The Mediterranean Diet Pattern and Its Main Components Are Associated with Lower Plasma Concentrations of Tumor Necrosis Factor Receptor 60 in Patients at High Risk for Cardiovascular Disease

Mireia Urpi-Sarda,1-6 Rosa Casas,5,6 Gemma Chiva-Blanch,5,6 Edwin Saúl Romero-Mamani,5,6 Palmira Valderas-Martínez,5,6 Jordi Salas-Salvadó,6-7 Maria Isabel Covas,6-8 Estefanía Toledo,6,9 Cristina Andres-Lacueva,10,11 Rafael Llorach,10,11 Ana García-Arellano,8,12 Monica Bulló,6,7 Valentina Ruiz-Gutierrez,6,13 Rosa M. Lamuela-Raventos,6,10 and Ramon Estruch5,6*

1 Department of Internal Medicine, Hospital Clinic, Institut d’Investigació Biomèdica August Pi i Sunyer, University of Barcelona, Barcelona, Spain; 2 CIBER 08/08: Fisiopatología de la Obesidad y la Nutrición and RD06/0045/1003 Alimentación Saludable, Instituto de Salud Carlos III, Spain; 3 Human Nutrition Unit, Hospital Universitari de Sant Joan de Reus, ISPV, University Rovira i Virgili, Reus, Spain; 4 Cardiovascular Risk and Nutrition Research Group, Institut Municipal d’Investigación Mèdica, Barcelona, Spain; 5 Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain; 6 Nutrition and Food Science Department, School of Pharmacy, University of Barcelona, Barcelona, Spain; 7 Ingenio-CONSOLIDER program, FUN-C-FOOD, CSD5009-088, Barcelona, Spain; 8 Cardiovascular Risk and Nutrition Research Group, Institut Municipal d’Investigació Mèdica, Barcelona, Spain; 9 Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain; 10 Nutrition and Food Science Department, School of Pharmacy, University of Barcelona, Barcelona, Spain; 11 Ingenio-CONSOLIDER program, FUN-C-FOOD, CSD5009-088, Barcelona, Spain; 12 Hospital Reina Sofia, Tudela, Navarra, Spain; and 13 Nutrition and Lipids Metabolism, Instituto de la Grasa, CSIC, Sevilla, Spain

Abstract

Adherence to a Mediterranean diet (MD) is associated with a reduced risk of coronary heart disease. However, the molecular mechanisms involved are not fully understood. The aim of this study was to compare the effects of 2 MD with those of a low-fat-diet (LFD) on circulating inflammatory biomarkers related to atherogenesis. A total of 516 participants included in the Prevention with Mediterranean Diet Study were randomized into 3 intervention groups [MD supplemented with virgin olive oil (MD-VOO); MD supplemented with mixed nuts (MD-Nuts); and LFD]. At baseline and after 1 y, participants completed FFQ and adherence to MD questionnaires, and plasma concentrations of inflammatory markers including intercellular adhesion molecule-1 (ICAM-1), IL-6, and 2 TNF receptors (TNFR60 and TNFR80) were measured by ELISA. At 1 y, the MD groups had lower plasma concentrations of IL-6, TNFR60, and TNFR80 (P < 0.05), whereas ICAM-1, TNFR60, and TNFR80 concentrations increased in the LFD group (P < 0.002). Due to between-group differences, participants in the 2 MD groups had lower plasma concentrations of ICAM-1, IL-6, TNFR60, and TNFR80 compared to those in the LFD group (P = 0.028). When participants were categorized in tertiles of 1-y changes in the consumption of selected foods, those in the highest tertile of virgin olive oil (VOO) and vegetable consumption had a lower plasma TNFR60 concentration compared with those in tertile 1 (P < 0.02). Moreover, the only changes in consumption that were associated with 1-y changes in the geometric mean TNFR60 concentrations were those of VOO and vegetables (P = 0.01). This study suggests that a MD reduces TNFR concentrations in patients at high cardiovascular risk.


