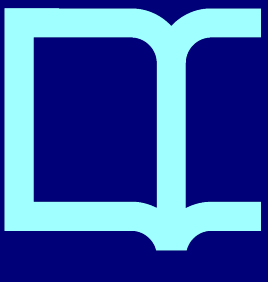

Virgin olive oil and nuts as key foods of the Mediterranean diet effects on inflammatory biomarkers related to atherosclerosis

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ABSTRACT

Previous epidemiological and feeding studies have observed that adherence to Mediterranean diet (Med- Diet) is associated with reduced cardiovascular risk. However, the molecular mechanisms involved are not fully understood. Since atherosclerosis is nowadays considered a low-grade inflammatory disease, recent studies have explored the anti-inflammatory effects of a Med-Diet intervention on serum and cellular biomarkers related to atherosclerosis. In two sub-studies of the PREDIMED (PREvencion con DIeta MEDiterranea) trial, we analyzed the effects at 3 months of two Med-Diet interventions supplemented with either virgin olive oil (VOO) or nuts compared with a control low-fat diet (LFD). Both Med-Diets showed an anti-inflammatory effect reducing serum C-reactive protein, interleukin-6 (IL6) and endothelial and monocytary adhesion molecules and chemokines ($P < 0.05$; all), whereas these parameters increased after the LFD intervention ($P < 0.05$; all). In another substudy, we evaluated the long-term (1 year) effects of these interventions on vascular risk factors in 516 high-risk subjects, as well as the effect of different Med-Diet components in the reduction of these biomarkers. At 1 year, the Med-Diet groups had significant decreases in the plasma concentrations of IL6, tumor necrosis factor receptor (TNFR) 60 and TNFR80 ($P < 0.05$), while intercellular adhesion molecule 1 (ICAM-1), TNFR60 and TNFR80 concentrations increased in the LFD group ($P < 0.002$). In addition, those allocated in the highest tertile of VOO and vegetables consumption had a significant diminution of plasma TNFR60 concentration com- pared with those in tertile 1 ($P < 0.02$). In conclusion, Med-Diet exerts an anti-inflammatory effect on cardiovascular system since it down-regulates cellular and circulating inflammatory biomarkers related to atherogenesis in subjects at high cardiovascular risk.

1. INTRODUCTION

Inflammation is essential in the development of atherosclerosis, the main cause of coronary heart disease (CHD), since appears to play a key role from the inception to the final lesions of this dis- ease [1]. Proinflammatory stimuli activate inflammation inducing the secretion of inflammatory cytokines and generating endothelial adhesion molecules and other chemoattractants. Cytokine production and up-regulation of adhesion molecules on endothelial cells and leukocytes facilitates the recruitment of inflammatory cells from the circulation, their adhesion to endothelium and finally migration to subendothelial space. Subsequent ongoing inflammation is also crucial in the development of instability and rupture of atheroma plaques and the subsequent appearance of ischemic events in advanced stages of the disease [1,2].

CHD is the main cause of death worldwide [3] and is higher in industrialized countries. Western countries, including the US, currently continue to exhibit unacceptably high absolute rates of cardiovascular morbidity and mortality. However, surprisingly, as compared to Northern European countries or the US, there is a low incidence of CHD in countries of Southern Europe, such as France, Spain, Greece and Italy [4,5]. The traditional Mediterranean food pattern has been the factor most frequently involved to explain this health advantage. The exact mechanism of this prevention is not fully understood, but could be caused by the functional compounds of main foods characteristics of Mediterranean diet (Med-Diet). These functional compounds could be phytochemicals such as polyphenols [6,7] or fatty acids [8,9].

In this review, we discuss the effects of the Med-Diet, its components and their functional compounds on inflammatory biomarkers related with atherosclerosis.

