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Olive Oil and Cardiovascular Health

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Abstract: The Mediterranean diet, in which olive oil is the primary source of fat, is associated with a low mortality for cardiovascular disease. Data concerning olive oil consumption and primary end points for cardiovascular disease are scarce. However, a large body of knowledge exists providing evidence of the benefits of olive oil consumption on secondary end points for the disease. Besides the classical benefits on the lipid profile provided by olive oil consumption compared with that of saturated fat, a broad spectrum of benefits on cardiovascular risk factors is now emerging associated with olive oil consumption. We review the state of the art concerning the knowledge of the most important biological and clinical effects related to olive oil and its minor components. The recent advances in human nutrigenomics associated with olive oil consumption will also be assessed. The wide range of benefits associated with olive oil consumption could contribute to explaining the low rate of cardiovascular mortality found in southern European–Mediterranean countries, in comparison with other westernized countries, despite a high prevalence of coronary heart disease risk factors.

Key Words: olive oil, phenolic compounds, monounsaturated, lipids, inflammation, oxidation, hypertension, gene expression

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INTRODUCTION

Olive oil is the primary source of fat in the Mediterranean diet. A high degree of adherence to the traditional Mediterranean diet has been associated with a reduced risk of overall and cardiovascular mortality, cancer incidence and mortality, and incidence of Parkinson and Alzheimer disease.[1,2] The most impressive benefits of this diet are, however, related to cardiovascular morbidity and mortality.[3] Despite this, data concerning olive oil consumption and primary end points for cardiovascular disease are scarce. A reduction in the relative risk of having a myocardial infarction was associated to a high exposure to olive oil consumption in a Spanish case-control study.[4] In a Greek cohort study, with a large sample size, the association between the ratio of monounsaturated (MUFA) to saturated (SFA) fatty acids, but not that of specific olive oil consumption, and cardiovascular and overall mortality was significant.[1] However, a large body of knowledge exists providing evidence of the benefits of olive oil consumption on secondary end points for cardiovascular disease.

The beneficial effects of olive oil on cardiovascular risk factors are now recognized, but are often attributed only to the high levels of MUFA present in olive oil.[5] Olive oil, however, is a functional food that, besides having a high-MUFA content, contains other minor components with biological properties.[6] Virgin olive oil (VOO), rich in phenolic compounds, is a natural product obtained directly from the fruit by mechanical procedures. Other natural oils, such as those obtained from the *Prunus cerasus* pits (sour cherry)[7] or the argan oil,[8] have also been shown to have bioactive properties. Thus, a high oleic acid intake could not be the only responsible factor for the health properties of olive oil. We review the state of the art concerning the knowledge of the most important biological and clinical effects related to the intake of olive oil and its phenolic compounds, as well as the extent to which we possess evidence of their health benefits.

OLIVE OIL COMPONENTS

The major components of olive oil are the fatty acids, of which the MUFA oleic acid represents from 55% to 83% of the total fatty acids, polyunsaturated fatty acids (PUFA) from 4% to 20%, and SFA from 8% to 14%. The minor components of olive oil constitute from 1% to 2% of the total content of an olive oil. They are classified into 2 types: (1) the unsaponifiable fraction that could be extracted with solvents after the saponification of the oil and contains squalene and other triterpenes, sterols, tocopherol, and pigments and (2) the soluble fraction that includes the phenolic compounds.[6]

The content of the minor components of an olive oil varies, depending on the cultivar, climate, ripeness of the olives at harvesting, and the processing system employed to produce the olive oils currently present on the market: extra virgin and virgin, olive oil (UE 1991), or pomace.[9] VOOs are obtained from the olive solely by physical means under conditions that do not alter the oil. Extra virgin olive oils are VOOs with a free acidity, expressed as grams of oleic acid per 100 grams of olive oil, less than 0.8 g of VOO and with an acidity less than or equal to 3.3 (International Olive Oil Council Regulation/T.15/NC.n3.Rev2.Nov24, 2006) ($\leq$2 in Europe, European Regulation N. 1513/0) that are submitted to a

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refining process in which some components, mainly phenolic compounds, and to a lesser degree squalene are lost.[9] By mixing virgin and refined olive oil, an ordinary olive oil (olive oil, UE 1991) is produced and marketed. After VOO production, the rest of the olive drupe and seed is processed and submitted to a refining process, resulting in pomace olive oil, to which a certain quantity of VOO is added before marketing.