Introduction

Coronary heart disease (CHD)14 is the main cause of death worldwide, claiming 17.1 million lives in 2004 (1). Atherosclerosis is the main cause of CHD, and inflammation plays a key role from its onset to its progression to final lesions (2). In the earliest stages of CHD, vascular inflammation is activated by...
proinflammatory stimuli such as saturated fat intake, hypercholesterolemia, obesity, hyperglycemia, hypertension, and smoking, which induce the secretion of inflammatory cytokines that promotes the generation of endothelial adhesion molecules and other chemoattractants. These molecules are subsequently released to the circulation where they mediate the adhesion of circulating monocytes and lymphocytes to the vascular endothelium (2,3), leading to the formation of atherosclerotic lesions. New insight into the central role of inflammation in atherogenesis has linked inflammatory biomarkers such as vascular cell adhesion molecule-1 (VCAM-1), TNF, IL-1, IL-6, IL-18, and proteases (matrix metallopeptidase-9) to this disease (3). IL-6, IL-1β, and TNFα have also been associated with an increased risk of developing CHD (4). However, few studies have analyzed the effects of food interventions such as the Mediterranean diet (MD) on TNFα receptors. TNFα is a pleiotropic cytokine produced by activated monocytes and other cells (5) and has shown an ambivalent role in relation to CHD (5). TNF expresses its activity through 2 membrane receptors: TNF receptor (TNFR) 60, the 55–60 kDa TNFR 1, and the TNFR80, the 75–80 kDa TNFR 2. The activation of TNFR60 induces adhesion molecule expression and activates NF-κB and TNFR80 plays a role in T cell proliferation (6).

The prevention of atherosclerosis at early stages is based on healthy dietary and lifestyle habits that may diminish its progression. Epidemiological studies have suggested that the MD pattern and consumption of certain healthy foods such as legumes, grains, fruit and vegetables, olive oil, and wine may protect against CHD (7–9). Although the exact mechanisms of this protection are not fully understood, it has been suggested that functional compounds of some nutrients from the MD such as polyphenols from plant products (10–14) and fatty acids from vegetables or olive oil (15–18) may play a key role in these protective effects.

We therefore embarked on a study to evaluate the 1-y changes in plasma inflammatory markers [TNFR60, TNFR80, IL-6, and intercellular adhesion molecule-1 (ICAM-1)] in a free-living population with high risk of CHD following a MD and to study the relationship between these changes and modifications in their food intake. We studied a subpopulation from a larger feeding trial [the Prevention with Mediterranean Diet (PREDIMED) Study] designed to analyze the effects of 2 MD, one supplemented with virgin olive oil (MD-VOO), and one with mixed nuts (MD-Nuts), compared with a low-fat diet (LFD) control.

Methods

Participants and study design. The PREDIMED Study is a parallel-group, single-blind, multicenter, randomized, controlled, 5-y feeding trial assessing the effects of the Mediterranean diet (MD) supplemented with VOO (MD-VOO) or mixed nuts (MD-Nuts) on the primary prevention of CHD compared with a low-fat diet (LFD). Details of the study protocol were previously published (19,20). This substudy is a post hoc analysis using data already collected from 516 participants entering consecutively into the PREDIMED trial (Barcelona-Hospital Clinic, Navarra and Reus centers) in whom we determined plasma inflammatory biomarker concentrations in frozen stored samples. The study protocols were approved by the Institutional Review Boards of the centers and participants provided signed informed consent.

Eligible participants were community-dwelling men aged 54–79 y and women aged 58–79 y with no documented CHD who either had type 2 diabetes or at least 3 of the following risk factors: smoking, hypertension [blood pressure ≥140/90 mm Hg or treatment with antihypertensive drugs], LDL-cholesterol concentration ≥4.14 mmol/L (or treatment with hypolipidemic drugs), HDL-cholesterol concentration ≤1.03 mmol/L, 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.

Diets and physical activity. Participants were randomly assigned to 3 diet groups: LFD or 2 MD groups, one supplemented with VOO and the other with mixed nuts. For the LFD group, participants were advised to follow the AHA guidelines (21), which are oriented at reducing the intake of all types of fat. Participants in both MD groups were recommended to increase the intake of vegetables (≥2 servings/d), fresh fruit (≥3 servings/d), legumes, nuts, fish or seafood (≥3 servings/wk), and the use of olive oil for cooking and dressings as previously described (19,20,22,23). Participants assigned to the 2 MD groups received free provisions of 2 Mediterranean foods: participants assigned to the MD-VOO were provided with VOO (1 L/wk) and those assigned to the MD-Nuts were provided with mixed nuts (30 g/d, as 15 g walnuts, 7.5 g almonds, and 7.5 g hazelnuts). The fatty acid compositions of VOO and mixed nuts were previously reported (22). No specific recommendations for physical activity were given.

