100 g of oil. The main sterols found in olive oil are β-*sitosterol*, Δ-*5-avenasterol*, and *campesterol* [21-24]. *Stigmasterol*, *cholesterol*, *24-methylene-cholesterol*, Δ-*5, 23-stigmastadienol*, *sitostanol*, Δ-*5, 24- stigmastadienol*, Δ-*7-stigmasterol*, and Δ-*7-avenasterol* are also present in olive oil but in smaller quantities [23-25].

Triterpene dialcohols, mainly *erythrodiol* and *uvaol*, are also found in olive oil, in a concentration ranging from 1 to 20 mg/100 g of oil [26].

Phenolic compounds

Phenols make up a part of the so-called “polar fraction” of virgin olive oil, which is usually obtained by extraction with methanol/water mixtures [27]. This fraction of the oil is very complex and many of its components remain unidentified.

Among the polyphenolic compounds identified in olive oils are *tyrosol* (4-hydroxyphenylethanol), *hydroxytyrosol* (3,4-dihydroxyphenylethanol), *oleuropein*, *caffeic acid*, *vanillic acid*, *syringic acid*, *p-coumaric acid*, *o-coumaric acid*, *protocatechuic acid*, *4-hydroxybenzoic acid*, *4-hydroxyphenylacetic acid* and *3,4-dihydroxyphenylacetic acid* [28, 29].

The absolute concentration of phenols in olive oils is the result of a complex interaction between several factors, including cultivar, degree of maturation, climate and other agronomic and technological factors, such as the extraction procedures [30, 31]. It usually decreases with overmaturation of olives, although there are some exceptions to this rule. For instance, olives grown in warmer climates, in spite of a more rapid

maturation, yield oils that are richer in phenols. Therefore, although the concentration of total phenols (alcohols, phenolic acids and others) normally ranges from 50 to 200 ppm, oils with contents up to 1000 ppm can be found [30].

These compounds, although minor constituents of olive oil, participate in the mechanisms involved in sensory organoleptic properties (flavour and aroma). Thus, most phenols confer a very bitter and pungent zest to the oil [30].

On the other hand, phenolic compounds exhibit antioxidant properties and contribute to the prevention of oil rancidity (high polyphenol content is associated with a high resistance to oxidation of the oils) [30, 31]. The major contribution to this effect has been attributed to hydroxytyrosol, an orthodiphenol that derives from oleuropein, the bitter principle of olives. On the contrary, tyrosol appears to contribute very little, if any, to the stability of olive oil [31].

In contrast to other crude oils, virgin olive oil produced from olives of good quality is consumed unrefined. Thus, virgin olive oils contain polyphenols which are usually removed from other edible oils in the various refining stages.

Volatile compounds

Aroma and flavour are distinctive features of olive oil in comparison with other edible oils. They are generated by a number of volatile compounds present at extremely low concentrations [7, 8]. The aromatic compounds chemical composition varies according to the variety of olive, pedoclimatic conditions, and quality of the oil [8].

CATEGORIES OF OLIVE OIL

In 1987, the European Community (EC) issued a directive (EC No. 2658/87) defining virgin olive oils. Another directive (EC No. 2568, September 5, 1991) defined the identity characteristics of olive oil and olive-pomace oil and also specified analytical methods. The initial specific methods and the limits adopted for each of the relevant criteria have been slightly changed or amended in successive directives (EC No. 2632/94, EC No. 656/95, and EC No. 2472/97). Tables 4 and 5 show the limits established for each criterion.

The designations and definitions of the various categories and subcategories of olive oil, according to EC legislation, are:

Olive Oil

This is the oil obtained solely from the fruit of the olive tree (*Olea europea sativa*), to the exclusion of oils obtained using solvents or reesterification processes and of any mixture with oils of other kinds. It is marketed in accordance with the following designations and definitions:

- *Virgin olive oil* is the oil 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, and which has not undergone any treatment other than washing, decantation, centrifugation, and filtration. Virgin olive oil fit for consumption as it is (which can also be called *natural*) includes extra virgin olive oil, virgin olive oil, and ordinary virgin olive oil.

- *Extra virgin olive oil* is a virgin olive oil that has a free acidity, expressed as oleic acid, of not more than 1 gram per 100 grams. This type of oil not only exhibits all the chemical elements characteristic of olive oil but it has also the best organoleptic features (minimum score in the panel test 6.5) with tastes and aromas which perfectly reproduce the fruit of origin. Due to the low stress which it has been subjected to during the extraction, it is the oil which keeps the highest unsaponifiable fraction of all the olive oils and therefore also the one with the highest amount of antioxidants.

- *Virgin olive oil*, which can be used at the production and wholesale stages, is a virgin olive oil that has a free acidity, expressed as oleic acid, of not more than 2 grams per 100 grams. This type of oil possesses good chemical characteristics, but it has suffered some organoleptic variations. The fundamental difference with the former type is the score of the panel test (minimum 5.5).