LIPIDS AND LIPOPROTEINS

The report of the First International Conference on Olive Oil and Health, held in Jaen, Spain, in 2004 highlighted the benefits of olive oil consumption on the lipid profile.[10] The plasma cholesterol–predictive equations, developed in the mid-1960s[11,12] from data of controlled diet studies, showed that consumption of MUFA did not affect total cholesterol levels, but the consumption of SFA raised them. Consumption of PUFA and MUFA lowered total cholesterol. Other studies have confirmed these findings, although there were some data that in MUFA consumption the low-density lipoprotein (LDL) cholesterol–lowering effect was less, whereas the high-density lipoprotein cholesterol (HDL) was higher than those observed for PUFA.[13–15] The results of a meta-analysis of 14 studies showed that replacement of SFA by oils enriched in MUFA versus PUFA had similar effects on total, LDL, and HDL cholesterol. The PUFA-enriched oil had a slight triglyceride-lowering effect, and there was an increase in HDL cholesterol after MUFA consumption in some studies.[16] Recent data confirm the HDL cholesterol–increasing effect of olive oil consumption both within[17] and out of[18] the frame of the Mediterranean diet. Both a high-fat diet (40% of energy) rich in MUFA and low in SFA and a low-fat carbohydrate-rich diet had similar cholesterol-lowering effects. However, a high-MUFA diet did not lower HDL cholesterol or increase triglycerides as did the carbohydrate-rich diet. A meta-analysis of 10 studies provided first level evidence of the benefits of MUFA-rich diets compared with the carbohydrate-rich diets not only for healthy individuals but also for individuals with diabetes.[19] Few data exist comparing the effect of olive oil–rich diets on lipoprotein (a) levels, with no changes[20] or a decrease[21] in the lipoprotein when comparing an olive oil–rich diet with a sunflower-rich one.

Postprandial lipemia and hyperglycemia are recognized risk factors for atherosclerosis.[22] Both the amount and the type of fat ingested influence the postprandial lipemia. A 25-mL single dose of olive oil does not promote postprandial lipemia,[23] whereas 40- and 50-mL doses of any type of olive oil do.[24,25] Controversial results on the magnitude of the postprandial lipemia have been obtained when comparing an oral olive oil fat load with other fats.[6] Increased and prolonged postprandial triglyceride concentrations are associated with numerous conditions related to insulin sensitivity. Recently, postprandial insulin sensitivity has been reported to progressively improve as the proportion of MUFA with respect to SFA in dietary fat increases.[26] It must be taken into account, however, that although fat intake is one of the major nutritional determinants of the postprandial triglyceride response, it is also highly influenced by other components, including fiber, glucose, and starch, present in a meal, as well as if the meal is accompanied by alcohol.[27]

OXIDATIVE DAMAGE

Oxidized LDL is currently thought to be more damaging to the arterial wall than native LDL due to the ability of oxidized LDL to promote the atherosclerotic process.[28] Elevated concentrations of circulating oxidized LDL are predictors for coronary heart disease development.[29] The MUFA component, the oleic acid, and the phenolic compounds from olive oil have shown in vivo antioxidant activity both in experimental models and in human studies.[6] The fact that phenolic compounds from olive oil are bioavailable in humans, even from low doses [25 mL (22 g)] of olive oil, reinforces their protective role in vivo.[18,23] Oleate-rich LDL has been shown to be less susceptible to oxidation than linoleate-rich LDL.[30] In agreement with this, of 14 studies comparing the resistance of LDL to oxidation, only 2 indicated that MUFA-rich diets do not promote a higher resistance of LDL to oxidation than the PUFA-rich ones.[31]

Postprandial oxidative stress is linked with postprandial lipemia and hyperglycemia.[22] Contradictory results have been obtained on the in vivo antioxidant effect of phenolic compounds from olive oil in postprandial studies.[6] In olive oil doses where oxidative stress occurs (equal to or greater than 40 mL),[24,25] the in vivo lipid oxidative damage was inversely modulated in a dose-dependent manner with the phenolic content of the olive oil administered.[26] Oxidative stress associated with postprandial lipemia also contributes to endothelial dysfunction, which shifts hemostasis to a more thrombogenic state. In this sense, a VOO with a high content of phenolic compounds changes the postprandial hemostatic profile to a less thrombogenic state compared with a low phenolic content olive oil.[32,33]

Concerning sustained olive oil consumption, controversial results were obtained in the studies performed in 2004.[10] Recent results of the EUROLIVE (The effect of olive oil consumption on oxidative damage in European populations) study have provided definitive evidence of the in vivo antioxidant role of phenolic compounds from olive oil in humans.[18] The EUROLIVE was a large, crossover, multicenter, clinical trial performed in 200 individuals from 5 European countries. Participants were randomly assigned to receive 25 mL/d of 3 similar olive oils, but with differences in their phenolic content, in intervention periods of 3 weeks preceded by 2-week washout periods in which olive oil and olives were avoided. Results of the study showed that all olive oils increased HDL cholesterol and the ratio between reduced and oxidized forms of glutathione and decreased triglycerides, total to HDL cholesterol ratio, and DNA oxidative damage.[18,34] Consumption of medium and high phenolic content olive oil decreased the LDL/HDL cholesterol ratio, and the in vivo plasma circulating oxidized LDL, serum uninduced conjugated dienes, and serum hydroxy fatty acids. The increase in HDL cholesterol and the decrease in lipid oxidative damage were observed in a dose-dependent manner of the phenolic content of the olive oil administered.[18] In the EUROLIVE study, no differences were observed in the