2. THE PREDIMED STUDY

The PREvencion con DIeta MEDiterranea (PREDIMED) study is a large, parallel-group, multicenter, randomized, controlled, 5-year clinical trial that aims to assess the effects of the Med-Diet on the primary prevention of cardiovascular disease (www.predimed.org) [10,11]. This trial was registered in the Current Controlled Trials at London, International Standard Randomized Controlled Trial Number, at www.controlled-trials.com, as ISRCTN35739639. Almost 7500 high-risk participants have been recruited for the study. Eligible participants had no documented CHD who

either had type 2 diabetes or had at least 3 of the following risk factors: smoking, hypertension (blood pressure 140/90 mmHg or treatment with antihypertensive drugs), LDL-cholesterol con- centration 160 mg/dL (or treatment with hypolipidemic drugs), HDL-cholesterol concentration 40 mg/dL, BMI 25 kg/m2, or a family history of early-onset CHD. Exclusion criteria were a history of previous CHD, any severe chronic illness, drug or alcohol abuse, history of allergy or intolerance to olive oil or nuts, or a low predicted likelihood of changing dietary habits according to the stages of change model. Participants were randomly assigned to 3 intervention groups: 2 Med-Diet groups, one supplemented with virgin olive oil (Med-diet with VOO) and the other supplemented with mixed nuts (Med-Diet with Nuts), and a low-fat diet (LFD) group whose participants received recommendations to reduce all types of fat according to the American Heart Association guidelines [12]. Participants in both Med-Diet groups were recommended to follow a Med-diet pattern through the use of olive oil for cooking and dressings, increase of intake of vegetables (2 servings/d), fresh fruit (3 servings/d), legumes, nuts and fish or seafood (3 servings/wk), reduce consumption of red meats, processed meats or meat products, and a moderate alcohol intake, usually in the form of red wine consumed with meals [10,11,13]. Participants assigned to the Med-Diet with VOO were provided with VOO (1 L/wk) and those assigned to the Med-Diet with Nuts were provided with mixed nuts (30 g/d, as 15 g walnuts, 7.5 g almonds, and 7.5 g hazelnuts). Otherwise, participants in the LFD group only received small non-food gifts. At baseline and at follow-ups, participants completed a validated 14-item questionnaire assessing adherence to the Med-Diet [14], a validated 137-item validated food frequency questionnaire [15], a validated version of the Minnesota Leisure Time Physical Activity Questionnaire for men [16] and women [17] and a 47- item questionnaire about education, lifestyle, history of illnesses, and medication use. The main outcome of the PREDIMED is an aggregate of cardiovascular events (cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke).

3. ANTI-INFLAMMATORY EFFECTS OF THE MEDITERRANEAN DIET

In 2006, we analyzed the 3-month effects of these 2 Med- Diet and a LFD interventions on 4 soluble adhesion molecules [intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), interleukine-6 (IL6) and C-reactive protein (CRP)] in a pilot study with the first 772 participants recruited in the

PREDIMED trial [10]. The plasma concentrations of IL6, VCAM-1 and ICAM-1 significantly decreased after 3 months in the Med-Diet intervention groups while plasma concentrations of CRP only decreased in the Med-Diet with VOO ($P < 0.05$; all). Other- wise, plasma concentrations of VCAM-1 and ICAM-1 increased after 3 months in the LFD group ($P < 0.05$; both) [10].

The anti-inflammatory effects of the Med-Diet have also been tested through the analysis of circulating inflammatory biomarkers and on immune cell activation biomarkers, all related to atherogenesis, in another sub-study of the PREDIMED trial [18,19]. We analyzed the changes in peripheral blood mononuclear cells expression of cell surface inflammatory mediators (adhesion molecules and proinflammatory ligand CD40 expression on T lymphocytes and monocytes) after 3 months of the 3 PRED-IMED interventions in 106 participants at high cardiovascular risk (Fig. 1). Both Med-Diet with VOO and Nuts down-regulate the following cellular inflammatory biomarkers related to atherogenesis: CD49d molecule in peripheral T-lymphocytes and CD11b, CD49d (crucial adhesion molecule for leukocyte homing) and CD40 (pro-inflammatory ligand) in monocytes [18]. The study of these mediators had not been previously investigated and we concluded that Med-Diet supplemented with VOO or Nuts modifies the process of firm adhesion of circulating monocytes and lymphocytes T to endothelial cells during inflammation, a crucial step linked to the appearance and development of atherosclerosis [1]. In this sub-study, we also analyzed the effects before and after 3 months of intervention on plasma concentrations of ICAM-1, VCAM-1, IL6, CRP, E-selectin and P-selectin in these 106 participants (Table 1). After the intervention period, ICAM-1 decreased in both Med-Diet groups, whereas VCAM-1, IL6 and CRP only decreased in Med- Diet group supplemented with VOO ($P < 0.05$). Interestingly, plasma concentrations of ICAM-1, VCAM-1 and IL6 increased in the LFD group. After 3 months, plasma concentrations of ICAM-1 and IL6 decreased in both Med-Diet groups and increased in the LFD group. These changes were different between Med-Diet groups and LFD group ($P < 0.02$). Plasma concentrations of VCAM-1 and CRP only decreased in the Med-Diet group with VOO and these changes were significantly different of the LFD group. No differences were observed for E- and P-selectin. According to these data, LFD may exert an inflammatory effect and, therefore, it seems not to be as healthy as it was supposed.