Measurements. At baseline and after 1 y of follow-up, participants completed a validated 14-item questionnaire assessing adherence to the MD (24), a validated 137-item validated FFQ (25), a validated version of the Minnesota Leisure Time Physical Activity Questionnaire for men (26) and women (27), and a 47-item questionnaire about education, lifestyle, history of illnesses, and medication use.

Trained personnel measured weight and height using calibrated scales and a wall-mounted stadiometer, respectively, waist circumference was determined midway between the lowest rib and the iliac crest using an anthropometric tape, and blood pressure was measured in triplicate with a validated semiautomatic oscillometer (Omron HEM-705CP) (19,20). In addition, fasting blood was collected and the plasma obtained was stored at −80°C until assay. Energy and nutrient intake estimates were obtained from Spanish food composition tables (20). All these procedures were repeated after 1 y of intervention.

The main outcome measurements were plasma concentrations of inflammatory biomarkers at baseline and after 1 y. ELISA assays were performed per participant in thawed plasma (kept at −80°C until analyzed) using commercial immunoassays kits for soluble ICAM-1, IL-6, TNFR60, and TNFR80 (Bender MedSystem). For the ELISA assays, the intra- and interassay CV ranged from 1.4 to 4.9% and from 2.0 to 8.6%, respectively.

Statistical methods. Statistical analyses were conducted using PASW Statistics 18 (version 18.0; SPSS). We estimated our sample size based on expected TNFR60 changes. Considering an expected decrease in TNFR60 of 0.15 μg/L in the intervention groups, an expected increase in TNFR60 of 0.15 μg/L in the LFD group, and an expected SD of 0.85 μg/L in all 3 groups, and assuming a 2-tailed α error of 0.05 and a statistical power of 0.8, our estimated sample size was 127 participants/group. Values for the baseline characteristics of the participants are expressed as means ± SD. Categorical variables are expressed as percentages. We transformed variables with a skewed distribution (ICAM-1, IL-6, TNFR60, and TNFR80) to their natural logarithm for analyses. Repeated-measures ANOVA was used to compare changes in inflammatory biomarkers and food variables, testing the effects of interaction of 2 factors: time as a within-participants factor with 2 levels (baseline and 1 y) and the groups of consumption (2 MD groups and LFD group), after adjustment for age, sex, BMI, smoking status, physical activity, research center, and drug use (aspirin and statins). To test the effects of individual factors, we calculated the differences between 1 y and baseline values for the molecules and then we applied an ANCOVA test after adjustment for the same variables as before. Participants from all groups were also categorized based on tertiles of 1-y changes in the consumption of 13 selected food groups (VOO, refined olive oil, nuts, vegetables, legumes, fruit, cereals, fish and seafood, meat and meat products, pastries, cakes or sweets, low-fat dairy products,
Concentrations of circulating inflammatory molecules at baseline and after 1 y of intervention with MD-VOO, MD-Nuts, or LFD in patients at high risk for cardiovascular disease.