protective effect of olive oil on DNA oxidation related to its phenolic content.[34] In a substudy with the Danish participants of the EUROLIVE, no changes on the etheno-DNA adduct formation, a lipid peroxidation–derived DNA damage, were observed among olive oils.[35] Protective effects of olive oil phenols on in vivo DNA oxidation, measured as 8-oxo-deoxyguanosine in peripheral blood mononuclear cells (PBMCs) and in urine, were found in healthy male subjects in a short-term study in which participants were previously submitted to a very low antioxidant diet.[36] In postmenopausal women, consumption of high-phenol extra virgin oil reduced the DNA oxidation, measured by the comet assay in peripheral blood lymphocytes, compared with low-phenol olive oil.[37] Further studies are warranted to investigate the role of phenolic compounds of olive oil on the different biomarkers for DNA oxidation.

INFLAMMATION AND VASCULOPROTECTIVE EFFECTS

Oxidation and inflammation are an intertwined process. Free radicals, besides promoting oxidative damage, activate pro- and anti-inflammatory cytokines. Major and minor olive oil components have been shown to be protective against inflammation and endothelial activation.[6] In animal models, a diet rich in olive oil suppressed the natural killer cell activity[38] and the expression of receptors for interleukin (IL)-2.[39] In human studies, LDL-induced monocyte adhesion to endothelial cells was lower after MUFA consumption than after that of SFA or PUFA in healthy individuals.[40] Human LDL enriched in oleic acid promoted less monocyte chemotaxis (52% lower) and reduced monocyte adhesion (77%) compared with linoleic-enriched LDL, when exposed to oxidative stress.[41] Consumption of an oleic acid–rich diet for 2 months promoted a decrease in the expression of the intracellular adhesion molecule-1 (ICAM-1) in PBMC of healthy subjects.[42] Inflammatory markers, such as the high-sensibility C-reactive protein and IL-6, and cell adhesion molecules, such as ICAM-1, have been reported to decrease after both short-term (3 months)[43] and long-term (2 years)[44] consumption of olive oil–rich diets, such as the Mediterranean diet. In postprandial studies, however, no differences were obtained in plasma levels of proinflammatory cytokines after the ingestion of different breakfasts rich in olive oil, walnuts, or butter, neither versus the baseline nor among treatments.[45]

Although the protective mechanism of oleic acid–rich diets on inflammation has been attributed to a decrease in the LDL linoleic acid content, oleic acid is not the single responsible factor for the anti-inflammatory properties of olive oil. Olive oil minor components have been shown to have anti-inflammatory, antihypertensive, and antiendothelial activation properties in experimental studies.[46] The olive oil phenolic compound named oleocanthal has been described as having similar properties to that of the anti-inflammatory molecule ibuprofene.[47] The bioavailability of oleocanthal in humans from olive oil ingestion remains, however, to be elucidated. Several studies have examined the anti-inflammatory and vasculoprotective effect of olive oil phenolic compounds in humans (Table 1). In these studies, olive oil phenolics have been shown to be effective in reducing the eicosanoid inflammatory mediators derived from arachidonic acid, such as thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$[48–51] and other inflammatory markers, such as high-sensibility C-reactive protein or IL-6.[52] Contradictory results have been obtained concerning the effect of olive oil phenolic compounds on cell adhesion molecules. A decrease of ICAM-1 and vascular cell adhesion molecule-1 serum levels at postprandial state after VOO when compared with refined olive oil ingestion has been reported.[53] However, no differences in ICAM-1 levels were reported after sustained virgin or refined olive oil consumption.[52] The anti-inflammatory role of olive oil and its minor compounds is promising, and further human studies are required to obtain full evidence.