In a third sub-study of the PREDIMED trial, [20] we analyzed the 1-year effects of the 2 Med-Diet supplemented with VOO or Nuts and a control LFD on 4 molecules related to systemic inflammation (TNFR60, TNFR80, ICAM-1 and IL6) (Fig. 2). The plasma concentrations of IL6, TNFR60 and TNFR80 decreased after 1 year in the Med-Diet intervention groups ($P < 0.05$; all). Otherwise, plasma concentrations of ICAM-1, TNFR60 and TNFR80 increased after 1 year in the LFD group ($P < 0.05$; all). Again, at 1-year plasma concentrations of ICAM-1, IL6, TNFR60 and TNFR80 were greater in the LFD group than in the Med-Diet groups ($P < 0.05$; all) (Fig. 2). To our knowledge, this is the first study in which TNFR concentrations were affected due to changes in the diet. Few studies have analyzed the effect of an intervention with foods with healthy compounds on TNFα receptors. Although the TNFα has an ambivalent role in relation to CHD [21], the activation of TNFR60 could induce expression of adhesion molecules and activates NF-kB, and TNFR80 play a role in T cell proliferation [22]. Several studies in humans have supported evidences that consumption of a Med-Diet pattern and its main components such as VOO and/or nuts provide beneficial anti-inflammatory effects [18,23], which are close related to the prevention of atherosclerosis, the main cause of coronary artery disease [1]. A recent study has demonstrated the association between adherence to the Med-Diet and systemic inflammation independent of sharing genetic and environmental factors [24]. Thus, diet is, indeed, one modified factor that could modulate inflammation [25,26].

In 2004, the participants of a population from the Attica area of Greece who were more adhered to the traditional Med-Diet had lower plasma concentrations of CRP, IL6, homocysteine and fibrinogen, as well as a lower white blood cell count and a border-line decrease of TNFα [27]. In addition, a group of patients with metabolic syndrome who followed a Med-Diet pattern showed reduced serum concentrations of CRP, IL6, IL7 and IL18, decreased insulin resistance and improved endothelial function [28]. In the Nurses' Health Study I cohort, different dietary patterns were related with markers of inflammation and endothelial dysfunction (CRP, IL6, ICAM-1 and VCAM-1). A prudent pattern, similar than a Med-Diet pattern, was inversely associated with plasma CRP and E-selectin concentrations, whereas a Western pattern, with higher intake of red meat, sweets, fries and refined grains, was positively associated with CRP, IL6, E-selectin, ICAM-1 and VCAM-1 concentrations [29]. A recent updated meta-analysis has observed that higher adherence to the traditional Med-Diet provides protection against major chronic degenerative diseases (cardiovascular, cancer and neurodegenerative

diseases) [30]. Until now, the PREDIMED study is the unique randomized trial that has evaluated the protective effect of a Med-Diet pattern vs a LFD in patients at high risk for cardiovascular disease on cardiovascular disease events and the possible mechanisms involved in this protection. In this study, we observed that those participants who reported a higher adherence to the traditional Med-Diet also showed a significant reduction in classical and novel risk factors, with a significant diminution of cellular and serum inflammatory parameters, compared to those participants with a low adherence top Med-Diet [10,19,20].

The Med-Diet is identified as the traditional dietary pattern found in olive-growing areas of Crete, Greece, and Southern Italy in the late 1950s and early 1960s. Its major characteristics are: (a) a high consumption of whole grains, legumes, nuts, vegetables, and fruits; (b) a relatively high-fat consumption (up to 40% of total energy intake), mostly from monounsaturated fatty acids (MUFA, up to 20% of energy) mainly provided by olive oil, the principal source of culinary and dressing fat; (c) moderate to high fish consumption; (d) poultry and dairy products (usually as yogurt or cheese) consumed in moderate to small amounts; (e) low consumption of red meats, processed meats, and meat products; and (f) moderate alcohol intake, usually in the form of red wine consumed with meals. VOO is one of the main components of the Med-Diet due to its almost exclusive production and consumption in the Mediterranean area. Nuts are also traditionally associated to the Med-Diet and mainly include walnuts (Juglans regia L.), almonds (Prunus dul- cis (Mill.) D.A.Webb) and hazelnuts (Corylus avellana L.). Both foods are rich in polyphenols and healthy fatty acids, i.e. MUFA in VOO and polyunsaturated fatty acids (PUFA) in mixed nuts. Most of the beneficial effects of their regular consumption in a Med-Diet pattern may be attributed to these components.