<table>
<thead>
<tr>
<th></th>
<th>MD-VOO</th>
<th>MD-Nuts</th>
<th>LFD</th>
<th>P value for differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 176)</td>
<td>(n = 175)</td>
<td>(n = 163)</td>
<td></td>
</tr>
<tr>
<td>ICAM-1, μg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>258 (245–271)</td>
<td>275 (261–290)</td>
<td>264 (251–279)</td>
<td></td>
</tr>
<tr>
<td>1 y</td>
<td>248 (237–259)</td>
<td>273 (261–285)</td>
<td>288 (275–301)*a</td>
<td>0.001</td>
</tr>
<tr>
<td>Change</td>
<td>−10 (−22 to −1)</td>
<td>−2 (−14 to −10)</td>
<td>24 (10–35)</td>
<td></td>
</tr>
<tr>
<td>IL-6, ng/L</td>
<td>0.50 (0.39–1.07)</td>
<td>0.98 (0.84–1.14)</td>
<td>0.53 (0.78–1.10)</td>
<td>0.001</td>
</tr>
<tr>
<td>1 y</td>
<td>0.67 (0.55–0.82)*b</td>
<td>0.65 (0.54–0.77)*b</td>
<td>1.06 (0.87–1.29)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change</td>
<td>−0.23 (−0.4 to −0.003)</td>
<td>−0.33 (−0.6 to −0.1)</td>
<td>0.13 (−0.1–0.4)</td>
<td></td>
</tr>
<tr>
<td>TNFR60, μg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.6 (1.3–1.8)</td>
<td>1.5 (1.3–1.6)</td>
<td>1.4 (1.3–1.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>1 y</td>
<td>1.4 (1.3–1.6)*b</td>
<td>1.3 (1.2–1.4)*b</td>
<td>1.8 (1.6–2.0)*b</td>
<td>0.001</td>
</tr>
<tr>
<td>Change</td>
<td>−0.2 (−0.4 to −0.1)</td>
<td>−0.2 (−0.3 to −0.1)</td>
<td>0.4 (0.2–0.5)</td>
<td></td>
</tr>
<tr>
<td>TNFR80, μg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>6.4 (6.0–6.8)</td>
<td>6.5 (6.1–6.9)</td>
<td>6.2 (5.8–6.6)</td>
<td>0.81</td>
</tr>
<tr>
<td>1 y</td>
<td>5.8 (5.4–6.1)*b</td>
<td>6.1 (5.8–6.5)*b</td>
<td>6.8 (6.4–7.3)*b</td>
<td>0.001</td>
</tr>
<tr>
<td>Change</td>
<td>−0.6 (−1.1 to −0.3)</td>
<td>−0.4 (−0.9 to −0.1)</td>
<td>0.6 (0.1–1.2)</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are geometric means (95% CI). Means in a row with superscripts without a common letter differ, *P < 0.05 (Bonferroni post hoc test). *Different from baseline, *P < 0.05 (Bonferroni post hoc test). ICAM-1, intercellular adhesion molecule-1; LFD, low-fat diet; MD, Mediterranean diet; MD-VOO, Mediterranean diet supplemented with virgin olive oil; MD-Nuts, Mediterranean diet supplemented with mixed nuts; TNFR, TNF receptor.

2 Data analyzed by repeated-measures 2-factor ANOVA (P < 0.05).

3 Data analyzed by ANCOVA (P < 0.05). Repeated measures and ANCOVA were adjusted for age, sex, energy intake, BMI, smoking status, physical activity, research center, and drug use (aspirin and statins).

Results

On average, participants were 66 y old and nearly one-half were men (Supplemental Table 1). Almost all the participants (>90%) were overweight or obese, 78.4% had hypertension, 62.0% had dyslipidemia, and 50.3% were diabetic. All these factors and characteristics were balanced among the 3 groups at baseline. We did not observe significant changes in medication treatments, body weight, or physical activity during the study period.

Food, energy, and nutrient intakes. The consumption of foods and nutrients in the participants of this substudy was similar to the overall PREDIMED population that followed a MD or a control LFD (23) (Supplemental Tables 2 and 3). We observed interactions between time and treatments (P < 0.05). The main dietary changes were the large increases in the consumption of VO0 and mixed nuts in the corresponding MD groups (P < 0.013) and reciprocal decreases in the consumption of common olive oil in the MD-VOO and LFD groups (P < 0.001) (Supplemental Table 2). The increase in the intake of VO0 was greater in the MD-VOO group than in the other 2 groups (P < 0.001). Compared with participants in the LFD group that decreased their consumption of nuts, those in the MD groups increased this intake (P ≤ 0.002). Moreover, we observed higher increases in the MD-Nuts group than in the MD-VOO group (P < 0.001). The consumption of meat or meat products decreased after 1 y of intervention in the 3 groups (P < 0.05). The MD score increased by 2.3 points in the MD-Nuts group that decreased their consumption of VOO and mixed nuts in the corresponding MD groups (P ≤ 0.013) or did not change in the LFD group, whereas the increase in the MD-VOO group was 0.4 points (P ≤ 0.013). When we compared changes between groups, the MD score increased more in the MD-Nuts group than in the LFD group (P < 0.001). Energy intake increased in the MD-Nuts group (P = 0.003). In contrast, total energy and protein intakes decreased in the LFD group (P < 0.001) (Supplemental Table 3). Both MD groups also increased MUFA and PUFA intakes (P < 0.001), whereas the LFD group decreased their SFA, MUFA, and PUFA intakes (P < 0.001), α-Linolenic acid and marine (n-3) fatty acid intakes increased after 1 y of intervention in the 2 MD groups (P ≤ 0.03) and decreased (P ≤ 0.013) or did not change in the LFD group, respectively. The estimated energy expenditure from physical activity was similar in the 3 groups at baseline and after 1 y (data not shown).

