Effect of a traditional Mediterranean diet on apolipoproteins B, A-I, and their ratio: A randomized, controlled trial

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A B S T R A C T

Objectives: Apolipoprotein (Apo)B, ApoA-I, and their ratio could predict coronary heart disease (CHD) risk more accurately than conventional lipid measurements. Our aim was to assess the effect of a traditional Mediterranean diet (TMD) on apolipoproteins.

Methods: High-cardiovascular risk subjects (n = 551, 308 women and 243 men), aged 55–80 years, were recruited into a large, multicenter, randomized, controlled, parallel-group, clinical trial (The PREDIMED Study) aimed at testing the efficacy of TMD on primary cardiovascular disease prevention. Participants assigned to a low-fat diet (control) (n = 177), or TMDs (TMD + virgin olive oil (VOO), n = 181 or TMD + nuts, n = 193) received nutritional education and either free VOO (ad libitum) or nuts (dose: 30 g/day). A 3-month evaluation was performed.

Results: Both TMDs promoted beneficial changes on classical cardiovascular risk factors. ApoA-I increased, and ApoB and ApoB/ApoA-I ratio decreased after TMD + VOO, the changes promoting a lower cardiometabolic risk. Changes in TMD + VOO versus low-fat diet were −2.9 mg/dL (95% CI, −5.6 to −0.08), 3.3 mg/dL (95% CI, 0.84 to 5.8), and −0.03 mg/dL (−0.05 to −0.01) for ApoB, ApoA-I, and ApoB/ApoA-I ratio, respectively.

Conclusions: Individuals at high-cardiovascular risk who improved their diet toward a TMD pattern rich in virgin olive oil, reduced their Apo B and ApoB/ApoA-I ratio and improved ApoA-I concentrations.

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1. Introduction

Adherence to the traditional Mediterranean diet (TMD) has been associated with a reduction in coronary heart disease (CHD), cancer, and overall mortality [1]. One classical feature of the TMD is to
improve the lipid cardiovascular risk profile [2]. Available evidence shows that apolipoprotein (Apo) B and ApoA-I, and the ApoB/ApoA-I ratio, could predict CHD and stroke risk more accurately than conventional lipid measures [3–5]. Recent cross-sectional data of the NHANES III study have shown a decrease of non-HDL cholesterol and ApoB as the adherence to a Mediterranean type diet increased in pre-menopausal women [6].

Among olive oils consumed within the frame of the Mediterranean diet, virgin olive oil (VOO) (produced by direct press or centrifugation methods) has the highest antioxidant polyphenol content. A significant decrease in the ApoB/ApoA-I ratio as the quantity of virgin olive oil administered increased has been reported [7]. Nuts are also typical of the Mediterranean diet. An increase in nut consumption was significantly associated with a lower risk of CHD and lower ApoB levels in a cohort of women with type 2 diabetes [8]. We designed a large-scale feeding trial in a high cardiovascular risk population in order to assess the effects of two Mediterranean diets, one enriched with virgin olive oil and the other with mixed nuts, compared with a low-fat diet on cardiovascular outcomes. Here, we report the results of a 3-month intervention on ApoB, ApoA-I, and the ApoB/ApoA-I ratio in 551 participants recruited into the trial.

2. Methods

2.1. Study design

The PREDIMED study (PREvención con Dileta MEDiterránea) is a parallel, multi-center, controlled, randomized clinical trial aimed at assessing the effects of the TMD on the primary prevention of cardiovascular disease (http://www.predimed.org). The trial is currently taking place with 7447 high-CHD risk participants assigned to 3 intervention groups: (1) a traditional Mediterranean Diet (TMD) with virgin olive oil (TMD + VOO); (2) a TMD with mixed nuts (TMD + nuts); and (3) a low-fat diet. The main outcomes are cardiovascular events (cardiovascular death, non-fatal myocardial infarction) or non-fatal stroke. The Institutional Review Board of the recruitment centers approved the study protocol and participants signed an informed consent. The trial is registered in Current Controlled Trials, London. Identifier: ISRCTN35739639. Before and after 3-month interventions, biological samples were obtained after an overnight fast, coded, shipped to central laboratories, and frozen at –80 °C until assay.