- *Ordinary virgin olive oil* is a virgin olive oil that has a free acidity, expressed as oleic acid, of not more than 3.3 grams per 100 grams. This type of oil exhibits serious organoleptic alterations (minimum score in the panel test 3.5).

Table 4. Characteristics of Olive Oil and its Chemical Methods. Directive EC Nº 2568/91. Last Modification Directive EC No 2472/97

Parameters	Extra virgin olive oil	Virgin olive oil	Ordinary virgin olive oil	Lampante virgin olive oil	Refined olive oil	Olive oil
Acidity (oleic acid, %)	M 1,0	M 2,0	M 3,3	> 3,3	M 0,5	M 1,5
Peroxide index (meq O_2/kg)	M 20	M 20	M 20	> 20	M 5	M 15
Halogenated solvents (mg/kg) (1)	M 0,2	M 0,2	M 0,2	> 0,2	M 0,2	M 0,2
Waxes (mg/kg)	M 250	M 250	M 250	M 350	M 350	M 350
Saturated fatty acids in position 2 (triglycerides, %)	M 1,3	M 1,3	M 1,3	M 1,3	M 1,5	M 1,5
Stigmastadienes (mg/kg) (2)	M 0,15	M 0,15	M 0,15	M 0,5	---	---
Erythrodiol + Uvaol (%)	M 4,5	M 4,5	M 4,5	M 4,5	M 4,5	M 4,5
Cholesterol (%)	M 0,5	M 0,5	M 0,5	M 0,5	M 0,5	M 0,5
Brassicasterol (%)	M 0,1	M 0,1	M 0,1	M 0,1	M 0,1	M 0,1
Campesterol (%)	M 4,0	M 4,0	M 4,0	M 4,0	M 4,0	M 4,0
Stigmasterol (%)	< Camp	< Camp	< Camp	---	< Camp	< Camp
β-Sitosterol (%) (3)	m 93	m 93	m 93	m 93	m 93	m 93
Delta-7-stigmastenol (%)	M 0,5	M 0,5	M 0,5	M 0,5	M 0,5	M 0,5
Total sterols (mg/kg)	m 1000	m 1000	m 1000	m 1000	m 1000	m 1000

(1) Total content of compounds detected by means of electron capture. For each component the maximum limit is 0.1 mg/kg.
(2) Sum of isomers that can (or cannot) be separated by capillary columns.
(3) Δ-5,23-stigmastadienol + Clerosterol + β-Sitosterol + Sitostanol + Δ-5-avenasterol + Δ-5,24-stigmastadienol.
M = maximum, m = minimum
Note: if one of the characteristics does not comply with the fixed limits, that will be enough to disqualify an oil.

- *Virgin olive oil not fit for consumption* as it is, known as *lampante virgin olive oil*, is a virgin olive oil that due to defective flavour or aroma and/or a free acidity, expressed as oleic acid, of more than 3.3 grams per 100 grams requires refining in order to be consumed.

- *Refined olive oil* is the olive oil obtained from virgin olive oils by refining methods that do not lead to alterations in the initial glyceridic structure. Its organoleptic characteristics are practically neutral, it lacks taste and aroma and it is used fundamentally in the composition of other commercial olive oils. It must not exceed a free acidity, expressed as oleic acid, of 0.5 grams per 100 grams and it exhibits oxidation indexes much higher than the ones of virgin olive oil. Moreover, its content of antioxidants is considerably lower than in the other groups.

- *Olive oil* is the oil consisting of a blend of refined olive oil and virgin olive oil fit for consumption as it is.

The physico-chemical characteristics of the different oil types and the organoleptic

Table 5. Characteristics of Olive Oil and its Analytical Methods. Directive EC Nº 2568/91. Last Modification Directive EC No 2472/97

Parameters	Extra virgin olive oil	Virgin olive oil	Ordinary virgin olive oil	Lampante virgin olive oil	Refined olive oil	Olive oil
Myristic (%)	M 0,05	M 0,05	M 0,05	M 0,05	M 0,05	M 0,05
Linolenic (%)	M 0,9	M 0,9	M 0,9	M 0,9	M 0,9	M 0,9
Arachidic (%)	M 0,6	M 0,6	M 0,6	M 0,6	M 0,6	M 0,6
Eicosanoic (%)	M 0,4	M 0,4	M 0,4	M 0,4	M 0,4	M 0,4
Behenic (%)	M 0,2	M 0,2	M 0,2	M 0,2	M 0,2	M 0,2
Lignoceric (%)	M 0,2	M 0,2	M 0,2	M 0,2	M 0,2	M 0,2
Total of trans-oleic Isomers (%)	M 0,05	M 0,05	M 0,05	M 0,1	M 0,2	M 0,2
Total of trans-linoleic and trans-linolenic isomers (%)	M 0,05	M 0,05	M 0,05	M 0,1	M 0,3	M 0,3
K 232	M 2,5	M 2,6	M 2,6	M 3,7	M 3.4	M 3.3
K 270	M 0,2	M 0,25	M 0,25	M 0,25	M 1,2	M 1,0
K270 after treatment With alumina (1)	M 0,1	M 0,1		M 0,11	---	---
ΔK	M 0,01	M 0,01	M 0,01	---	M 0,16	M 0,13
Overall grading of panel test	m 6,5	m 5,5	M 3,5	< 3,5	---	---
Diffecence between theorical and empirical ECN 42 (2)	M 0,2	M 0,2	M 0,2	M 0,3	M 0,3	M 0,3

