

# Olive Oil, the Mediterranean Diet, and Cardiovascular Health

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## The Mediterranean Diet

Inhabitants of Southern European and North African regions surrounding the Mediterranean Sea have a longer life expectancy and lower risk of chronic diseases than in other regions of the world.<sup>1</sup> It is believed that the diet and lifestyle of these Mediterranean populations have led to decreased rates of cancer, diabetes, and heart disease. The 1968 Seven Countries Study concluded that coronary heart disease was not a major indicator of mortality in men who inhabited the Greek island of Crete.<sup>2</sup> The occurrence of myocardial infarction, fatal and nonfatal, was 26 in 10,000 Cretans, in contrast to the Northern Finland cohort, where the rate was 1,074 in 10,000.<sup>3</sup> Although the Mediterranean diet varies somewhat regionally, its nutritional model of whole grains, vegetables, fruits, red wine, and olive oil is believed to contribute to decreased rates of coronary heart disease. A recent survey in the region of Girona, Spain, of 3,179 subjects found that adherence to the traditional Mediterranean diet was inversely associated with body mass index and obesity, major risk factors for heart disease.<sup>4</sup> Health benefits of the Mediterranean diet have precipitated studies on the effects of its various components, specifically extra virgin olive oil (EVOO).

Mediterranean countries have maximized their use of olive oil, becoming the largest consumers, producers, and exporters of both olives and olive oil. Together they manufacture approximately 90% of the total olive oil produced. Because of olive oil's role as the primary source of fat intake, the Mediterranean diet is high in monounsaturated fatty acids (MUFA), specifically oleic acid, and low in saturated fatty acids (SFA).<sup>5</sup> Studies affirm that dietary *cis*-MUFA have a greater antiatherosclerotic effect than SFA and are comparable with the effects of polyunsaturated fatty acids (PUFA) on cardiovascular risk factors.<sup>6,7</sup>

Worldwide, the Japanese and Cretan populations have the lowest SFA intake, 3% to 8% and 8% of total fat consumption, respectively. The Japanese and Cretan co-

horts in the Seven Countries Study had the lowest ischemic heart disease (IHD) mortality rates.<sup>8</sup> Cretans exhibited a low rate of IHD and low plasma cholesterol levels despite their high fat diet (33% to 40% of caloric intake).<sup>9</sup> For Cretans, olive oil provides approximately 29% of the total dietary energy and this accounts for the island's unusually high MUFA-to-SFA ratio.<sup>10</sup> Conversely, Finland, with the world's highest SFA intake at 20% of their diet, has the highest rates of IHD mortality, with a rate of 132.2 IHD deaths per 100,000 deaths, and Japan and Greece have a rate of 28.6 and 64.7, respectively<sup>11</sup> (Fig. 1A, 1B). Per capita olive oil consumption can be loosely correlated to rates of IHD mortality, with the exception of France and Japan.

Although a variety of factors play a role in heart disease mortality rates, including differing regional diets, health care quality, and socioeconomic status, it is generally established that the Finnish diet of fatty red meats, butter, and bread is highly conducive to heart disease when compared with the Mediterranean diet. Fat- and calorie-dense foods support the physically demanding Finnish lifestyle of living and laboring in cold, wet conditions. Other factors, including genetic differences, stress levels, and Finland's arduous work environments, can contribute to these observed health disparities. In contrast, whole grains, fresh vegetables, red wine, and olive oil are sufficient in the temperate Mediterranean basin and, as research now shows, this dietary complex might contribute to a decreased risk of cardiovascular disease. Epidemiologic and biologic evidence also suggest that moderate consumption of red wine has an important role in low rates of IHD because of the antioxidative nature of its resveratrol component. It should be noted also, that although diet does influence cardiovascular health, a host of confounding variables, such as lifestyle, exercise, stress level, environment, and genetics, play a role in the health of those in the Mediterranean region.

The Japanese and Mediterranean diets and lifestyle are remarkably similar, and both emphasize physical activity. The core components of the Japanese and Mediterranean diets are cereals; vegetables such as beans and nuts; and lean meat, such as fish (Fig. 2A, 2B). Although the Japanese population only has a per capita consumption of olive oil of 0.24 kg per year, vegetables and vegetable oils are integral to the diet.