Replacement of SFA by MUFA in the diet led to a decrease in blood pressure.[54] An inverse relationship between arterial blood pressure and olive oil consumption has been reported in a large cross-sectional study.[55] Data from EUROLIVE study showed a decrease in the systolic blood pressure in northern and central European participants after olive oil ingestion.[56] Olive oil was more effective in reducing blood pressure, and the antihypertensive treatment, than PUFA-rich diets.[57,58] VOO, but not high-oleic sunflower oil, reduced blood pressure in hypertensive women,[59] suggesting a role for the minor olive oil components on blood pressure levels. Phenolic compounds from olive oil have been shown to be able to improve the endothelium-dependent vasodilation at postprandial state.[60] Also, a decrease in the systolic blood pressure after high-phenolic olive oil consumption, in comparison with low phenolic olive oil, in hypertensive stable patients with coronary heart disease has been reported.[61]

NUTRIGENOMIC EFFECTS

Besides their role as scavengers of reactive oxygen species and chain-breaking antioxidants, olive oil components can exert their health effects by acting at the genomic level, directly or through a decrease in reactive oxygen species, by modulating the expression of key genes for disease processes. Some of the gene expression–mediated mechanisms underlying the beneficial health effects of particular components of olive oil have been examined in experimental models. In endothelial cell models, oleic acid inhibited the expression of the vascular cell adhesion molecule-1 messenger RNA (mRNA) levels and the nuclear factor κB.[62,63] Pomace olive oil, rich in unsaponifiables, upregulated the extracellular nitric oxide synthase expression in spontaneously hypertensive rat aortic rings.[64] Also, an olive oil enriched with the unsaponifiable fraction upregulated the expression of obesity- and insulin sensitivity–related genes in Apo E–deficient mice.[65] In agreement with the previously described cyclooxygenase-2 (COX-2) inhibitory activity of oleocanthal,[47] a reduction in COX-2 expression in cell cultures, via inhibition of p38/cyclic adenosine monophosphate response element–binding protein phosphorylation, by olive oil phenolic compounds has also been reported.[66] The COX-2-765C allele is associated with a low degree of inflammation in human studies. However, the polymorphism COX-2-765G>C has been reported as not modifying the anti-inflammatory effect of an olive oil–rich Mediterranean diet.[67] In experimental studies, the antioxidant

TABLE 1. Vasculoprotective and Anti-inflammatory Effects of Olive Oil Phenolic Compounds in Human Studies (Weeks)

Reference	Subjects	Study Design	Intervention	End Points	Effects
Ruíz-Gutiérrez et al[59]	Hypertensive women (16)	Randomized, crossover, controlled	Virgin versus high-oleic sunflower oil (4 wk ad libitum; washout 4 wk, usual diet)	SBP DBP	Decrease with the phenolic content of the oil
Oubiña et al[49]	Postmenopausal women (12)	2 consecutive periods, no washout	VOO versus oleic acid-rich sunflower oil ad libitum	TXB_2 in PRP TXB_2 in urine 6-keto-$PGF_{1\alpha}$	Lower in VOO No differences No differences
Visioli et al[48]	Hyperlipidemic patients (12 men and 10 women)	Randomized, crossover	Virgin versus refined olive oil (40 mL/d, 7 wk, washout 4 wk with usual diet)	Serum TBX_2	Decrease with the phenolic content of the olive oil
Fitó et al[61]	Hypertensive men with CHD (n = 19)	Randomized, crossover, controlled	Virgin versus refined olive oil (3 wk, raw 50 mL/d; washout, 2 wk; refined olive oil for cooking in all the study)	SBP DBP	Decrease with the phenolic content of the olive oil No changes
Lèger et al[50]	Patients with type I diabetes (5)	Linear intervention	Olive mill waste water (HT: 25 mg first day; 12.5 mg/d during 3 d)	Serum TBX_2	Decrease at day 4
Bogani et al[51]	Healthy men (12)	Randomized, crossover, postprandial	Virgin versus refined olive oil (50 mL with potatoes)	Plasma LTB_4 Plasma TBX_2	Decrease with the phenolic content of the olive oil
Pacheco et al[53]	Healthy (14) and hypertriglyceridemic (14) men	Randomized, crossover, postprandial	Fat meal with virgin versus refined olive oil, after 1 wk of each olive oil (50 mg/m^2 body surface)	Serum ICAM-1 and VCAM-1 area under curve	Decrease with the phenolic content of the olive oil
Fitó et al[52]	Men with CHD (n = 28)	Randomized, crossover, controlled	Virgin versus refined olive oil (3 wk, raw 50 mL/d, washout, 2 wk; refined olive oil for cooking during all the study)	hs-CRP IL-6 Serum ICAM-1	Decrease with the phenolic content of the olive oil No changes

CHD, coronary heart disease; hs-CRP, high-sensibility C-reactive protein; HT, hydroxytyrosol; SBP, systolic blood pressure; TXB_2, thromboxane B_2; VCAM-1, vascular cell adhesion molecule-1; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$.

scavenger enzyme transcriptome response has been shown to be upregulated by olive oil phenolic compounds. They activated the mRNA transcription of glutathione-related enzymes in murine J774 A.1 macrophage-like cells.[68] In agreement with this, olive oil feeding increased catalase and glutathione peroxidase expression in the islets of Langerhans in mice.[69]