(1) For purity determination, in case that K270 exceeds the established limit for the corresponding cathegory a new determination must be carried out after treatment with alumina.
(2) ECN = equivalent carbon
M = maximum, m = minimum
Note: if one of the characteristics does not comply with the fixed limits, that will be enough to disqualify an oil.

characteristics of olive oils are listed in Tables 4 and 5.

Olive – Pomace Oil

This is the oil obtained by treating olive pomace with solvents, to the exclusion of oils obtained by reesterification processes and of any mixture with oils of other kinds.

BIOLOGICAL IMPORTANCE OF OLIVE OIL

Olive Oil and Oxidative Stress

Oxygen radicals are recognized mediators of various degenerative diseases and inflammatory diseases, including rheumatoid arthritis, diabetes, cancer, cataract formation, immune and brain dysfunctions, and aging. Consequently, organisms have evolved not only antioxidant defense systems to protect against them, but also repair systems that prevent the accumulation of oxidatively damaged molecules [32, 33].

Cell membranes require unsaturated fatty acids to maintain their structure, fluidity and function [34]. Many studies have shown that, after the period of adaptation, the lipid profile of the diet affects membrane lipid content and phospholipid fatty acyl composition [35, 36]. Hence a diet rich in saturated fatty acids decreases membrane fluidity, which on the contrary increases if the diet is rich in PUFA.

However, PUFA are constantly exposed to the hazard of lipid peroxidation. It has been demonstrated that membrane lipid peroxide levels also increase as membrane polyunsaturated fatty acids content and membrane unsaturation index increase [37, 38].

Lipid peroxidation is initiated by the attack on a fatty acid or fatty acyl side chain of any chemical species that has sufficient reactivity to abstract a hydrogen atom from a methylene carbon in the side chain. The greater the number of double bonds in a fatty acid side chain, the easier is the removal of a hydrogen atom, which is why PUFA are particularly susceptible to peroxidation. By contrast, both monounsaturated and saturated fatty acids are more resistant to free-radical attack.

In aerobic cells, the resulting carbon-centered lipid undergoes molecular rearrangement, followed by reaction with oxygen to give a peroxyl radical. Peroxyl radicals can combine with each other if they meet, or they can attack membrane proteins, but they are also capable of abstracting hydrogen from adjacent fatty acid side chains and so propagating the chain reaction of lipid peroxidation. Hence, a single initiation event can result in conversion of hundreds of fatty acid side chains into lipid hydroperoxides [32].

A number of decomposition products of lipid hydroperoxides, such as malonaldehyde and hydroxynonenal, are known to be cytotoxic. The integrity of cell membranes provides an important protective mechanism by separating unsaturated lipids from biological oxidants and activated oxygen species and by maintaining a low intracellular concentration of oxygen. Any factors that affect this structural separation will cause damaging lipid peroxidation. Therefore, the choice of alimentary fats should be in such a way that the MUFA, which allow an optimal fluidity without risk of peroxidation, prevail.

Recently, antioxidants from extra-virgin olive oil have received special attention for their potential protective effects against the damage from biological oxidants. Relatively low concentrations of β-*carotene* are effective in preventing lipid peroxidation by single oxygen [39 – 41].

α-*Tocopherol* is considered the major lipid-soluble antioxidant in membranes, acting by several mechanisms, including: by scavenging free radicals (superoxide, hydroxyl radicals), which can initiate and propagate lipid peroxidation, by reacting with nitric oxide, and by deactivating single oxygen [40 – 43].

Among the phenolic constituents of olives and extra virgin olive oil, of special interest for their biological activities are hydroxytyrosol [44, 45], oleuropein [45] or caffeic acid [46]. Several studies of possible mechanisms of phenol action indicate that these compounds are able to scavenge free radicals [45, 47, 48] and to break peroxidative chain reactions. Olive oil phenols can prevent lipid peroxidation by metal chelation [46, 47, 49]. The antioxidant activity of these compounds depends on the hydrogen-donating capacity of the hydroxyl group. The oxidation of a hydroquinone such as hydroxytyrosol to an orthoquinonic structure normally occurs via a radical intermediate, the resonance-stabilized phenoxyl radical. Moreover, the stabilization of this radical is further enhanced by its dismutation equilibrium with quinonic and hydroquinonic forms. In contrast, the tyrosol-derived phenoxyl radical cannot dismutate, which probably explains the lack of a protective effect of this molecule against oxygen radicals.