Several studies have demonstrated the anti-inflammatory effects of the consumption of diets rich in these foods. Twenty- eight stable CHD patients receiving 50 mL of VOO and refined olive oil sequentially administered over two periods of 3 weeks showed a significant decrease in plasma IL6 and CRP concentrations after VOO intervention with respect to refined olive oil intervention [31]. The anti-inflammatory effect of VOO and cod liver oil has also been demonstrated after an intervention study where healthy humans received 50 mL of VOO and cod liver oil and their ICAM-1 and TNFα plasma concentrations decreased after 3 h of both interventions [8].

In healthy Tehran women, it was observed that the intake of olive oil also decreased plasma concentrations of TNFα, ICAM-1 and CRP [32]. Three month intervention with a traditional Med-Diet supplemented with VOO decreased the gene expression related with inflammation [INF-gamma, Rho GTPase-activating protein 15, and IL-7 receptor] and oxidative stress [adrenergic beta(2)-receptor] in peripheral blood mononuclear cells [33]. In line with this study, Camargo et al. [34] also reported that the intake of a breakfast enriched in VOO with high content in polyphenols, decreased the expression of NF-kB and COX-2, genes involved in inflammation. Diets supplemented with olive oil or walnuts, rich in MUFA and PUFA, respectively, had a larger diminution in the RNA expression of TNFα messenger than a butter breakfast diet [35]. When compare two Med-Diets in hypercholesterolemic subjects, a regular Med-Diet and the same diet replacing MUFA by walnuts, walnut diet improved endothelium-dependent vasodilation and decreased VCAM-1 concentrations while endothelium-independent vasodilation and concentrations of ICAM-1, CRP, homocysteine, and oxidation biomarkers did not differ between both Med-Diets [26]. In another controlled intervention study where patients with metabolic syndrome were supplemented with mixed nuts (including walnuts, almonds and hazelnuts) during 12 weeks, only the inflammatory biomarker IL6 decreased after the nuts intervention compared with the control diet [36]. In this line, a cross-sectional Multi-Ethnic Study of Atherosclerosis with 6080 participants from USA showed that regular nut and seed consumption was associated with lower levels of inflammatory markers (CRP and IL6) [37].

There are consistent epidemiological evidences to support a cardioprotective effect of nut consumption. In a large Californian cohort, the Adventists Health Study, the frequency of nut intake was inversely associated with CHD incidence [38]. More recently, the results of 3 additional observational studies, the Iowa Women's Health Study, the Nurses' Health Study, and the Physician's Health Study, have confirmed that frequent nut consumption is associated with a lower risk for incident CHD [39–41]. However, the results of the Iowa cohort did not reach statistical significance, and the Physician's Health Study only found protection for sudden cardiac death, but not for non-sudden coronary death or myocardial infarction.

Part of these protective effects has been attributed to their actions on lipid profile. Several small feeding trials (<50 subjects) have shown consistent decreases in total and LDL cholesterol with diets enriched with a variety of nuts in comparison with other

healthy diets. Almonds and walnuts have been the nuts most frequently investigated in this regard [42,43]. The hypocholesterolemic effect is achieved with intakes of 1.5–3 servings per day. Effects on HDL cholesterol have been inconsistent. When evaluated, the ratio total/HDL cholesterol was found to decrease [44]. How- ever, more recently, an anti-inflammatory effect of nut intake has been reported. Thus, a walnut diet has been reported to improve endothelium-dependent vasodilation and to reduce VCAM-1 levels in hypercholesterolemic subjects [26]. These results suggest mechanistic explanations for the observed CHD risk reduction associated with nut intake. Some studies have also demonstrated the effects of nuts when they are used in manufactured functional foods. Canales et al. [45] compared the effects of the consumption a functional food created with walnuts (walnut-enriched meat) versus a low- fat meat on adhesion molecules and leukotrienes (LTB4) in patients at increased cardiovascular risk. They observed that patients that consumed walnut-enriched meat had higher levels of paraoxonase activity, lower levels of ICAM-1, VCAM-1 and leukotriene B4 and lower ratios of paraoxonase-1/HDLc and paraoxonase-1/Apo A1 than those with low-fat meat consumption. In conclusion, follow a Med-Diet pattern together with some of its main components such as VOO and nuts generally reduce the cardiovascular risk factors and down-regulate cellular inflammatory pathways related to atherosclerosis, thus recommendations to follow-up a healthy Med-Diet pattern is adequate in all stages of the disease. The results suggest that learning healthy diets and obtain dietary habits are important keys in the prevention of cardiovascular diseases in any period of life.