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### Abbreviations and Acronyms

CM	= chylomicrons
EVOO	= extra virgin olive oil
ICAM-1	= intercellular adhesion molecule-1
IHD	= ischemic heart disease
IMT	= intima-media thickness
MUFA	= monounsaturated fatty acids
NO	= nitric oxide
PAI-1	= plasminogen activator inhibitor-1
PUFA	= polyunsaturated fatty acids
SFA	= saturated fatty acids
VCAM-1	= vascular cell-adhesion molecule-1

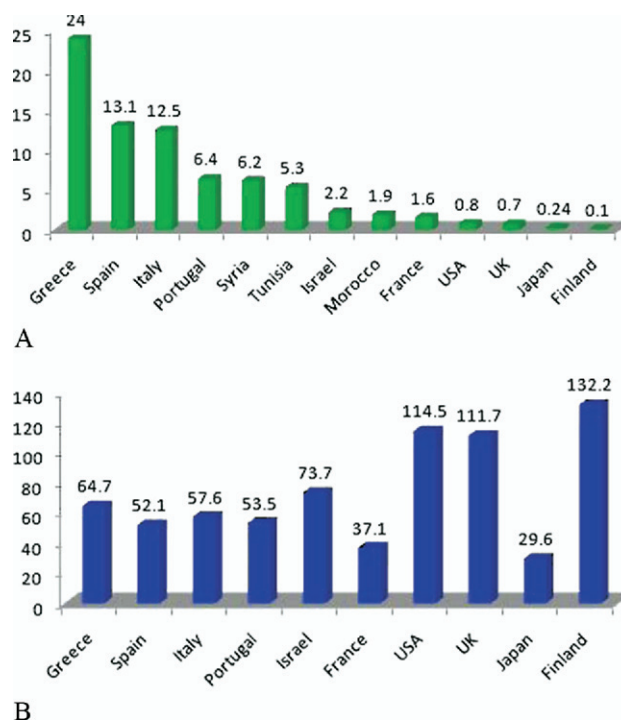
approved a new pyramid incorporating elements of the Japanese and Mediterranean diets (Fig. 2C). The previous 1992 Food Pyramid was revised to emphasize regular moderate consumption of olive oil and red wine, encourages daily exercise, and distinguishes between good and bad fats, and whole and processed grains. This article intends to review the data that links olive oil to decreased rates of heart disease risk factors, including arteriosclerosis, high blood pressure, and hypercholesterolemia.

### From olive to oil

The Mediterranean region is positioned at the convergence of the hot Saharan and the cool Atlantic climates. This results in dry summers and mild winters, which provide a favorable extended growing season for vegetables and fruits.<sup>12</sup> The region's substantial sun exposure has been correlated to the high antioxidant content in plants. Vegetation native to the area has augmented its production of antioxidants to defend against reactive oxygen species produced during photosynthesis. Recent epidemiologic studies have established an inverse relationship between intake of fruit and vegetable-based antioxidants and mortality rates from chronic diseases.<sup>13</sup>

The most widespread species of olive is the *Olea europaea* and its genus includes 35 species of evergreen shrubs and trees.<sup>14</sup> Olive trees have an unusual ability to develop roots from temporary buds at the lower end of their trunks, are resistant to severe weather conditions, and are able to grow in infertile soil. Olive fruit maturation spans several months and its taste and chemical composition is dependent on growing conditions, including latitude, water availability, and temperature. The maturation, harvesting, and developing process of olives and olive oil is heavily dependent on regional techniques.

Olive oil extraction is conducted through pressure, centrifugation, and percolation. Nonedible olive oil undergoes a refining process and is blended with edible oils to obtain regular olive oil.<sup>14</sup> Virgin olive oil is obtained under me-

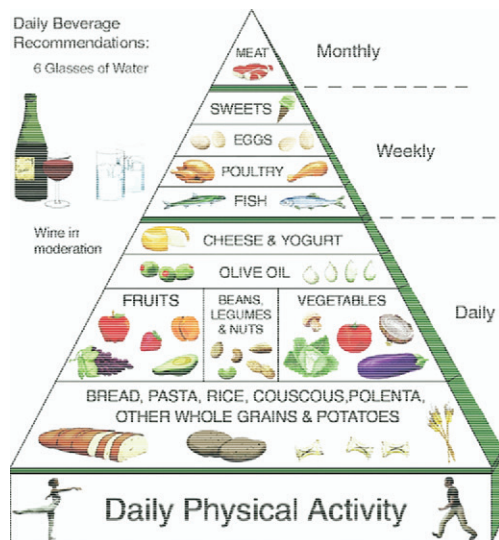


**Figure 1.** (A) Per capita consumption of olive oil in selected countries (Olive Oil Council Data). (B) Rates of coronary heart disease mortality according to the World Health Organization standard (Cardiovascular Disease Infobase).