Olive Oil and Cardiovascular System

Several hypotheses have been advanced to explain the development of cardiovascular disease and the lipid hypothesis has been given the most attention.

Even though cholesterol and saturated fatty acid intake may be primary determinants of serum cholesterol, the role of dietary fat in the development of atherosclerosis still remains controversial and not well understood. The question arises whether or not dietary saturated fats should be replaced by unsaturated fats. Unsaturated fats, especially n-3 PUFA, may be beneficial to human health [50]. In patients with hyperlipidemia, only at high doses of n-3 fatty acids results in a decrease of low density lipoproteins (LDL). However, these fatty acids consistently lower serum triacylglycerols in normal subjects and in patients with hypertriacylglycerolemia. Diets high in n-6 and n-3 PUFA may lead to a decrease in serum cholesterol, but replacing saturated with

unsaturated lipids may not be desirable because of their ability to oxidize easily.

Diets high in oleic acid are now recognized to be as effective in lowering serum cholesterol as diets rich in linoleic acid [51, 52]. However, in contrast to polyunsaturated fats, monounsaturated fats have the important advantages of not only lowering LDL, but also of being much less susceptible to oxidation. Experiments with rabbits and humans fed diets rich in oleic acid (MUFA-rich diet) compared to linoleic acid (PUFA-rich diet), showed that LDL was much less suceptible to copper-catalysed oxidation and underwent less degradation by macrophages after incubation with endothelial cells [53]. Several other studies showed that the linoleate content of LDL strongly correlated with the extent of oxidation and the reverse trend was observed with the oleate content of LDL. In studies with hypercholesterolemic subjects, diets rich in oleic acid produced LDL more resistant to oxidation [54]. And in subjects with impaired glucose tolerance, a PUFA-diet with a moderate amount of fat tended to increase the susceptibility of LDL to oxidation as compared to a higher fat diet rich in MUFA [55].

Furthermore, Mattson and Grundy [56] published a study conducted with 20 patients (12 normo-lipidemic and 8 hypertriglyceridemic individuals) under metabolic control, fed hyperlipidic diets (40 percent of total calories) in which the predominant fatty acids were either saturated, monounsaturated, or polyunsaturated. The results of this study showed that oleic acid was as effective as linoleic acid in lowering LDL-cholesterol levels in normo-triglyceridemic patients, and oleic acid seemingly reduced HDL-cholesterol levels less frequently than did linoleic acid.

Therefore, olive oil diets, with lower linoleic acid and higher oleic acid contents, have a clear advantage by lowering plasma cholesterol by the same degree as PUFA and at the same time decreasing the risk of LDL oxidation [33].

Recent findings suggest that LDL oxidation is inhibited by polyphenolic compounds found in olive oil [57-59]. The protective effect of oleuropein has been demonstrated on *in vitro* oxidation of human LDL by measuring vitamin E disappearance, thiobarbituric acid-reactive substances formation and lipid hydroperoxide quantification [60, 61]. Wiseman and coworkers [62] reported that dietary antioxidants in unrefined virgin olive oil may significantly increase the resistance of rabbit LDL particles to an in vitro oxidative stress measured by malondialdehyde formation. Thereby, the low incidence of atherosclerosis in populations utilizing olive oil as their primary source of fat, such as the Mediterranean population, may be ascribable not only to the high unsaturated/saturated fatty acid ratio characteristic of olive oil, but also to the unique antioxidant properties of its phenolic compounds [57].

Finally, different studies have demonstrated that olive oil yields a higher amount of trienoic prostaglandins than the administration of sunflower oil [63]. In man, dienoic prostaglandins derived from arachidonic acid (20:4*n*6) prevail. Monoenoic prostaglandins, which derive from dihomo-γ-linolenic acid (20:3*n*6), scarcely appear in nature, whereas trienoic prostaglandins derived from the eicosapentanoic acid (20:5*n*3), are proportional to the amount of fatty acids of the n-3 series present in the diet [64] "Fig. (**1**)".

The increase in the formation of trienoic prostaglandins is considered favourable as it implies an amelioration of the lipidic profile (decrease in triglyceridemia and increment in high density lipoproteins, HDL) and especially a descent in platelet aggregation because thromboxane A_3 is less active than thromboxane A_2. A correct lipidic supply should therefore contain an adequate amount of α-linolenic acid (18:3*n*3) and that amount is precisely found in olive oil.

Other non-lipidic effects could also be involved in MUFA-diet protective effects. One of these involves the decrease in plasma levels of plasminogen activator inhibitor type 1 (PAI-1), the main inhibitor of fibrinolisis. Pérez-Jiménez and coworkers [65] showed that consumption of a MUFA diet produced a decrease in plasma levels of endothelial products such as von Willebrand factor, thrombomodulin and tissue factor pathway inhibitor; and PAI-1 plasma levels in young healthy males. These results suggested that the MUFA of the diet had a beneficial effect on endothelial function resulting in protective changes against thrombogenesis.