Few studies have analyzed the in vivo changes in gene expression associated with olive oil consumption in humans. In an ex vivo study, triglyceride-rich lipoproteins isolated from healthy volunteers after ingestion of a meal enriched with refined olive oil promoted a fewer atherogenic gene expression in smooth muscle cell of human coronary artery than triglyceride-rich proteins isolated after meals rich in butter or a mixture of vegetable and fish oils.[70] In some in vivo studies, the gene expression response in human PBMCs after breakfasts rich in butter, walnuts, or olive oil has been compared.[45,71] Butter elicited a higher increase in tumor necrosis factor-α mRNA than olive oil or walnuts.[45] Butter and walnuts, but not olive oil, elicited a nuclear factor κB postprandial activation in PBMCs of healthy volunteers.[71] The increase in IL-6 mRNA expression, however, was greater after butter and olive oil than after walnuts.[45] A single dose of 50 mL of olive oil can elicit a rapid response of insulin sensitivity–related genes at postprandial time.[72] Insulin sensitivity after an olive oil–rich diet has been shown to be modulated by the polymorphism exon 1 variants of the scavenger receptor class B type I (*SCARB1*).[73] Recently, the mononuclear transcriptome response in human PBMCs after sustained VOO consumption (3 weeks) has been reported.[74] In agreement with the previous reports concerning the protective effects of sustained olive oil consumption on lipid and DNA oxidative damage,[18,34,36,37] some related genes such as lipoic acid synthase (*LIAS*), aldehyde dehydrogenase A1 (*ALDH1A1*), and genes corresponding to the DNA repairing proteins such as the excision repair cross complementation group (*ERCC5*) and X-ray repair complementing defective repair in Chinese hamster cells 5 (*XRCC5*) were upregulated.[74] Although further studies are needed, nutrigenomic responses could be key mechanisms to explain the benefits associated with olive oil consumption.

COMMENTS

On the basis of the information discussed above, olive oil–rich diets can be a useful tool against risk factors for cardiovascular disease. The benefits of olive oil consumption are beyond a mere reduction of the LDL cholesterol. Olive oil–rich diets increased HDL cholesterol and insulin sensitivity and decreased lipid and DNA oxidative damage. Moreover, oxidative lipid damage was inversely related to the phenolic content of the olive oil in a dose-dependent manner. Directly, or through a reduction in the oxidative status, dietary olive oil reduces the inflammatory and thrombogenic status, the endothelial dysfunction, and the blood pressure levels. Olive oil elicits changes in the expression of genes associated with the atherosclerosis process. The wide range of antiatherogenic effects associated with olive oil consumption could contribute

to an explanation of the low rate of cardiovascular mortality found in southern European–Mediterranean countries, in comparison with other westernized countries. The mechanisms by which olive oil exerts its beneficial effects merit deeper investigation. Further studies are required to obtain evidence of the benefits of olive oil consumption on primary end points for cardiovascular disease.