2.2. Study population

From October 2003 to March 2004, a total of 930 asymptomatic subjects at high risk for CHD, aged 55–80 years, were initially selected in 10 Spanish Primary Care Centers. They fulfilled at least one of the two following criteria: (1) type 2 diabetes mellitus or (2) three or more CHD risk factors (smoking, hypertension, dislipidemia, obesity, or family history of CHD). Exclusion criteria were: history of cardiovascular disease; severe chronic illness; drug or alcohol addiction; difficulties with or low predicted likelihood of following the Prochaska and DiClemente stages of change in behaviour; history of food allergy or intolerance to olive oil or nuts; and any condition that may impair participation in the study. Participants’ eligibility was based on the review of clinical records and a screening visit in the Primary Care Center by the physician. After the screening visit, each center randomly assigned eligible participants to 1 of 3 diet groups by using a computer-generated random-number sequence. The coordinating center constructed the randomization table, and participants were randomly assigned into blocks of 50 participants balanced by center, sex, and age group. We concealed allocation into the intervention groups by using closed envelopes with correlative numbers by pre-specified subgroups of sex and age.

2.3. Baseline assessments and interventions

The baseline examination included the administration of: (1) a 14-item questionnaire, an extension of a questionnaire designed to assess the degree of adherence to the TMD [9], (values of zero or one were assigned to each of 14 items); (2) a 137-item food frequency questionnaire [10]; (3) the Minnesota Leisure Time Physical Activity questionnaire [11]; and (4) a 47-item general questionnaire assessing life-style, health conditions, socio-demographic variables, medical diagnoses, and medication use. On the basis of the baseline TMD-14-item questionnaires, each participant was given personalized dietary advice by the dietician during a 30 min session. Participants allocated to a low-fat diet were advised to reduce all types of fat, and were given written recommendations according to the American Heart Association guidelines. The detailed protocol including study design, rationale, and organization has been previously published [2,12].

2.4. Evaluation of the intervention

After the 3-month interventions, all baseline procedures were repeated. Biological assessment of the intervention compliance was performed in 275 (49.5%) participants (96, 87, and 92 from the TMD + VOO, TMD + nuts, and low-fat groups, respectively) selected at random and matched by age and sex. Tyrosol and hydroxytyrosol, the main phenolic compounds present in virgin olive oil, were measured in urine by gas chromatography–mass spectrometry in order to assess the compliance of the TMD + VOO group. The plasma α-linolenic content, measured by gas chromatography, was used as a biomarker of compliance for the TMD + nuts group.

2.5. Outcome measures

Anthropometric data were measured by standardized procedures [2,12]. Serum glucose, cholesterol, and triglyceride concentrations were measured using standard enzymatic reagents (Trinder, Bayer Diagnostics, Tarrytown, NY, USA) adapted to a Cobas Mira automated analyzer (Hoffmann-La Roche, Basel, Switzerland). HDL cholesterol was quantified after precipitation with phosphotungstic acid and magnesium chloride. LDL cholesterol was calculated by the Friedewald formula. Intrassay coefficients of variation (CV) were 0.5%, 0.6%, 0.6%, and 1.1% for glucose, total cholesterol, triglycerides, and HDL cholesterol, respectively. Inter-assay CVs were 1.2%, 0.9%, 2.5%, and 3.1% for glucose, total cholesterol, triglycerides, and HDL cholesterol, respectively. Serum ApoB and ApoA-I were determined by PEG enhanced immunoturbidimetry (Siemens Health Care Diagnostic Inc., Tarrytown, New York, USA) in a ADVIA 2400 Chemistry System analyzer (Siemens, Tarrytown, NY, USA). Intrassay CVs were 1.1% for both apolipoproteins. Inter-assay CVs were 3.6% and 3.3% for ApoB and ApoA-I, respectively. Changes in the Framingham risk score and in the calibrated Framingham risk score for the Spanish population (the REGICOR score) [13] were evaluated. We also assessed the changes in the estimated risk of developing myocardial infarction (MI) according to the ApoB/Apo A-I from data of the joint results of AMORIS and INTERHEART [14]. We evaluated the percentage of individuals who decreased ApoB levels under the recommended values for their cardiometabolic risk according to the Consensus Conference Report from the American Diabetes
2.6. Statistical analyses

Normality of continuous variables was assessed by normal probability plots. One-factor analysis of variance or Kruskal–Wallis test were used to assess differences in basal characteristics among the three interventions as appropriate. Sex differences at baseline were assessed by Student’s t or Mann–Whitney tests as appropriate. Area under the curve for ApoB was estimated using the observed mean and standard deviation to obtain normal quantiles. In order to examine 3-month changes in clinical, dietary, and laboratory variables, we performed multivariate analyses of covariance (ANCOVA) with the intervention group as fixed factor. We controlled potential confounding by age, sex, center, and baseline values of the examined variable by also entering these variables into the multivariable model as covariates. Statistical significance was defined as $P<0.05$. These statistical analyses were performed using the SAS System for Windows release 8.02.