4. RELATIONSHIP BETWEEN MEDITERRANEAN DIET KEY FOODS AND ITS FUNCTIONAL COMPOUNDS AND INFLAMMATION

In another sub-study of the PREDIMED trial, we analyzed the relationship between the 1-year change in consumption of 13 foods and 4 plasma inflammatory biomarkers in 516 participants at high cardiovascular risk [20]. We observed a clear relationship between the consumption of VOO and vegetables and the receptor 60 of TNF. Concretely, we observed that participants who increased more than 24 g/d their consumption of VOO after 1 year decreased their plasma concentration of TNFR60 from 1.8 µg/L (baseline levels) to 1.5 µg/L (1-year levels) ($P < 0.05$) [20]. In line with this result, participants who increased more than 62.7 g/d their consumption of

vegetables after 1 year decreased their plasma concentration of TNFR60 from 1.7 µg/L to 1.5 µg/L ($P < 0.05$). We also observed that participants who increased their adherence to the Med-Diet in more than 2.4 points had decreases in plasma concentration of TNFR80 from 6.5 µg/L to 5.9 µg/L ($P < 0.05$) [20].

The healthy effects on the prevention of inflammation derived of VOO consumption have been described above. In this section, we analyze the health effects of functional components of VOO and nuts. The beneficial effects of VOO could be due to their components such as phenolic compounds, α-tocopherol, carotenoids and to the high unsaturated/saturated fatty acid ratio with oleic acid (MUFA) as its main fatty acid [46,47]. The polyphenols in VOO have been described as the main components that attributed the anti-inflammatory properties after VOO consumption and thus contribute to the lower incidence of CHD [48,49]. Up to 36 polyphenols from different groups have been identified and described in VOO [7]: (1) secoiridoids: the group with higher quantity of polyphenols in VOO (from 0.8 to 522.2 mg/kg) includes oleuropein aglycone (3,4-DHPEA-EA), oleuropein-aglycone di-aldehyde (3,4-DHPEA- EDA), ligstroside aglycon and deacetoxy-ligstroside aglycone (oleocanthal); (2) flavonoids, mainly dihydroflavonols such as taxifolin (up to 129.4 mg/kg) and flavones (apigenin and luteolin); (3) phenolic alcohols, mainly tyrosol and hydroxytyrosol (from 0.5 to 14.4 mg/kg); (4) lignans, mainly (+)-1-acetoxypinoresinol and (+)-pinoresinol (from 0.2 to 36.2 mg/kg); (5) phenolic acids- benzoic acid derivatives, up to 1.8 mg/kg include protocatechuic, gentisic, and gallic acids, between others; (6) cinnamic acid derivatives, up to 0.4 mg/kg, include caffeic, o-coumaric and p-coumaric acids; (7) hydroxy-isocromans include 1-phenyl-6,7- dihydroxy-isochroman and 1-(3r-methoxy-4r-hydroxy) phenyl- 6,7-dihydroxy-isochroman. The content of polyphenols in VOO could vary by variety of olive fruits, region of production, agricultural techniques, fruit maturity at harvest and processing methods during extraction [7]. The cardioprotective role and the anti- inflammatory effects attributed to polyphenols of VOO depend on their bioavailability. Several studies have demonstrated the human bioavailability of olive oil polyphenols after VOO consumption through determination of tyrosol and hydroxytyrosol conjugates in plasma and its derived metabolites such as homovanillic acid and vanillin conjugates [50], as well as analyzing urinary excretion of tyrosol and hydroxytyrosol [51]. Recently, a exploratory analysis of human urine using a time-of-flight analyzer has identified more than 60 metabolites of olive oil polyphenols, including mainly those derived from secoiridoids, phenolic alcohols (mainly

hydroxytyrosol) and flavonoids, after a single intake of 50 mL of VOO in healthy volunteers [52].