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REFERENCES

1. Trichopoulou A, Costacou T, Bamia C, et al. Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med*. 2003;348: 2599–2608.
2. Sofi F, Cesari F, Abbate R, et al. Adherence to Mediterranean diet and health status: meta-analysis. *BMJ*. 2008;337:a1344.3.
3. Parikh P, McDaniel MC, Ashen MD, et al. Diets and cardiovascular disease. An evidence-based assessment. *J Am Coll Cardiol*. 2005;45: 1379–1387.
4. Fernández-Jarné E, Martínez-Losa E, Prado-Santamaría M, et al. Risk of first non-fatal myocardial infarction negatively associated with olive oil consumption: a case-control study in Spain. *Int J Epidemiol*. 2002;31: 474–480.
5. US Food and Drug Administration.Press Release P04-100. November 1, 2004. Available at: http://www.fda.gov/bbs/topics/news/2004/NEW01129.htlm. Accessed March 30, 2009.
6. Covas MI, Ruiz-Gutiérrez V, de la Torre R, et al. Minor components of olive oil: evidence to date of health benefits in humans. *Nutr Rev.* 2006; 64(Suppl 1):20–30.
7. Bak I, Lekli I, Juhasz B, et al. Cardioprotective mechanisms of *Prunus cerasus* (sour cherry) seed extract against ischemia-reperfusion-induced damage in isolated rat hearts. *Am J Physiol Heart Circ Physiol*. 2006;291: H1329–H1336.
8. Cherki M, Berrougui H, Drissi A, et al. Argan oil: which benefits on cardiovascular diseases? *Pharmacol Res*. 2006;54:1–5.
9. Gimeno E, Castellote AI, Lamuela-Raventós RM, et al. The effect of harvest and extraction methods on the antioxidant content (phenolics, α-tocopherol, and β-carotene) in virgin olive oil. *Food Chem*. 2002;78: 207–211.
10. Pérez-Jiménez F, Alvarez de Cienfuegos G, Badimon L, et al. International Conference on the Healthy Effect of Virgin Olive Oil. *Eur J Clin Invest*. 2005;35:421–424.
11. Keys A. Effects of different dietary fats on plasma-lipid levels. *Lancet*. 1965;17:318–319.
12. Hegsted DM, McGandy RB, Myers ML, et al. Quantitative effects of dietary fat on serum cholesterol in man. *Am J Clin Nutr*. 1965;17:281–295.
13. Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. *Arterioscler Thromb*. 1992;12: 911–919.
14. Yu S, Derr J, Etherton TD, et al. Plasma cholesterol-predictive equations demonstrate that stearic acid is neutral and monounsaturated fatty acids are hypocholesterolemic. *Am J Clin Nutr*. 1995;61:1129–1139.
15. Howard BV, Hannah JS, Heiser CC, et al. Polyunsaturated fatty acids result in greater cholesterol lowering and less triacylglycerol elevation than do monounsaturated fatty acids in a dose-response comparison in a multiracial study group. *Am J Clin Nutr*. 1995;62:392–402.
16. Gardner CD, Kraemer HC. Monounsaturated versus polyunsaturated dietary fat and serum lipids. A meta-analysis. *Arterioscler Thromb Vasc Biol*. 1995;15:1917–1927.
17. Fitó M, Guxens M, Corella D, et al; for the PREDIMED Study Investigators. Effect of a traditional Mediterranean diet on lipoprotein oxidation: a randomized controlled trial. *Arch Intern Med*. 2007;167: 1195–1203.
18. Covas MI, Nyyssonen K, Poulsen HE, et al. The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. *Ann Intern Med*. 2006;145:333–341.
19. Garg A. High-monounsaturated-fat diets for patients with diabetes mellitus: a meta-analysis. *Am J Clin Nutr*. 1998;67(Suppl 3):S577–S582.
20. Casasnovas Lenguas JA, Crussells Canales MJ, Pelegrín Díaz J, et al. Changes in lipid profile of young persons after substitution of sunflower oil by olive oil in the diet. *Rev Esp Cardiol*. 1997;50:843–850.
21. Chan YM, Demonty I, Pelled D, et al. Olive oil containing olive oil fatty acid esters of plant sterols and dietary diacylglycerol reduces low-density lipoprotein cholesterol and decreases the tendency for peroxidation in hypercholesterolaemic subjects. *Br J Nutr*. 2007;98:563–570.