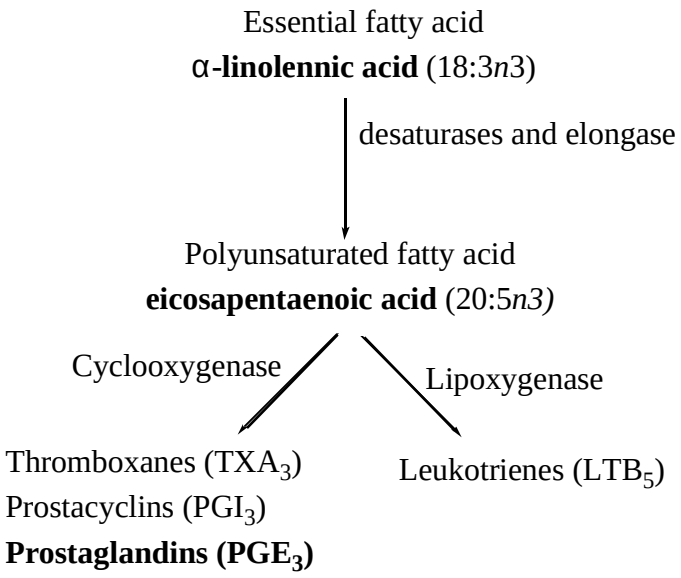

Fig. (1). Trienoic eicosanoids biosynthesis.

Recently, in an *in vitro* model of early atherogenesis based on cultured endothelial cells stimulated by cytokines, Massaro and coworkers [66] observed that oleic acid may contribute to the prevention of atherosclerosis also through a modulation of gene expression for endothelial leukocyte adhesion molecules. In this respect, Yaqoob and coworkers [67], showed that there is a significant decrease in the expression of intercellular adhesion molecule 1 (ICAM-1) by peripheral blood mononuclear cells from subjects consuming a MUFA diet and this diet also tended to decrease the expression of a monocyte- and macrophage-associated adhesion molecule, Mac-1.

Olive Oil and Diabetes

Type 2 diabetes (non insulin dependent diabetes) is associated with metabolic abnormalities such as central obesity, hypertension and dyslipidemia, which contributes to the very high rate of cardiovascular morbidity and mortality. Initiation of nonpharmacologic therapy should be started as soon as the diagnosis is made. The cornerstone of therapy consists of a regular exercise routine along with a diet consisting of 40 to 50 percent complex carbohydrates, 10 to 20 percent protein, and monounsaturated fats such as olive oil [68]. Different studies show that a diet with a high content in fatty acids represents a valid option to the classic diet rich in carbohydrates, as it ameliorates glucose tolerance and exerts a positive effect on HDL and LDL cholesterol and on the levels of triglycerides [69, 70]. In a 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, similar lowering effects on total cholesterol, LDL-cholesterol, and triglyceride levels were seen after both diets. And slightly higher levels of HDL-cholesterol were found in the MUFA diet. Furthermore, the insulin sensitivity and the first response insulin areas were similar, as were the 24-hours blood pressures and the von Willebrand Factor levels [71].

Several studies have compared the effects of different dietary fatty acids on cardiovascular risk factors in diabetic animal or human. Thomsen and coworkers [72] compared the effects on the diurnal blood pressure, glucose, and lipid levels of a diet rich in MUFA with a diet rich in PUFA in type 2 diabetic subjects. The two diets had similar, beneficial effects on glycaemic control, and cholesterol and lipoproteins concentrations. But a diet rich in MUFA had beneficial effects on the blood pressure (reduced systolic and diastolic blood pressure), while similar effects on glucose and lipid levels were observed in normotensive type 2 diabetic subjects. Recently, Giron and coworkers [73] examined lipid metabolism in diabetic rats fed a basal diet supplemented with olive oil, sunflower oil (rich in n-6 PUFA), or fish oil (rich in n-3 PUFA), respectively. In diabetes, olive oil fed rats showed the lowest levels of triglycerides, and plasma phospholipids were significantly higher in olive oil fed rats versus fish oil fed rats. These findings suggested that olive oil

contributes to a better control of the hypertriglyceridemia accompanying diabetes as compared with the other two diets.

Olive Oil and Immune System

Olive oil has classically been used as a placebo treatment in studies investigating the effects of fish oils on immune function because MUFA were typically regarded as being neutral fatty acids [74]. However, some of these studies found that the beneficial effects of olive oil in inflammatory diseases were often as potent as those of fish oil [75].

Thus, a smaller number of studies suggested that there may be beneficial effects of olive oil consumption on autoimmune diseases such as rheumatoid arthritis [76]. In particular, a much-cited study by Linos and coworkers [77] showed that frequent consumption of olive oil decreases the relative risk for developing rheumatoid arthritis in a Greek population. It is proposed that the suppressive effect of olive oil on the development of rheumatoid arthritis may be exerted via an effect on the immune system. Comparison of the effects of feeding olive oil (rich in oleic acid), safflower oil (rich in linoleic acid), and a high-oleic acid sunflower oil on these immune cell functions suggested that the effects were due to oleic acid rather than to a non lipid component of olive oil [78].