The anti-inflammatory and anti-atherogenic properties of individual VOO polyphenols have been observed in several experimental studies [53–55]. An in vitro study has observed that oleuropein inhibited vascular smooth muscle cell proliferation through a cell cycle block between the G1 and the S phases, which may be regulated by ERK1/2 [56], increased in a dose-dependent manner the nitrite production and the inducible nitric oxide synthase (iNOS) expression in mouse macrophages challenged with lipopolysaccharide (LPS) [54], and inhibited lipoxygenase activity and production of leukotriene B4 [57]. It has also been reported that oleuropein exerts a cardioprotective effect against acute adriamycin cardiotoxicity [58] and exhibit anti-ischemic and hypolipidemic activities [59]. Pretreatment of ischemic hearts with oleuropein significantly reduced the prompt release of oxidized glutathione and prevent membrane lipid peroxidation [60]. Oleuropein and an olive oil extract also prevented the stimulation of matrix metalloproteinase 9 (MMP-9) expression and secretion in TNFα-treated THP-1 cells in a monocyte cell line [61]. The incubations of nutritionally concentrations of oleuropein and hydroxytyrosol with human umbilical vein endothelial cells (HUVEC) for 30 min, followed by co-incubation with bacterial LPS or cytokines to trigger adhesion molecule expression, showed that these polyphenols reduced monocytoid cell adhesion to stimulated endothelium, as well as VCAM-1 mRNA and protein [53]. The effect of a phenolic extract from VOO and separate pure polyphenols from VOO (oleuropein aglycone, hydroxytyrosol and homovanillyl alcohol) were evaluated on cell surface and mRNA expression in HUVEC of 3 crucial adhesion molecules (ICAM-1, VCAM-1 and E-selectin). Oleuropein aglycone and hydroxytyrosol were the responsible compounds for the reduced expression of ICAM-1 and VCAM-1 obtained by VOO phenolic extract. Otherwise, homovanillyl alcohol decreased the expression of the 3 molecules, but the effect on mRNA expression was weaker [62]. Oleocanthal possesses a relatively similar chemical structure than ibuprofen and acts inhibiting the same cyclooxygenase enzymes in the prostaglandin- biosynthesis pathway than this well-known anti-inflammatory drug [63]. In vitro hydroxytyrosol has been reported to attenuate the TNF-α, iNOS, and COX-2 in LPS-induced human monocytic (THP-1) cells [64]. Hydroxytyrosol inhibited the production of nitric oxide and prostaglandin E (PGE) and both hydroxytyrosol and an olive aqueous extract decreased secretion of cytokines (IL1α, IL1β, IL6, TNFα and IL12) and chemokines

(CXCL10/IP10 and CCL2/MCP- 1), reduced the expression of genes of iNOS, IL1α, CXCL10/IP-10, MIP-1 β, matrix MMP-9, and inhibited PGE synthase in murine macrophages (RAW264.7 cells) [65]. The hydroxyisocroman 1- phenyl-6,7-dihydroxy-isochroman inhibited in a dose-dependent manner the production of prostanoid and TNF-α in LPS-primed human monocytes in vitro [66].

Otherwise, only few studies have demonstrated the healthy properties of olive oil fatty acids (mainly oleic acid) linked to Med- Diet on inflammatory biomarkers [67]. Oleic acid was able to reduce the inflammatory effects of saturated fatty acids in human aortic endothelial cells by reducing the incorporation of stearic acid into phospholipids and by the reduction of NF-kB activation [67]. In other studies, it reverses the in vitro inhibitory effect of the inflammatory cytokine TNFα on insulin production in the rat pancreatic β cell line INS-1 [9] and decreased membrane expression of VCAM-1 and NF-kB activation in endothelial cells [68].

In summary, to follow a Med-Diet pattern together with the intake of some of its main components (i.e. VOO and nuts), which contained functional compounds (polyphenols) generally reduce the cardiovascular risk factors and down-regulate cellular inflammatory pathways related to atherosclerosis; thus, it seems adequate to recommend a Med-Diet pattern and intake of polyphenol-rich foods are adequate in patients in all stages of atherosclerosis disease. Learning healthy diets and upgrade adherence to the traditional Med-Diet are important keys in the prevention of cardiovascular diseases in all periods of life.