22. Roche HM, Gibney MJ. The impact of postprandial lipemia in accelerating atherothrombosis. *J Cardiovasc Risk*. 2000;7:317–324.
23. Weinbrenner T, Fitó M, Farré-Albaladejo M, et al. Bioavailability of phenolic compounds from olive oil and oxidative/antioxidant status at postprandial state in healthy humans. *Drugs Exp Clin Res*. 2004;30: 207–214.
24. Fitó M, Gimeno E, Covas MI, et al. Postprandial and short-term dietary intervention effects of virgin olive oil ingestion on the oxidative/antioxidative status. *Lipids*. 2002;37:245–251.
25. Covas MI, de la Torre K, Farré-Albaladejo M, et al. Postprandial LDL phenolic content and LDL oxidation is modulated by olive oil phenolic compound in humans. *Free Radic Biol Med*. 2006;40:608–616.
26. López S, Bermúdez B, Pacheco YM, et al. Distinctive postprandial modulation of beta cell function and insulin sensitivity by dietary fats: monounsaturated compared with saturated fatty acids. *Am J Clin Nutr*. 2008;88:638–644.
27. Rivellese AA, Iovine C, Ciano O, et al. Nutrient determinants of postprandial triglyceride response in a population-based sample of type II diabetic patients. *Eur J Clin Nutr*. 2006;60:1168–1173.
28. Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest*. 1991;88:1785–1792.
29. Meisinger C, Baumert J, Khuseyinova N, et al. Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population. *Circulation*. 2005;112:651–657.
30. Parthasarathy S, Khoo JC, Miller E, et al. Low density lipoprotein rich in oleic acid is protected against oxidative modification: implications for dietary prevention of atherosclerosis. *Proc Natl Acad Sci U S A*. 1990;87: 3894–3898.
31. Lapointe A, Couillard C, Lemieux S. Effects of dietary factors on oxidation of low-density lipoprotein particles. *J Nutr Biochem*. 2006;17: 645–658.
32. Pacheco YM, Lopez S, Bermudez B, et al. Extra-virgin vs. refined olive oil on postprandial hemostatic markers in healthy subjects. *J Thromb Haemost*. 2006;4:1421–1422.
33. Ruano J, López-Miranda J, de la Torre R, et al. Intake of phenol-rich virgin olive oil improves the postprandial prothrombotic profile in hypercholesterolemic patients. *Am J Clin Nutr*. 2007;86:341–346.
34. Machowetz A, Poulsen HE, Gruendel S, et al. Effect of olive oils on biomarkers of oxidative DNA stress in north and south Europeans. *FASEB J*. 2007;21:45–52.
35. Hillestrøm PR, Covas MI, Poulsen HE. Effect of dietary virgin olive oil on urinary excretion of etheno-DNA adducts. *Free Radic Biol Med*. 2006;41: 1133–1138.
36. Weinbrenner T, Fitó M, de la Torre R, et al. Olive oils high in phenolic compounds modulate oxidative/antioxidative status in men. *J Nutr*. 2004; 134:2314–2321.
37. Salvini S, Sera F, Caruso D, et al. Daily consumption of a high-phenol extra-virgin olive oil reduces oxidative DNA damage in postmenopausal women. *Br J Nutr*. 2006;95:742–751.
38. Yaqoob P, Newsholme EA, Calder PC. Inhibition of natural killer cell activity by dietary lipids. *Immunol Lett*. 1994;41:241–247.
39. Yaqoob P, Newsholme EA, Calder PC. The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation. *Immunology*. 1994;82:603–610.
40. Mata P, Alonso R, López-Farre A, et al. Effect of dietary fat saturation on LDL oxidation and monocyte adhesion to human endothelial cells in vitro. *Arterioscler Thromb Vasc Biol*. 1996;16:1347–1355.
41. Tsimikas S, Philis-Tsimikas A, Alexopoulos S, et al. LDL isolated from Greek subjects on a typical diet or from American subjects on an oleate-supplemented diet induces less monocyte chemotaxis and adhesion when exposed to oxidative stress. *Arterioscler Thromb Vasc Biol*. 1999; 19:122–130.