3. Results

3.1. Study population

Of the 930 participants, 772 met the eligibility criteria and were randomized to one of the 3 interventions. The effect of the 3 interventions on Apo B. Apo A-I, and their ratio was assessed in a subpopulation of 551 individuals (308 women and 243 men) selected at random, with similar characteristics to those of the whole group (Supplementary Appendix 1). Fig. 1 shows the flow of participants throughout the study. Baseline characteristics of participants by groups of intervention are shown in Table 1.

3.2. Energy balance and dietary adherence

Adherence to supplemental foods was good in both TMD groups. Compared with the changes observed in the low-fat diet group,
participants assigned to the TMD + VOO group showed an increase in urinary tyrosol of 21 ng/mL (95% CI, 6–34 ng/mL) (P = 0.013) and in hydroxytyrosol of 87 ng/mL (95% CI, 31–142 ng/mL) (P = 0.008). Participants allocated to the TMD + nuts group showed an increase of plasma α-linolenic acid level from baseline of 0.16% (of total fatty acids) (95% CI, 0.08–0.22%) (P = 0.039). Physical activity did not change during the intervention periods in any group. Participants in the TMD + nuts group increased the intake of total fat, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), and reduced saturated fatty acids (SFA) and carbohydrate intake (P < 0.05) [Supplementary Appendix 2]. In the TMD + VOO group a decrease in total and SFA fat without an increase in MUFA consumption, was observed. In agreement with this, total olive oil consumption was similar, whereas significant changes were observed in virgin and ordinary (a mix of refined and virgin) olive oils consumption in the TMD + VOO group [Supplementary Appendix 2]. These data, together with the observed increase of urinary tyrosol and hydroxytyrosol, indicated a replacement of the ordinary olive oil by the virgin one in the TMD + VOO group. In both TMD groups consumption of pulses and fruit increased. Participants in the TMD + nuts group decreased their intake of dairy products. Consumption of meat diminished in all groups. The global dietary pattern of adherence to the Mediterranean diet increased in the TMD groups after the intervention, as was reflected in the changes in the 14-item score [Supplementary Appendix 2].

3.3. Classical cardiovascular risk factors and apoproteins

No changes were observed in body mass index or waist circumference in the three interventions. Compared with the low-fat diet, the mean changes in TMD + VOO and TMD + nuts groups were −5.8 mmHg (95% CI, −8.2 to −2.9 mmHg) and −6.9 mmHg (CI, −10.2 to −3.9 mmHg), respectively, for systolic blood pressure; and −1.58 mmHg (CI, −2.96 to −0.03 mmHg) and −2.8 mmHg (CI, −4.1 to −0.89 mmHg), respectively, for diastolic blood pressure (P < 0.05). As shown in Table 2, participants in the TMD + nuts group showed reductions in total cholesterol and triglycerides, the changes being significant versus that of the low-fat group (P < 0.05). LDL cholesterol increased in both TMD groups, the changes reaching significance versus that of the low-fat group (P < 0.05). LDL cholesterol decreased in both TMD groups versus their baseline (P < 0.05). Non-HDL cholesterol, and total/HDL cholesterol and LDL/HDL cholesterol ratios decreased in both TMD groups, the changes reaching significance versus those of the low-fat diet group (P < 0.05). After both TMD interventions ApoA-I increased and ApoB and the ApoB/ApoA-I decreased, the changes reaching significance after TMD + VOO (P < 0.01) (Table 2). In this group,
Changes were also significant versus those observed in the low-fat group ($P<0.05$).

### 3.4. Changes in cardiovascular risk and treatment goal achievements

Changes in the Framingham risk score after 3-month interventions were (mean ± SD): −5.1% (8.2%), −4.2% (8.6%), and −3.2% (9.6%) for the TMD + VOO, TMD + nuts, and low-fat diet groups, respectively ($P<0.001$ in all groups). The change in the TMD + VOO group was significant versus that of the low-fat diet group ($P=0.035$). Changes in the REGICOR score (Framingham risk score adapted for Spanish populations) were −2.3% (3.8%), −1.9% (3.9%), and −1.5% (4.8%) for the TMD + VOO, TMD + nuts, and low-fat diet groups, respectively ($P<0.001$ in all cases). The change in the TMD + VOO group was significant versus that of the low-fat diet group ($P=0.028$). Changes in the estimated risk for developing MI according to the ApoB/ApoA-I ratio [14] by sex are shown in Table 3. After the TMD + VOO intervention, there was a fall in the male population of 7.8% from the high to low MI risk categories, whereas 16.6% of females fell from the high-medium to low MI risk. In the TMD + nuts group, 5.6% of the male population dropped from high to medium-low MI risk. Changes in other groups were less than 4%.