Circulating inflammatory biomarkers. We observed interactions between time and treatment (P ≤ 0.001) in the molecules whole-fat dairy products, and wine), some nutrients (MUFA, and MD score. To study the interaction (time × treatment) between baseline and 1-y concentrations in plasma inflammatory molecules (TNFR60, TNFR80, ICAM-1, and IL-6) across tertiles, we used repeated-measures 2-factor ANOVA, and to study the effects of the individual factors we used an ANCOVA test, both performed after adjustment for age, sex, energy intake, BMI, smoking status, physical activity, research center, and drug use (aspirin and statins). In addition, to compare changes between baseline and 1-y concentrations of TNFR60 across tertiles in the 30 food groups, we fit a multivariate linear regression model obtaining the ratio of the geometric means of 1-y TNFR60 to baseline TNFR60 according to tertiles of changes in each of the 13 food groups after adjustment for the aforementioned variables. Then, we calculated the significance (P-trend) and the CI for the between-tertile differences. Significant interactions were analyzed by the simple-effect analysis. The multiple contrasts were adjusted by a Bonferroni post hoc test. Within- and between-group differences were expressed as estimated means and 95% CI. The significance level was set at P < 0.05.
analyzed (Table 1). After the intervention in the MD groups, the plasma concentrations of IL-6, TNFR60, and TNFR80 decreased ($P < 0.05$), whereas that of ICAM-1 tended to decrease in the MD-VOO group ($P = 0.09$) and did not change in the MD-Nuts group ($P = 0.82$). In the LFD group, the plasma concentrations of ICAM-1, TNFR60, and TNFR80 increased ($P = 0.002$) and the concentration of IL-6 tended to increase ($P = 0.05$). Plasma concentrations of the molecules analyzed at baseline did not differ among the 3 intervention groups ($P > 0.29$). We compared the effects of between-group differences by the ANCOVA test (Table 1). Compared with the LFD group, the 2 MD groups had 1–34% lower plasma concentrations of ICAM-1, IL-6, TNFR60, and TNFR80 ($P = 0.028$).

**Relationship among changes in food intake, body weight, and inflammatory markers.** In this study, we mainly focused on the changes in plasma inflammatory molecules across tertiles of 1-y changes in the intake of selected foods and in the MD score (Table 2). We observed interactions between time and treatment ($P \leq 0.016$). Participants in the highest tertile of VOO consumption of selected foods and nutrients had lower plasma TNFR60 concentrations after 1 y ($P = 0.009$) (Table 2). Participants in tertile 3 had a decrease of 17% in the plasma concentration of TNFR60 and this diminution was greater than in those in tertiles 1 and 2 ($P \leq 0.008$). The decrease in the TNFR60 concentration (−12%) in participants in tertile 3 of vegetable consumption was greater than in those in tertile 1 ($P = 0.013$). Again, the only significant 1-y change in food intake was in that of VOO and vegetables. A greater increase in the consumption of VOO and vegetables was associated with a lower plasma TNFR60 concentration after 1 y ($P = 0.01$) (Table 3). Participants in the lowest tertile of changes in alcohol intake, i.e., those who reduced their alcohol consumption, had a higher plasma ICAM-1 concentration ($P = 0.016$). In addition, participants who were more adherent to the MD according to the 14-point score did not differ from tertile 2 ($P = 0.006$) and tended to differ from tertile 1, in which the concentration did not change ($P = 0.07$) (Table 2). Changes in the participants’ body weight were

---

**TABLE 2** Concentrations of circulating inflammatory molecules at baseline and after 1 y in all participants by tertile of change in consumption of selected foods and nutrients