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TABLE 1. Concentrations of circulating inflammatory molecules at baseline and after 3 months of intervention with Med-Diet supplemented with virgin olive oil (Med-Diet with VOO), Med-Diet supplemented with nuts (Med-Diet with Nuts) or LFD in patients at high risk for cardiovascular disease.

	Med-diet with VOO ($n = 35$)	Med-diet with nuts ($n = 35$)	LFD ($n = 36$)	Repeated-measures ANOVA[a]	*P* value for differences[b]		
				Time × treatment	MD-VOO vs MD-nuts	MD-VOO vs LFD	MD-nuts vs LFD
ICAM-1, μg/L							
Baseline	290 ± 104	270 ± 113	239 ± 103	<0.001	1.00	<0.001	<0.001
3 months	212 ± 93b[c]	208 ± 85b[c]	315 ± 148a[c]				
Change	−78 ± 122	−63 ± 118	76 ± 85				
VCAM-1, μg/L							
Baseline	1033 ± 311	962 ± 363	1023 ± 298				
3 months	857 ± 252b[c]	883 ± 329b	1147 ± 318a[c]	0.003	0.85	0.002	0.08
Change	−176 ± 275	−79 ± 440	124 ± 325				
IL6, ng/L							
Baseline	6.8 ± 4.6	6.8 ± 6.0	5.9 ± 5.3				
3 months	5.7 ± 3.7[c]	5.9 ± 5.5	7.3 ± 5.8[c]	0.002	1.00	0.004	0.019
Change	−1.2 ± 2.5	−0.9 ± 0.9	1.4 ± 2.7				
CRP, μg/L							
Baseline	4.0 ± 4.9	2.2 ± 1.9	2.8 ± 2.7				
3 months	2.5 ± 2.4[c]	2.5 ± 3.2	3.9 ± 5.7	0.024	0.20	0.025	1.00
Change	−1.5 ± 4.9	0.3 ± 2.5	1.1 ± 4.1				

Modified from Mena et al. [19].

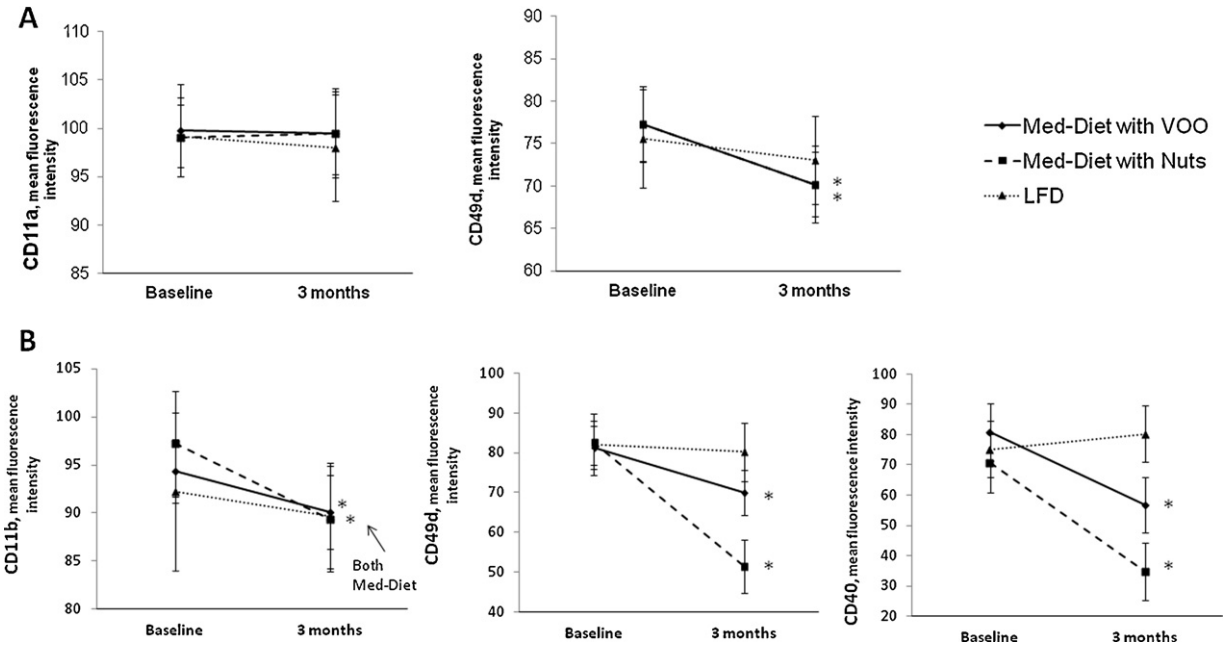
Values are means ± SD. Means in a row with online letter (a,b) without a common letter differ, P < 0.05 (Bonferroni post hoc test).