chanical conditions that do not alter its composition and it is not mixed with other oils. EVOO is the highest quality olive oil and accounts for only 10% of oil produced. It has a free acidity, expressed as oleic acid, of not  $>0.8\%$ . Experts judge it for taste, mouth feel, and aroma; the oil tends to be most delicate in flavor. Refined olive oil has a free acidity of  $0.3\%$ . Regular olive oil, a blend of refined and virgin olive oils has a free acidity of  $0.1\%$ .<sup>14</sup>

The major components of olive oil are known as the saponifiable or glyceride fraction. Glycerols represent  $>98\%$  of total oil weight and are composed mainly of triacylglycerols (Table 1). Oleic acid makes up 70% to 80% of the fatty acids in olive oil. Minor components are present in about 2% of oil weight and include  $>230$  chemical compounds. These minor components are present almost exclusively in virgin olive oil because the refining process expunges these compounds. Considerable research has centered on extra virgin and virgin olive oil with the belief that these minor components contain important cardiovascular protective effects.

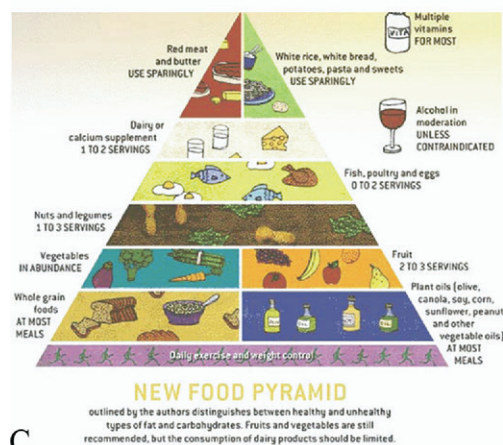
Several components of olive oil have beneficial health effects on the atherosclerotic and thrombotic pathways, which include lipid oxidation, hemostasis, platelet aggregation, coagulation, and fibrinolysis. Oleic acid, a major component, and the polyphenols—tocopherol, hydroxyty-



A



B



C

**Figure 2.** (A) Mediterranean Diet Food Pyramid ([neac.eat-online.net](http://neac.eat-online.net)). (B) Asian Diet Food Pyramid (<http://www.neac.eat-online.net>). (C) United States 2005 US Department of Agriculture—approved Food Pyramid (<http://www.mypyramid.gov>).

rosol, and oleuropein—exert the most substantial antiatherosclerotic effects.

### Oleic acid and heart disease

#### LDL, HDL, oxidation, and chylomicrons

Oleic acid is preventive in the development of atheromas and subsequent thrombi through their establishment of larger MUFA-to-PUFA and MUFA-to-SFA ratios, increased resistance to oxidation, and induction of larger hydrolysable chylomicrons (CM). Increased levels of LDL are important factors in arteriosclerosis, as they facilitate transport of cholesterol to arteries. LDL, which carries about two-thirds of plasma cholesterol, can infiltrate the arterial wall and attract macrophages, smooth muscle cells, and endothelial cells. Once embedded in the intima, LDL undergoes oxidation to oxLDL, contributing to foam-cell formation. Circulating oxLDL induces transcription of adhesion factors and is chemotactic for monocytes and leukocytes, thereby inhibiting egression of macrophages from plaques.<sup>15</sup> Conversely, HDL are antiatherogenic. Unlike their larger counterpart, HDL primarily deliver cholesterol to the liver to be metabolized and excreted or reused. It is also hypothesized that HDL are able to dislodge cholesterol molecules from atheromas in arterial walls.

LDL are less susceptible to free radical oxidation in a diet enriched by MUFA. MUFA are more stable than PUFA and more resistant to oxidation.<sup>16</sup> A supplemental diet of EVOO was found to decrease LDL oxidation in rabbits with experimentally induced arteriosclerosis.<sup>17</sup> It also led to lower atherosclerotic lesions in all aortic fragments isolated from the rabbits.<sup>18</sup> In addition, MUFA consumption, specifically that of oleic and linoleic acids, has been linked to a decrease in human plasma levels of LDL and an increase in serum HDL.<sup>19</sup> In one trial, 24 human subjects diagnosed with peripheral vascular disease were fed EVOO or refined olive oil for 3 months. It was found that LDL susceptibility to oxidation was considerably lower after the EVOO period.<sup>20</sup>

Additional studies suggest that in a meal enriched with MUFA, larger, more beneficial CM are secreted and rapidly cleared. CM transport dietary cholesterol and fats to the liver and periphery and are strongly atherogenic. They can penetrate the artery wall and facilitate foam-cell formation. One trial found statistically significant differences between olive oil's ability to increase the entry of CMs after ingestion of a meal more so than fish, safflower, and palm oils.<sup>21</sup> Oleic acid sustained a large CM size for the longest postprandial period of time in comparison with sunflower oil, mixed oil, and beef tallow.<sup>22</sup>