<table>
<thead>
<tr>
<th>Foods and Nutrients Tertiles</th>
<th>Repeated-measures ANOVA$^2$</th>
<th>$P$ value for differences$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time x treatment</td>
<td>1 vs. 2</td>
</tr>
<tr>
<td><strong>VOD consumption tertiles, g/d</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 ($n = 131$) ($\leq -0.3$)</td>
<td>2 ($n = 125$) ($-0.3$ to $-24$)</td>
<td>3 ($n = 128$) ($\geq 24$)</td>
</tr>
<tr>
<td><strong>TNFR60, $\mu$g/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.4 (1.2–1.5)$^b$</td>
<td>1.4 (1.3–1.6)$^b$</td>
</tr>
<tr>
<td>1 y</td>
<td>1.4 (1.3–1.6)</td>
<td>1.5 (1.3–1.7)</td>
</tr>
<tr>
<td>Change</td>
<td>0 ($-0.1$ to $-0.2$)</td>
<td>0.1 ($-0.03$ to $-0.2$)</td>
</tr>
<tr>
<td><strong>TNFR80, $\mu$g/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1 ($n = 132$) ($\leq -3.7$)</td>
<td>2 ($n = 127$) ($-3.7$ to $-9.8$)</td>
</tr>
<tr>
<td><strong>Vegetable consumption tertiles, g/d</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 ($n = 101$) ($\leq -24.5$)</td>
<td>2 ($n = 112$) ($-24.5$ to $-62.7$)</td>
<td>3 ($n = 121$) ($\geq 62.7$)</td>
</tr>
<tr>
<td><strong>MUFA consumption tertiles, g/d</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 ($n = 135$) ($\leq 22.5$)</td>
<td>2 ($n = 122$) ($22.5$ to $38.1$)</td>
<td>3 ($n = 127$) ($\geq 38.1$)</td>
</tr>
<tr>
<td><strong>MD score tertiles</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 ($n = 124$) ($\leq 9.9$)</td>
<td>2 ($n = 127$) ($9.9$ to $2.4$)</td>
<td>3 ($n = 134$) ($\geq 2.4$)</td>
</tr>
<tr>
<td><strong>TNFR80, $\mu$g/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>6.6 (6.1–7.0)$^a$</td>
<td>6.1 (5.7–6.6)</td>
</tr>
<tr>
<td>1 y</td>
<td>6.6 (6.2–7.0)$^h$</td>
<td>6.4 (6.0–6.8)$^{ab}$</td>
</tr>
<tr>
<td>Change</td>
<td>0 ($-0.5$ to $-0.7$)</td>
<td>0.3 ($-0.4$ to $-0.7$)</td>
</tr>
</tbody>
</table>

$^1$ Values are geometric means (95% CI). Means in a row with superscripts without a common letter differ, $P < 0.05$ (Bonferroni post hoc test). *Different from baseline, $P < 0.05$ (Bonferroni post hoc test). Dif, differences between 1 y and baseline; MD, Mediterranean Diet; TNFR, TNF receptor; VOO, virgin olive oil.

$^2$ Data analyzed by repeated-measures 2-factor ANOVA ($P < 0.05$).

$^3$ Data analyzed by ANCOVA ($P < 0.05$). Repeated measures and ANCOVA were adjusted for age, sex, energy intake, BMI, smoking status, physical activity, research center and drugs (aspirin and statins).
not associated with changes in the plasma inflammatory biomarkers studied (data not shown).

**Discussion**

In the current study, we observed that the 2 MD interventions supplemented with either VOO or nuts had an antiinflammatory effect, inducing significant reductions in the plasma concentrations of TNFR, IL-6, and ICAM-1. The latter 2 have been widely related to cardiovascular disease (28) and TNFR signaling has been implicated in both the development and consequences of atherosclerosis (29,30). Results from controlled feeding trials or in free-living participant studies have suggested that consumption of VOO and nuts decreases plasma ICAM, VCAM, E-selectin, IL-6, and CRP concentrations (31–34). However, the most outstanding and novel result of our study is the effect observed with VO

Olive oil is the most remarkable food of the MD due to its high production and consumption in the Mediterranean area and its reported beneficial effects on a wide range of cardiovascular risk factors (7,36). The antiinflammatory properties of VOO have been attributed to its content of polyphenols such as tyrosol, hydroxytyrosol, and oleuropein (10,11,14,37,38) and a recently discovered phenolic compound with high antiinflammatory activity, oleocanthal (13). Moreover, other polyphenols in olive oil such as 1-phenyl-6,7-dihydroxy-isochroman inhibit the activity of COX-2 in vitro and thus inhibit TNFα production in LPS-primed human monocytes in a dose-dependent manner (39).