Many studies have investigated the effects of including high levels of n-9 MUFA or n-6 or n-3 PUFA in the diet of laboratory animals on *ex vivo* lymphocyte functions [78-80]. These studies show that feeding high levels of n-3 PUFA or n-9 MUFA results in markedly diminished lymphocyte proliferation, lymphocyte-mediated cytotoxicity and cell-mediated immunity; diets rich in n-6 PUFA are less suppresive [78, 79, 81]. Moussa and coworkers [82] compared the effect of a olive oil-based lipid emulsion containing 18 percent linoleic acid, and an emulsion based on soybean oil containing 52 percent linoleic acid on lymphocyte functions. A greater proportion of cells expressed the interleukin (IL) 2 receptor a-chain, CD25, after administration of olive oil-based lipid emulsion when compared to soybean oil-based emulsion. Moreover, the CD25 expression was positively correlated with oleic acid content on spleen lymphocyte phospholipids.

Several investigations have revealed that feeding laboratory rodents on diets rich in oleic acid suppresses natural killer (NK) cell activity [83]. Later, Yaqoob and coworkers [67] showed that NK cell activity in healthy humans was not significantly affected by chronic consumption of the MUFA diet. This observation contrasts with animal studies and the difference may be attributable to the higher amount of MUFA fat used in the animal studies.

Some studies have reported that diets rich in n-3 PUFA such as fish oil decrease pro-inflamatory cytokine production by macrophages, whereas other studies report that such diets increase production of these cytokines [32]. It has been shown that the presence of macrophages in different activation states may explain some of the contradictions in the literature [84]. In an interesting paper, Mulrooney and Grimble demonstrated that inclusion of oleic acid in the diet of rats suppresses responses to tumour necrosis factor (TNF) [85].

Tappia and Grimble [86] reported that olive oil has immunosuppressive activity because it has anti-inflammatory effects. These authors described a slight reduction in TNF production in response to a lipopolysaccharide stimulus from peritoneal macrophages of rats fed a diet containing olive oil after 8 weeks. However, this reduction did not show significant differences. After 4 weeks of feeding, olive oil significantly reduced IL-1 and enhanced IL-6 production in response to a TNF challenge, but after 8 weeks, olive oil enhanced IL-1 production.

De Pablo and coworkers [87] investigated the effect of a diet containing olive oil on the phagocytic activity and cytokine production by murine peritoneal cells. These results were compared with those obtained from mice fed diets containing sunflower oil or hydrogenated coconut oil (rich in saturated fatty acids). Phagocytic activity and IL-1 production were increased in olive oil fed mice as compared to the other groups. On the contrary, no significant differences were observed in the levels of TNF production, although the levels of this cytokine were slightly increased in mice fed the olive oil diet.

Recently, Wallace and coworkers [88] investigated the effects of dietary fats on macrophage-mediated cytotoxicity towards tumour cells. They found that lipopolysaccharide-stimulated TNF-alpha production by macrophages decreased with increasing unsaturated fatty acid content of the diet (fish oil < safflower oil < olive oil < coconut oil < a low fat diet).

These observations suggest that olive oil is able to modify the immune response and therefore, it may be used as an immunomodulatory agent.

Olive Oil and Cancer

Excessive consumption of dietary fat is recognized as a crucial factor in the increased risk for several common types of cancers, including breast, colon, pancreatic and prostatic cancers [89, 90]. However, the potential mechanisms of the cancer-promoting effects of different fats have not been identified completely. Moreover, the influence of the various types of fat on carcinogenesis may differ depending on the organ affected. Studies in animal models [91, 92] and recent observations in humans, have provided evidence that a high intake of n-6 PUFA stimulates several stages in the development of mammary and colon cancer. Several mechanisms have been proposed in order to explain the promoting effects of n-6 PUFA (mainly linoleic acid) on mammary carcinogenesis. They may be related to effects exerted by these fatty acids on the endocrine system, the immune system, eicosanoid metabolism, cell membrane fluidity, cell-cell interactions, or lipid peroxidation process.

In rodents, diets enriched with n-6 PUFA effectively shortened the latency period for tumour appearance, promoted growth and increased the incidence of mammary tumours as compared to diets with a high content of saturated fatty acids [93]. In contrast, diets enriched with n-3 PUFA, seem to prevent cancer by influencing the activity of enzymes and proteins related to intracellular signalling and, ultimately, cell proliferation [94, 95].