**Table 1.** The Major Glyceride Fraction and Minor Nonglyceride Fraction of Olive Oil

		Extra virgin	Virgin	Refined*†
Glyceride fraction				
Fatty acids (g/100 g)				
16:0	Palmitic	6.6	8.6	9.1
16:1n-7	Palmitoleic	0.4	1.1	0.6
18:0	Stearic	2.8	1.9	3.4
18:1n-9	Oleic	83.1	78.7	78.6
18:2n-6	Linoleic	5.1	8.3	6.2
18:3n-3	$\alpha$ -Linoleic	0.6	—	0.4
18:3n-	$\gamma$ -Linoleic	0.4	—	0.5
Nonglyceride fraction*				
Component (mg/kg)				
Aliphatic alcohols				
C <sub>18</sub> –C <sub>30</sub> alcohols		≤200	≤200	≤200
Triterpene alcohols		500–3,000	500–3,000	500–3,000
Total sterols‡		1,260.8	687.4	1,366.6
Cholesterol		1.9	2.8	2.0
$\Delta^5$ -Avenasterol		91.5	35.1	82.7
$\beta$ -Sitosterol		1,124.4	640.9	1,268.8
Sitostanol		7.3	2.3	1.1
Stigmasterol		8.2	6.4	12.0
Proteins (μg/kg)		1.76	1.76	1.26
Nonglyceride esters		100–250	100–250	100–250
Waxes		≤250	≤250	≤350
Hydrocarbons				
Squalene <sup>§</sup>		4,277	ND	2,598
$\beta$ -carotene		0.33–4.0	ND	ND
Polyphenols				
Lipophilic				
$\alpha$ -tocopherols <sup>¶</sup>		300	ND	200
Tocotrienols		ND	ND	None
Hydrophilic		40–1,000	40–1,000	None
Hydroxytyrosol $p$ -Tyrosol		Considered to be the major constituents of polyphenols, although data is inconsistent in quantifying the total concentrations, which depend on irrigation and harvesting techniques.		

\*All data from *Olive Oil and Health*<sup>10</sup> unless otherwise noted.

†Phenols are removed completely during the refining process and so are not present in refined oil. Currently there is no standard measurement for quantification of phenols in olive oil and present values cannot be compared with total polyphenol content or that of individual compounds.

§Squalene can make up to 40% of the weight of minor components.

¶ $\alpha$ -tocopherols make up to 95% of total tocopherols.

ND, No data.

### Hemostasis, platelet aggregation, and fibrinolysis

Oleic acid consumption decreases platelet sensitivity and aggregation, lowers levels of the coagulation factor VII (FVII), and increases fibrinolysis.<sup>23</sup> A full mechanistic explanation of the ability of dietary MUFA to decrease platelet aggregation has yet to be determined. Studies suggest that changes in membrane lipid fluidity and long-chain n-3 fatty acid from oleic acid can reduce platelet sensitivity to collagen and other coagulatory factors, induce a hypersen-

sitivity to aggregation antagonist, ADP, and inhibit thromboxane and prostaglandin synthesis.<sup>24,25</sup>

Fifty-one healthy adults participated in a 4-month trial with diets of high and moderate MUFA intake (18% and 15% of caloric intake, respectively) and a diet high in saturated fats (16% intake). At 8 weeks, those on the high and moderate MUFA diets demonstrated a decrease in platelet aggregation when exposed to platelet agonists, ADP, arachidonic acid, and collagen. The reduction in aggregatory



response to ADP and arachidonic acid was sustained in the high-MUFA group for the entire 16-week trial.<sup>26</sup>

Several animal studies confirmed olive oil's correlation to a reduction in thrombogenic factors. Rats fed an EVOO-enriched diet had a lower rate of thrombotic occlusion in an "aortic loop" model, a lower incidence of venous thrombosis, and an extended bleeding time relative to a control group on a normal diet.<sup>27</sup> Hypercholesterolemic rabbits fed a virgin olive oil diet compared with those on a SFA diet had a substantial decrease in platelet hyperactivity, subendothelial thrombogenicity, and platelet lipid peroxide production. In addition to marked changes in cholesterol, triglycerides, and HDL, the olive oil supplement stimulated endothelial synthesis of prostacyclin, and lowered thromboxane B2 plasma levels.<sup>28</sup>