Although no studies to our knowledge have directly related the consumption of healthy diets and fatty acids to plasma concentrations of TNFR, several studies have shown healthy effects of fatty acids on various antiinflammatory markers, including TNF and IL (16,40). Thus, oleic acid reverses the in vitro inhibitory effect of the inflammatory cytokine TNFα on insulin production (18). Furthermore, type 2 diabetic mice fed an oleic acid diet derived from peanut oil had lower plasma glucose concentrations than those fed a high-fat diet without oleic acid (18). Recently, healthy humans receiving 50 mL of VOO and cod liver oil had significant reductions in plasma ICAM-1 and TNFα concentrations measured 3 h after the treatment, demonstrating the antiinflammatory effect of these oils (17). Mice treated with different oil-enriched diets such as fish oil, refined olive oil, and pomace olive oil for 8 wk showed that refined olive oil and fish oil diets reduced TNFα, IL-1, and IL-6 and PG E2 production (41). Chrysohou et al. (42) studied the effect of adherence to a MD in a population from the Attica area of Greece and observed that the participants most adherent to this diet had lower plasma concentrations of CRP, IL-6, homocysteine, and fibrinogen as well as a lower white blood cell count and a borderline association with TNFα. Regular diets supplemented with olive oil (rich in MUFA) or with walnuts (rich in PUFA) induced a greater diminution in TNFα mRNA expression in peripheral blood cells than those diets rich in SFA such as butter (43). The intake of vegetable oils such as olive oil by healthy Tehran women was associated with lower plasma concentrations of TNFα, ICAM-1, and CRP (44). In a recent interventional study with VOO in humans, the expression of genes related to atherosclerosis was downregulated, with
polyphenols of VOO having a significant impact on the changes in the genetic expression of the disease (12). All these mechanisms may help to explain the observation that a dietary intervention to enhance a MD rich in VOO can contribute to a reduction in the risk of type 2 diabetes mellitus (45).

On the other hand, participants who reduced alcohol consumption had a significant increase in the plasma ICAM-1 concentration. Several studies have shown the antiinflammatory effects of moderate alcohol consumption (46). Finally, variations in the body weight of the participants did not mediate changes in the plasma inflammatory biomarkers studied.

The main limitations of our study are the higher age of the participants and their high cardiovascular risk factors, which do not allow extrapolation of the results to the general population. Ensuring adherence to dietary instructions is difficult in a diet trial. However, adherence to recommended dietary patterns and supplemental foods was good, as judged by self-report and objective measurements (23). On the other hand, the strengths of our study are the robust epidemiological design (randomized, controlled feeding trial), the reproduction of real-life conditions with home-prepared foods that reflect usual practice, the high completion rates, the adherence to the MD, and the compliance with supplemented foods.

In conclusion, this is the first time, to our knowledge, that a diminution in TNFR concentrations has been related to a MD in addition to the diabetes risk factors. However, further investigations should be performed to identify the molecular mechanisms underlying these relationships.

Acknowledgments
The authors are grateful to the Fundación Patrimonio Comunal Olivarero, California Walnut Commission, Borges SA, and Morella Nuts SA for generously donating the olive oil and nuts used in this study. The authors thank Emilio Corbella from the Hospital Universitari de Bellvitge (Barcelona, Spain) and Joan Vila from IMIM (Barcelona, Spain) for their support in the statistical analysis. R.E., J.S-S., M.C., E.T., C.A-L., R.L., V.R-G., and R.M.L-R. designed research; M.U-S., G.C-B., R.C., and P.V-M. conducted research; E.S.R-M., J.S-S., M.C., E.T., C.A-L., R.L., A.G-A., M.B., V.R-G., R.M.L-R., and R.E. provided essential materials; M.U-S. and R.E. analyzed data and performed statistical analysis; M.U-S. and R.E. wrote the paper; E.T., J.S-S., M.B., A.G-A., G.C-B., V.R-G., and P.V-M. critically reviewed the manuscript; and R.E. had primary responsibility for the final content. All the authors read and approved the final manuscript.

Literature Cited