The MUFA have received less attention than their n-6 and n-3 fatty acid counterparts. Recently, several case-control epidemiologic studies from Spain [96], Italy [97] and Greece [98, 99], have demonstrated that increased dietary intake of olive oil is associated with a small decreased risk or no increased risk of breast cancer, despite a higher proportion of overall lipid intake. Experimental animal model studies of high dietary fat and cancer also indicate that olive oil has either no effect or a protective effect on the prevention of a variety of chemically induced tumours [100]. However, Cohen, L.A. and coworkers [101], in an induced rat mammary tumour model study, determined whether the level of oleic acid in olive oil is a key determinant of its protective effects. They compared the inhibitory effects among three different types of olive oil containing 54, 70 and 88 percent oleic acid and 20, 15 and 5 percent linoleic acid, respectively. Olive oil containing 80 percent oleic acid and 5 percent linoleic acid exhibited the lowest level of adenocarcinomas and the highest level of the more benign adenocarcinoma arising from within a fibroadenoma. These results show that oleic acid may reduce the risk of breast cancer and suggest that future studies on the health benefits of olive oil should take into account the type as well as the amount of olive oil. Furthermore, Newmark [100] proposed that the high squalene content of olive oil, as compared to other human foods, is a major factor in the cancer risk-reducing effect of olive oil. Later, Rao and coworkers [102] showed that squalene significantly suppresses colonic aberrant crypt foci formation and crypt multiplicity.

Currently, there is growing evidence that reactive oxygen species are involved in the aetiology of fat-related neoplasms such as cancer of the breast and colorectum. Owen and coworkers [103] have demonstrated that the antioxidant phenolic compounds present in olive oil are potent inhibitors of free radical generation by the faecal matrix. This indicates that the study of the interrelation between reactive oxygen species and dietary antioxidants is an area of great promise for elucidating mechanisms of colorectal carcino-genesis and possible future chemopreventive strategies.

Olive Oil and Digestive System

Cholelithiasis is one of the most common diseases in Western countries. Its principal features are the production of cholesterol stones or biliary pigments. The precipitation of cholesterol

crystals and the subsequent formation of gallstones occurs due to the hypersaturation of bile with cholesterol and as a consequence of a faster nucleation time.

It has been demonstrated that oleic acid enhances gallbladder emptying consequently reducing cholelithiasis risk, which is justified as the mentioned acid is the substance with the highest capacity to stimulate choleycistokinin [2]. Moreover, olive oil has been proven to dilate the Oddi sphincter keeping it open for a longer time, while the synthesis and excretion of bile salts is stimulated [104, 105].

Recently, Bravo and coworkers [106] have studied the influence of dietary saturated and unsaturated fat on hepatic cholesterol metabolism and the biliary excretion of chylomicron cholesterol in the rat. Their results indicated that feeding rats MUFA or n-6 PUFA as compared to saturated fat in the diet promotes the storage of cholesteryl ester in the liver and leads to increased bile acid synthesis, resulting in the more rapid excretion of cholesterol originating from the diet via the bile.

Recent studies have confirmed that the type of dietary fat supplied (especially the oleic acid content) leads to differences in the pancreatic response to food. This fact may be related to the balance between factors that stimulate and inhibit pancreatic exocrine secretion. In this context, Ballesta and coworkers [107] have demonstrated in dogs that long-term adaptation to two diets that only differed in the quality of dietary fat (olive and sunflower oil) leads to very different patterns in the pancreatic response to food, in terms of both volume of exocrine secretion and electrolyte and enzyme content in the juice secreted. Lipase and amylase outputs, and protein concentration were significantly higher in the group fed on the diet rich in sunflower oil. Food intake was not followed by any change in flow-rate or electrolyte or protein content in the group given the diet rich in olive oil. Amylase activity and output were also lower in this group, as was lipase output, whereas activity and specific activity of chymotrypsin were lower in dogs fed on the diet containing sunflower oil.

Later, Yago and coworkers [108] investigated in human subjects whether or not the ingestion of two liquid meals that differed only in their fatty acid composition (due to the addition of olive oil (group O) or sunflowerseed oil (group S) as the source of dietary fat) would lead to differences in the exocrine pancreatic secretion, in the basal period and in response to food. No significant differences were revealed in postprandial enzyme activities, except for lipase activity, which was higher in group O, probably in relation to the greater plasma cholecystokinin concentrations observed in this group.

The functional repercussion of these findings is evident, because of the possible use of diets rich in olive oil in pathologies that require pancreatic rest, like pancreatitis and others, and even in newborn where a physiological pancreatic immaturity exists.

Peptic ulcer disease currently includes a group of pathological processes of great importance due to their prevalence and incidence. Moreover, it still represents a high index of hospitalization and even of mortality. The origin of gastroduodenal ulcus is multifactorial with several factors coexisting: genetic, infections (fundamentally due to *Helicobater pylor*i), environmental, psychological and nutritional. Basically, it is directly related to the rupture of the defence/aggression balance, that is an increase in the acid-pepsin secretory response and alterations of the defensive mechanisms of the mucosa [109].

Currently the aim of the peptic ulcer treatment consists in reasonably managing the mechanisms which affect its pathogenesis, reducing acid secretion, eradicating Helicobater pylori, increasing the quantity and quality of different components of the mucus-bicarbonate barrier or in promoting the action of different useful elements on the digestive epithelium.