Human trials found that 3 weeks of a MUFA-enriched diet resulted in a substantial reduction in von Willebrand Factor levels. von Willebrand Factor induces irreversible binding of platelets to the subendothelial collagen layer.<sup>29</sup> In a study of 25 people on low-fat, high-MUFA, or high-SFA diets, the MUFA diet induced a statistically significant decrease in von Willebrand Factor activity, 71.8% compared with 78.6% for the SFA diet.<sup>5</sup>

In the case of plaque rupture, tissue factor and FVII proteins are released as a component in the coagulation cascade. High FVII levels increase the risk of fatal coronary heart disease because of coronary thrombosis.<sup>30</sup> Tissue-factor complexes with FVII to activate the fibrin cascade. Research has found varying results on the effects of oleic acid on tissue factor and its inhibitor, tissue factor pathway inhibitor. A high MUFA diet has been linked to decreased levels of FVII.<sup>31</sup> A recent study with an isocaloric replacement of a MUFA-enriched Mediterranean diet found a reduction in plasma tissue factor pathway inhibitor. Although this can be seen as detrimental, it has been suggested that low tissue factor pathway inhibitor levels indicate the presence of the protease in the endothelium, which has a regulatory effect on thrombogenesis.<sup>5</sup>

Human trials have examined the effect of MUFA, oleic acid, and other vegetable oils on tissue plasminogen activator and plasminogen activator inhibitor-1 (PAI-1). When 21 young healthy males were given two low-fat diets and two oleic acid-enriched diets from virgin olive oil with the same dietary cholesterol as the low-fat diet, there was a decrease in PAI-1 plasma levels with both oleic acid-enriched diets. Substantial decreases in insulin levels and PAI-1 activity were observed, suggesting an improvement in insulin sensitivity during high-MUFA olive oil diets.<sup>32</sup> Another intervention compared the isocaloric substitution of a palmitic acid diet for a low-fat or a MUFA diet in 25 healthy male subjects. Both diets decreased

PAI-1 plasma levels with a higher reductive effect with the Mediterranean diet.<sup>5</sup>

A study involving urban and rural populations in Western Sicily found that conversion from an urban diet to a Mediterranean diet for 8 weeks substantially reduced FVIIc and PAI-1 activity. Conversely, a rural Mediterranean diet population that switched to an urban diet developed substantial increases in FVIIc, t-PA antigen, PAI-1 activity, and fibrinogen.<sup>33</sup> In a study with 15 volunteers ingesting either an SFA-rich diet or an MUFA-rich diet, the diet with MUFA from high oleic acid sunflower oil resulted in a lower concentration of FVIIc, LDL cholesterol, and triglycerides.<sup>34</sup> A study was comprised of 69 students in a controlled feeding environment, where they were fed sunflower, rapeseed, or olive oil. The sunflower oil decreased FXIIa, FXIIc, and FIXc after 4 weeks. Rapeseed oil induced zero change. The olive oil diet induced a decrease in FVIIc, FXIIc, FXIIa, and FXc.<sup>35</sup>

### Polyphenols and heart disease

The minor constituents of olive oil also have substantial vascular and cardioprotective effects. The unsaponifiable or nonglyceride fraction of EVOO is rich in hydrocarbons, nonglyceride esters, tocopherols, flavonoids, sterols, and phenolic constituents (Table 2). The proportions of these minor compounds depend on the manufacturing processes of oil. Because these processes vary by oil mill, it is difficult to quantify the dietary intake of these components; and Mediterranean countries tend to consume EVOO, which is much richer in phenolic compounds than refined oils.

The main antioxidants in olives are carotenoids and polyphenolic compounds. The primary polyphenols are oleuropein, hydroxytyrosol, and  $\alpha$ -tocopherol. Oleuropein, the major polyphenol, consists of up to 14% of the total net weight.<sup>36</sup> Hydroxytyrosol is a byproduct of oleuropein.<sup>37</sup>  $\alpha$ -tocopherol, also known as an active form of vitamin E, is highly resistant to oxidative degradation.<sup>38</sup> Although it exists in relatively low concentrations in olive oil, its daily consumption augments the overall antioxidant content in the human body and protects against free radicals and lipid peroxidation in humans.<sup>39</sup>

Polyphenols interfere with the chain of reactions initiated and supported by free radicals.<sup>40</sup> This prevents DNA damage, lipid hydroperoxide formation, and lipid peroxidation.<sup>41</sup> In addition, exogenous antioxidants increase the concentration of antioxidants present in the body and protect against degenerative diseases.<sup>42</sup> Flavonoids contribute by sparing the basal levels of  $\beta$ -carotene, urate, and vitamins C and E activity.<sup>15</sup> The phenolic compounds decrease the presence of cell-adhesion molecules, increase nitric oxide (NO) disposability, suppress platelet aggregation, and boost total phenolic