42. Yaqoob P, Knapper JA, Webb DH, et al. Effect of olive oil on immune function in middle-aged men. *Am J Clin Nutr*. 1998;67:129–135.
43. Estruch R, Martínez-González MA, Corella D, et al. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. *Ann Intern Med*. 2006;145:1–11.
44. Esposito K, Marfella R, Ciotola M, et al. Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the Metabolic syndrome: a randomized trial. *JAMA*. 2004;292: 1440–1446.
45. Jiménez-Gómez Y, López-Miranda J, Blanco-Colio LM, et al. Olive oil and walnut breakfasts reduce the postprandial inflammatory response in mononuclear cells compared with a butter breakfast in healthy men. *Atherosclerosis*. 2009;204:e70–e76.
46. Perona JS, Cabello-Moruno R, Ruíz-Gutíerrez V. The role of virgin olive oil components in the modulation of endothelial function. *J Nutr Biochem*. 2006;17:429–445.
47. Beauchamp GK, Keast RS, Morel D, et al. Phytochemistry: ibuprofen-like activity in extra-virgin olive oil. *Nature*. 2005;437:45–46.
48. Visioli F, Caruso D, Grande S, et al. Virgin olive oil study (VOLOS): vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients. *Eur J Nutr*. 2005;44:121–127.
49. Oubiña P, Sanchez-Muniz FJ, Rodenas S, et al. Eicosanoid production, thrombogenic ratio, and serum and LDL peroxides in normo- and hypercholesterolaemic post-menopausal women consuming two oleic acid-rich diets with different content of minor components. *Br J Nutr*. 2001;85:41–47.
50. Lèger CL, Carbonneau MA, Michel F, et al. A thromboxane effect of a hydroxytyrosol-rich olive oil wastewater extract in patients with uncomplicated type I diabetes. *Eur J Clin Nutr*. 2005;59:727–730.
51. Bogani P, Galli C, Villa M, et al. Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. *Atherosclerosis*. 2007;190: 181–186.
52. Fitó M, Cladellas M, de la Torre R, et al. Anti-inflammatory effect of virgin olive oil in stable coronary disease patients: a randomized, crossover, controlled trial. *Eur J Clin Nutr*. 2008;62:570–574.
53. Pacheco YM, Bermúdez B, Lopez S, et al. Minor compounds of olive oil have postprandial anti-inflammatory effects. *Br J Nutr*. 2007;98:260–263.
54. Rasmussen OW, Thomsen C, Hansen KW, et al. Effects on blood pressure, glucose, and lipid levels of a high-monounsaturated fat diet compared with a high-carbohydrate diet in NIDDM subjects. *Diabetes Care*. 1993; 16:1565–1571.
55. Psaltopoulou T, Naska A, Orfanos P, et al. Olive oil, the Mediterranean diet, and arterial blood pressure: the Greek European prospective investigation into cancer and nutrition (EPIC) study. *Am J Clin Nutr*. 2004;80:1012–1018.
56. Bondia-Pons I, Schröder H, Covas MI, et al. Moderate consumption of olive oil by healthy European men reduces systolic blood pressure in non-Mediterranean participants. *J Nutr*. 2007;137:84–87.
57. Perona JS, Canizares J, Montero E, et al. Virgin olive oil reduces blood pressure in hypertensive elderly subjects. *Clin Nutr*. 2004;23:1113–1121.
58. Ferrara LA, Raimondi AS, d'Episcopo L, et al. Olive oil and reduced need for antihypertensive medications. *Arch Intern Med*. 2000;160:837–842.
59. Ruíz-Gutiérrez V, Muriana FJ, Guerrero A, et al. Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources. *J Hypertens*. 1996;14:1483–1490.
60. Ruano J, López-Miranda J, Fuentes F, et al. Phenolic content of virgin olive oil improves ischemic reactive hyperemia in hypercholesterolemic patients. *J Am Coll Cardiol*. 2005;46:1864–1868.
61. Fitó M, Cladellas M, de la Torre R, et al. Antioxidant effect of virgin olive oil in patients with stable coronary heart disease: a randomised, crossover, controlled, clinical trial. *Atherosclerosis*. 2005;181:149–158.
62. Carluccio MA, Massaro M, Bonfrate C, et al. Oleic acid inhibits endothelial activation: a direct vascular antiatherogenic mechanism of a nutritional component in the Mediterranean diet. *Arterioscler Thromb Vasc Biol*. 1999;19:220–228.
63. Massaro M, Carluccio MA, Paolicchi A, et al. Mechanisms for reduction of endothelial activation by oleate: inhibition of nuclear factor-kappaB through antioxidant effects. *Prostaglandins Leukot Essent Fatty Acids*. 2002;67:175–181.
64. Rodríguez-Rodríguez R, Herrera MD, De Sotomayor MA, et al. Pomace olive oil improves endothelial function in spontaneously hypertensive rats by increasing endothelial nitric oxide synthase expression. *Am J Hypertens*. 2007;20:728–34.
65. Acín S, Navarro MA, Perona JS, et al. Microarray analysis of hepatic genes differentially expressed in the presence of the unsaponifiable fraction of olive oil in apolipoprotein E-deficient mice. *Br J Nutr*. 2007; 97:628–638.
66. Corona G, Deiana M, Incani A, et al. Inhibition of p38/CREB phosphorylation and *COX-2* expression by olive oil polyphenols underlies their anti-proliferative effects. *Biochem Biophys Res Commun*. 2007;362: 606–611.
67. Corella D, González JI, Bulló M, et al. Polymorphisms cyclooxygenase-2 −765G>C and interleukin-6 −174G>C are associated with serum inflammation markers in a high cardiovascular risk population and do not modify the response to a Mediterranean diet supplemented with virgin olive oil or nuts. *J Nutr*. 2009;139:128–134.
68. Masella R, Vari R, D_Archivio M, et al. Extra virgin olive oil biophenols inhibit cell-mediated oxidation of LDL by increasing the mRNA transcription of glutathione-related enzymes. *J Nutr*. 2004;134:785–791.
69. Oliveras-López MJ, Berná G, Carneiro EM, et al. An extra-virgin olive oil rich in polyphenolic compounds has antioxidant effects in OF1 mice. *J Nutr*. 2008;138:1074–1078.
70. Bermúdez B, López S, Pacheco YM, et al. Influence of postprandial triglyceride-rich lipoproteins on lipid-mediated gene expression in smooth muscle cells of the human coronary artery. *Cardiovasc Res*. 2008;79:294–303.
71. Bellido C, López-Miranda J, Blanco-Colio LM, et al. Butter and walnuts, but not olive oil, elicit postprandial activation of nuclear transcription factor kappaB in peripheral blood mononuclear cells from healthy men. *Am J Clin Nutr*. 2004;80:1487–1491.
72. Konstantinidou V, Khymenets O, Covas MI, et al. Time course of changes in the expression of insulin sensitivity-related genes after an acute load of virgin olive oil. *OMICS*. 2009;13:431–438.
73. Pérez-Martínez P, Pérez-Jimenez F, Bellido C, et al. A polymorphism exon 1 variant at the locus of scavenger receptor class B type 1 (*SCARB1*) gene is associated with differences in insulin sensitivity in healthy people during the consumption of an olive oil-rich diet. *J Clin Endocrinol Metab*. 2005;90:2297–2300.
74. Khymenets O, Fitó M, Covas MI, et al. Mononuclear cell transcriptome response after sustained virgin olive oil consumption in humans: an exploratory nutrigenomics study. *OMICS*. 2009;13:7–19.