Since 1886, when Ewald and Boas [110] 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 in different segments of the gastrointestinal tract inhibits acid secretion, with olive oil (or oleic acid) being used in most of them [111–114]. Serrano and coworkers [110] examined the effects of adaptation for 30 days to two diets that only differ in the type of dietary fat (olive and sunflower oil) on the gastric acid secretory

response to food and on the circulating levels of gastrin, somatostatin and peptide YY (PYY) in humans. In this study, the subjects fed the olive oil-enriched diet showed lower values of intragastric acidity when compared with those fed the sunflower oil-enriched diet. These results complement the findings of Taits [116] in gastric and duodenal ulcer patients. He observed that the substitution of animal fat for olive oil in the diet was associated with a significant reduction in the size of the ulcer as well as an important increase in the percentage of healing.

The effect of olive oil on gastric acid secretion involves the suppression of serum gastrin and higher levels of PYY. Circulating somatostatin does not mediate the attenuation of acid response or the suppression of postprandial gastrin. A role for somatostatin as a paracrine mediator in the inhibition of gastrin release cannot be excluded [109]. On these bases, it appears that consumption of olive oil may be very useful in the nutritional therapy of those gastrointestinal diseases requiring a limitation of acid secretion.

In contrast, other studies that investigated the effect of fish oil and olive oil on the gastric mucosal damage in rats induced by cold-restraint stress [117] and in promoting the healing of indomethacin induced gastric lesions [118] suggested that olive oil had no effect in protecting from gastric ulceration or in promoting the healing of gastric lesions.

Among the possible peptic lesions in the gastrointestinal mucosa, the ones induced by non steroidal antiinflammatories, aspirine and alike drugs (NSAID) still constitute one of the most important sanitary challenges. The incidence of this iatrogenia and even worse, of its complications such as digestive haemorrhage or perforation, is increasing due to the progressive increment in the consumption of these drugs in the Occidental world, especially in elderly people. Therefore it is only natural that the scientific community and the pharmaceutical industry spare no efforts in order tho obtain pharmacological strategies designed to minimize this important untoward effect.

Although many aspects remain unclear, classically the damage induced by NSAID on the digestive mucosa is understood as the result of a topical and direct action and another indirect or systemic action depending precisely on the inhibition of prostaglandin synthesis. In the last years other etiopathogenic factors have been evaluated, especially the underlying vascular alterations. Leukocytes after being activated, are transformed into secretory cells producing numerous damaging substances, especially oxygen free radicals. In fact, these radicals modify cellular integrity affecting structural constituents and endangering cellular viability.

The results obtained in a recent experimental study using rats fed for six weeks diets enriched with extra virgin olive oil (5 and 20 percent), have demonstrated that the presence of this type of olive oil in the diet affords a higher resistance against NSAID-induced ulcerogenesis. In this sense, rats were shown to be less sensitive to indomethacin, NSAID of elevated gastrointestinal toxicity, and the damage reduction was statistically significant and dependent on the olive oil content. Regarding the data of associated leukocyte inflammatory response and the generated oxidative stress, those animals fed olive oil diets exhibited an important descent in inflammation levels. Parallel results were obtained in the production of oxygen free radicals and in those parameters related to vascular lesions of ischaemic type. Complementarily, in the glutathion determination interesting increases in the endogenous thiol and potent antioxidant were detected, particularly in those animal groups which had been fed olive oil enriched diets.

Currently, there has been an increasing interest in the possible role of reactive oxygen metabolites (ROM) as mediators of cellular damage in several gastrointestinal diseases, and ROM have been implicated in ischaemia-induced permeability changes of the intestine, in Crohn's disease and in ulcerative colitis [119]. Manna and coworkers [120] investigated the injurious effects of ROM on the intestinal epithelium and the possible protective role played by two olive oil phenolic compounds, hydroxytyrosol and tyrosol, using the Caco-2 human cell line. They observed that the H_2O_2-induced alterations were completely prevented by preincubating Caco-2 epithelial intestinal cells with hydroxytyrosol (250 μmol/L) and when the oxidative stress was induced by xanthine oxidase, complete protection was obtained at a concentration of polyphenol as small as 100 μmol/L. In contrast, tyrosol was ineffective. These results demonstrated that

hydroxytyrosol can act as a biological antioxidant in a cell culture experimental model and suggested that dietary intake of olive oil polyphenols could exert a protective effect against those intestinal pathologies whose aetiology has been related to ROM-mediated injuries, especially those characterized by changes in epithelium permeability such as inflammatory diseases.

ABBREVIATIONS

EC = European Community

HDL = High density lipoproteins

ICAM-1 = Intercellular adhesion molecule 1

IL = Interleukin

LDL = Low density lipoproteins

MUFA = Monounsaturated fatty acids

NK = Natural killer

NSAID = Non steroidal antiinflammatory drugs

PAI-1 = Plasminogen activator inhibitor type 1

PUFA = Polyunsaturated fatty acids

PYY = Peptide YY

ROM = Reactive oxygen metabolites

TNF = Tumour necrosis factor

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