**Table 2.** Composite Results from Research on Oleic Acid and Polyphenol Effects on Cardiovascular Function

	Oleic acid effects	Polyphenol effects
Lipoproteins	↑ Serum HDL <sup>16</sup> ↓ Plasma LDL ↓ Oxidation of LDL <sup>17, 19</sup>	↑ Lag phase before oxidation <sup>46</sup> Maintain vitamin E basal levels <sup>40</sup> ↓ Oxidation of LDL <sup>40</sup> ↑ Inhibition of thiobarbituric acid reactive substances <sup>49</sup> ↑ Phenolic concentration
Chylomicrons	↑ CM secretion ↑ CM size <sup>22</sup>	
Platelets	↓ Collagen sensitivity ↓ Platelet aggregation High-MUFA diet ↓ vWF <sup>25</sup> ↓ Venous thrombosis ↓ Thrombotic occlusion ↑ Bleeding time <sup>27</sup> ↓ Platelet hyperactivity <sup>28</sup> ↓ Platelet aggregation ↑ Prostacyclin ↓ Sensitivity to ADP and arachidonic acid ↓ FVIIc response <sup>26</sup>	↓ Collagen-induced thromboxane production by 94% <sup>57</sup> ↓ C-reactive protein and interleukins, markers of inflammation <sup>60</sup> ↓ Inhibited platelet aggregation and camp-PDE <sup>58</sup> ↓ Aggregation when exposed to APD and collagen agonists <sup>57</sup>
Coagulation	↓ FVII <sup>31</sup> ↓ FVIIc <sup>34</sup> ↓ LDL cholesterol, triglycerides ↓ FXIIc, FXIIa, FXc <sup>35</sup>	↓ Postprandial increase in FVIIa <sup>53</sup>
Fibrinolysis	↓ Plasma PAI-1 ↑ t-PA <sup>5</sup> ↑ Insulin sensitivity <sup>32</sup>	↓ PAI-1 plasma concentration and activity <sup>53</sup>
Vasodilation		↑ Postprandial vasodilation ↑ Final products of NO Reversal of cholesterol-induced vasoconstriction <sup>53</sup> ↑ LDL oxidation resistance <sup>50</sup> ↑ Nitric oxide ↓ Lipoperoxides <sup>53</sup>
Adhesion molecules	↓ VCAM-1 and E-selection expression <sup>5</sup> ↓ Monocyte chemotaxis and cell adhesion	↓ Monocyte cell adhesion to endothelium ↓ VCAM-1 levels <sup>62</sup> ↓ Cell surface expression of ICAM-1 and VCAM-1 <sup>61</sup>

CM, chylomicrons; ICAM, intercellular adhesion molecule; MUFA, monounsaturated fatty acids; PAI-1, plasminogen activator inhibitor-1; PDE, phosphodiesterase; t-PA, tissue plasminogen activator; VCAM, vascular cell-adhesion molecule; vWF, von Willebrand Factor.

content of LDL to delay arteriosclerosis, reduce inflammation, and inhibit oxygen use in neutrophils.<sup>43</sup>

### LDL and oxidation

Free radicals are responsible for oxidation of plasma LDL into atherogenic oxLDL. They have deleterious effects on cellular membranes and internal structures, which can bring about the onset of cardiovascular disease.<sup>15</sup> The orthodiphenolic structure of hydroxytyrosol and oleuropein confers an especially strong antioxidant property.<sup>44</sup> It is believed that these polyphenols exert their antioxidant activity by chelating free metal ions, such as copper and iron, and also by scavenging free radicals.<sup>45</sup>

The effects of polyphenols on LDL susceptibility to

copper-mediated oxidation in rabbits with normalized levels of vitamin E was reported. Rabbits were fed diets of refined olive oil, EVOO, and high oleic sunflower oil. The EVOO rabbits demonstrated no change in cholesterol or vitamin E levels after 6 weeks. They had a 30% longer lag phase before oxidation compared with the refined olive oil and sunflower oil group.<sup>46</sup> Rats fed diets of olive oil had a decreased concentration of lipoproteins and thiobarbituric acid-reactive substances, end products of lipid peroxidation.<sup>47</sup>