[a]Data were analyzed by repeated-measures 2-factor ANOVA (P < 0.05).

[b]Data were analyzed by ANOVA (P < 0.05).

[c]Different from baseline, P < 0.05 (Bonferroni post hoc test).

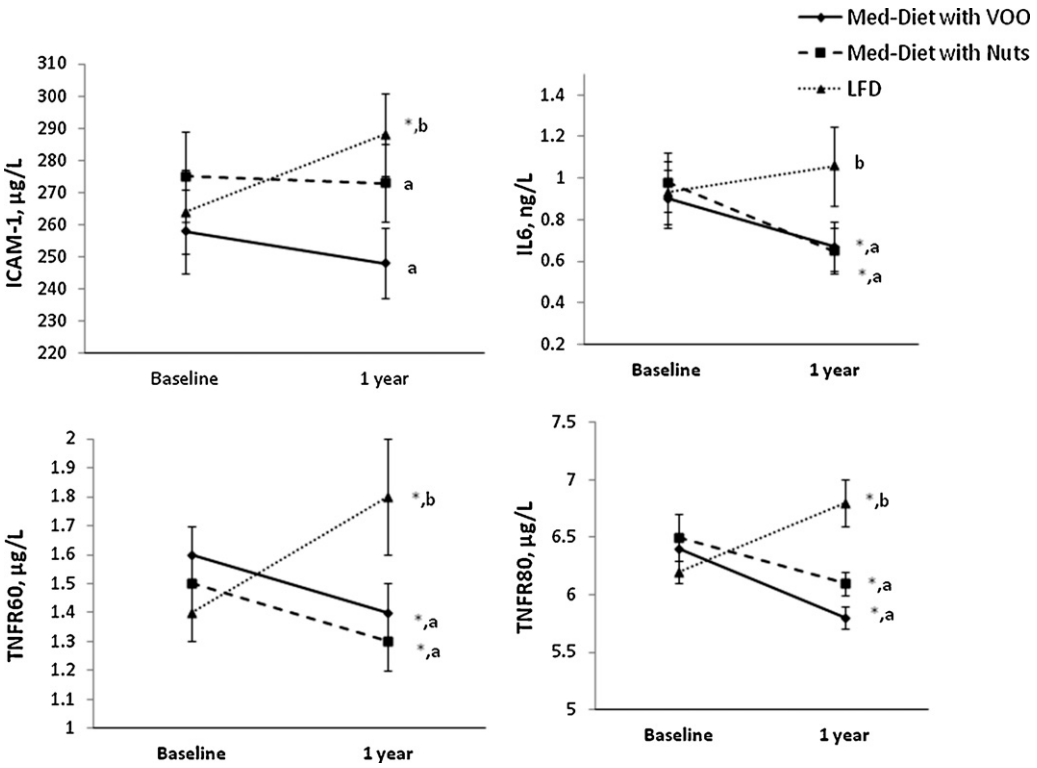
FIGURE 1.



Changes in adhesion molecules and proinflammatory ligand CD40 expression on T-lymphocytes (A) and monocytes (B). Values are mean ± SD; *different from baseline, $P < 0.05$ (Bonferroni post hoc test).

Data from Estruch [18] and modified from Mena et al. [19].

FIGURE 2.



Concentrations of circulating inflammatory molecules at baseline and after 1 year of intervention with Med-Diet supplemented with VOO, Med-Diet supplemented with nuts or LFD in patients at high risk for cardiovascular disease. Values are geometric means (95% CI). Data were analyzed by repeated-measures 2-factor ANOVA ($P < 0.05$). Changes between 1 year and baseline in response to the intervention treatment were analyzed by ANCOVA ($P < 0.05$). Repeated measures and ANCOVA were adjusted for age, gender, BMI, smoking status, physical activity and drugs (aspirin and statins). *Different from baseline, $P < 0.05$ (Bonferroni post hoc test). Changes in response to the intervention without a common letter differ ($P < 0.05$).

Modified from Urpi-Sarda et al. [20].