The Attica epidemiologic study conducted in the primarily urban Greek province of Attica examined the correlation between Mediterranean diet adherence and levels of antioxidants and oxLDL. Those with the highest Mediterranean diet score, on average, had 11% higher levels of total

antioxidant capacity than those in the lowest tertile. In addition, the study found that those with the strongest adherence to the Mediterranean diet, on average, had 19% lower oxLDL-cholesterol levels than those with the lowest dietary score.<sup>48</sup>

A multicenter study, part of the Prevención con Dieta Mediterránea Studies (PREDIMED), conducted a randomized controlled trial of asymptomatic subjects at high risk for cardiovascular disease. Three-hundred and seventy-two participants from 10 Spanish Primary Care Centers were placed on a low-fat diet or a traditional Mediterranean diet supplemented with either nuts or virgin olive oil. A 3-month followup found substantial decreases in oxLDL levels in both Mediterranean diet groups.<sup>49</sup>

### NO activity and endothelial dysfunction

In hypercholesterolemia, the production of superoxide anions and other free radical species is increased in endothelial cells, smooth muscle cells, and monocytes when compared with that of normocholesterolemic controls.<sup>50</sup> These species degrade NO and a damaged endothelium cannot produce sufficient NO, which can lead to monocyte recruitment, platelet aggregation, and thrombosis. Diminished NO bioactivity can cause constriction of coronary arteries during exercise and vascular inflammation leading to lipoprotein oxidation and foam-cell formation.<sup>51</sup>

On the other hand, other studies have found that oxLDL stimulates NO synthase transcription and synthesis in bovine aortic cells.<sup>52</sup> Studies also demonstrated increased expression of NO and NO synthase in atherosclerotic rabbit aorta tissue and human atherosclerotic plaques. It is postulated that this overproduction accompanies rapid oxidative inactivation or conversion of the NO to toxic nitrogen oxides because of accumulation of superoxide anions and free radicals.<sup>51</sup>

Studies suggest that vascular dysfunction can be reversed through intake of agents able to scavenge these radicals.<sup>39</sup> Consumption of a meal with high phenolic EVOO improved endothelium-dependent microvascular vasodilation during the first 4 hours of the postprandial period in hypercholesterolemic volunteers. Subjects fed the phenol-rich meal displayed a higher concentration of NO and lower lipoperoxide levels than those fed a low-phenol meal. This improvement is linked with a decrease in oxidative stress and increase in the final products of NO.<sup>53</sup> In addition, oleuropein stimulated NO production in mouse macrophages and activated the inducible form of NO synthase.<sup>54</sup>

Rabbits with cholesterol-induced impaired endothelial dilation were given a supplement of  $\alpha$ -tocopherol or  $\beta$ -carotene. The  $\alpha$ -tocopherol led to complete reversal of vasoconstriction. Additionally, increased concentrations of

$\alpha$ -tocopherol were detected in the plasma, aorta, and LDL. The  $\alpha$ -tocopherol was able to increase the resistance to LDL ex vivo oxidation after exposure to copper.<sup>50</sup>

A survey of 20,343 subjects, as part of the Greek cohort of the EPIC (European Prospective Investigation into Cancer and Nutrition) study, found that adherence to the Mediterranean diet was inversely related to systolic and diastolic blood pressure. Olive oil, vegetables, and fruits were the principal factors responsible for the overall effect of the Mediterranean diet on arterial blood pressure. Interestingly, cereals, generally considered to be beneficial to health, were correlated to increases in arterial blood pressure.<sup>55</sup>

The PREDIMED study investigated the effect of the low fat and two Mediterranean diet diets on 772 adults with high risk for cardiovascular disease. The Mediterranean diet participants with hypertension showed statistically significant reductions from baseline values in systolic blood pressure, and the low-fat group showed a mean increase in blood pressure. The Mediterranean diet participants demonstrated improved lipid profiles, decreased insulin resistance, and reduced concentrations of inflammatory molecules compared with the low-fat group.<sup>56</sup>

### Platelet aggregation

An in vitro study examined the effects of hydroxytyrosol and oleuropein on platelet aggregation. Hydroxytyrosol completely inhibited ADP (2  $\mu$ M) and collagen (2  $\mu$ g/mL) induced platelet aggregation in platelet-rich plasma.<sup>57</sup> The aggregation inhibition potency of hydroxytyrosol was found to be equivalent to that of aspirin. In the same study, hydroxytyrosol was also found to inhibit collagen and thrombin-induced thromboxane B2 production. In human volunteers, it was also reported that the pure olive leaf extract with 5.40 mg/mL polyphenol oleuropein, was capable of inhibiting in vitro platelet activation in healthy, nonsmoking male individuals.<sup>58</sup>

Dell'Agli and colleagues<sup>59</sup> examined the effects of oil extracts of high and low phenol content and single phenols on platelet aggregation to prove that cAMP and cGMP-phosphodiesterases might be a biologic target of platelet aggregation inhibition. Of the polyphenols examined—oleuropein, hydroxytyrosol, tyrosol, and the flavonoids quercetin, luteolin, and apigenin—oleuropein had the most substantial effect on platelet aggregation inhibition followed by luteolin. The oil extracts and the single phenols exhibited inhibition in a concentration-dependent manner on aggregation and on cAMP-phosphodiesterases.

In a randomized controlled trial in Naples, Italy, 180 subjects with a metabolic disorder were instructed to follow a Mediterranean-style diet supplemented with olive oil. After 2 years, there was marked improvement in endothe-

lial function, with statistically significant decreases in blood pressure, cholesterol, insulin and glucose levels and platelet aggregation response to L-arginine. There were substantial reductions in markers of systemic vascular inflammation, including C-reactive protein and interleukins. Sixty of 90 participants in the intervention group experienced reductions in risk factors, such that they were no longer classified as having metabolic syndrome.<sup>60</sup> It is still unclear through what exact pathway polyphenols inhibit platelet aggregation. It is hypothesized to be mediated by a phenol-induced decrease in eicosanoid production or through the degradation of cAMP in conjunction with inhibition of the arachidonic acid cascade.<sup>59</sup>

### Endothelial adhesion molecules

The inflammatory response during atherogenesis includes adhesion of leukocytes, monocytes, and lymphocytes to the endothelium. Adhesion of these molecules is facilitated by intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selection.<sup>61</sup> One study reported that physiologically relevant dosages of phenolic extract from EVOO reduced cell surface expression of ICAM-1 and VCAM-1.<sup>62</sup> A mixture of olive oil polyphenols, including oleuropein, hydroxytyrosol, and tyrosol, also induced a decrease in VCAM-1 mRNA levels and promoter activity and ICAM-1 and E-selection expression.<sup>62</sup> The PREDIMED study on human subjects found that the Mediterranean diet supplemented with olive oil resulted in statistically significant reductions in inflammatory markers including C-reactive protein, interleukin-6, ICAM-1, and VCAM-1 when compared with a low-fat diet.<sup>56</sup>

Lastly, little research has examined the effect of the Mediterranean diet on the vascular wall. The PEDIMED study examined the effect of olive oil on morphologic changes in the vascular wall. Measurements of carotid intima-media thickness (IMT) have been used as surrogate markers to evaluate the severity of atherosclerotic disease. An increase of 0.2 mm in IMT has been reported to imply a 28% and 31% increase in risk for stroke and myocardial infarction, respectively.<sup>63</sup> Data collected on 190 participants found that those who consumed the least amount of olive oil have the highest IMT. The study found a statistically significant difference in IMT between those who consumed 6 to 34 g and 35 to 74 g of olive oil per day.<sup>64</sup>

### SUMMARY

In recent years, the Mediterranean diet has become increasingly popular, gaining widespread attention among the nutrition and research communities. Increasing awareness of the role of fats, sugars, and processed foods

in obesity, cardiovascular disease, diabetes, and cancer is generating modifications in dietary habits. Many of those interested in weight loss, cardiovascular disease prevention, and other health issues are largely inclined to adopt the Mediterranean diet. The Mediterranean diet encourages a balanced intake of a broad range of foods and does not require exclusive adherence to a single nutrient or type of food. The popularity of pizza, pasta, and beans, elements of the Mediterranean diet, suggests that it can satisfy many dietary preferences. Ultimately, it is pivotal that Mediterranean diet foods are prepared without SFA, and regularly incorporate olive oil, fresh vegetables, and lean meats.

Olive oil is a type of food that can easily replace commonly used animal oils, lard, and butters that are detrimental to one's health. Research has demonstrated the advantageous effects of olive oil on health on the epidemiologic and cellular levels. Much more research needs to be conducted especially at the cellular level, to more fully understand the pathways by which oleic acid and the polyphenols in olive oil help to reduce cardiovascular disease risk factors. In addition, clinical studies, prospective or randomized, with hard outcomes such as death or myocardial infarction, are still lacking and difficult to conduct. Nevertheless, current evidence suggests that the components within EVOO exert a beneficial effect on cardiovascular health. As such, it seems that integrating olive oil as a dressing, condiment, and cooking lubricant would be a positive health benefit in light of increasing rates of cardiovascular disease and obesity rates within the United States, the Mediterranean regions, and worldwide.

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