Concerning treatment goals recommended by the ADA/AC [15], the percentage of individuals at high cardiovascular risk who decreased their ApoB concentrations under 90 mg/dL were 7.9% and 5.2% in the TMD + VOO and TMD + nuts groups, respectively. After the TMD + VOO intervention, 8.8% of the individuals at the highest risk decreased their ApoB concentrations below 80 mg/dL. Changes in other groups were below 1.5%. Fig. 2 shows the area under the curve for ApoB values pre- and post-intervention in the TMD + VOO group.

### 4. Discussion

The present study is, to our knowledge, the first large, randomized, controlled, clinical trial focused on the effect of a Mediterranean type diet on apolipoproteins. Here, individuals at CHD high-risk who improved their diet toward a TMD pattern with richness in virgin olive oil had significant reductions in plasma ApoB and ApoB/ApoA-I ratio, with an increase in plasma ApoA-I, in comparison with individuals assigned to a low-fat diet. A 3-month consumption of both TMDs (rich in VOO and rich in nuts) decreased by over 5% the percentage of male individuals at high cardiovascular risk according to their ApoB/ApoA-I ratio [14]. It is also increased by more than 5% the percentage of individuals of both sexes achieving the therapeutic goal for high MI risk individuals (ApoB < 90 mg/dL) [15]. Consumption of the TMD + VOO also promoted that 16.6% of women fell from the high-medium to low cardiometabolic risk, as a consequence of the changes in their ApoB/ApoA-I ratio [14], and increased by 8.8% the percentage of individuals achieving the therapeutic goal for higher MI risk individuals (ApoB < 80 mg/dL) [15].

In this study, and in agreement with our previous results [2] and others [16], a 3-month consumption of a TMD improved the classical cardiovascular risk lipid profile. The usefulness of LDL cholesterol or lipid ratios as predictors for CHD has been questioned as an incomplete measure of other atherogenic lipoproteins such as very low density (VLDL) and intermediate density (IDL) lipoproteins. Recently, expert panels have proposed using ApoB, in conjunction with standard lipids, to address the previously mentioned limitations [15,17,18]. ApoB is considered to be particularly appropriate in monitoring settings such as diabetes or statin therapy [15,19]. The usefulness of the ApoB/ApoA-I ratio as a predictor for CHD risk remains in elderly people in whom conventional lipid

### Table 2

<table>
<thead>
<tr>
<th>Variables</th>
<th>Changes from baseline at 3 months</th>
<th>TMD vs. VOO vs. low-fat diet</th>
<th>Mean (%)</th>
<th>TMD + nuts vs. low-fat diet</th>
<th>Mean (%)</th>
<th>TMD vs. VOO vs. low-fat diet</th>
<th>Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>−6.8 (−12.4 to −1.3)</td>
<td>0.016</td>
<td>−6.8 (−12.4 to −1.3)</td>
<td>0.016</td>
<td>−6.8 (−12.4 to −1.3)</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>−8.4 (−10.0 to 0.4)</td>
<td>0.005</td>
<td>−8.4 (−10.0 to 0.4)</td>
<td>0.005</td>
<td>−8.4 (−10.0 to 0.4)</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>2.1 (−0.9 to 5.0)</td>
<td>0.000</td>
<td>2.1 (−0.9 to 5.0)</td>
<td>0.000</td>
<td>2.1 (−0.9 to 5.0)</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>LDL/HDL cholesterol ratio</td>
<td>0.43 (−0.39 to −0.39)</td>
<td>0.000</td>
<td>0.43 (−0.39 to −0.39)</td>
<td>0.000</td>
<td>0.43 (−0.39 to −0.39)</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>ApoB/ApoA-I ratio</td>
<td>0.03 (−0.14 to 0.02)</td>
<td>0.11</td>
<td>0.03 (−0.14 to 0.02)</td>
<td>0.11</td>
<td>0.03 (−0.14 to 0.02)</td>
<td>0.11</td>
<td></td>
</tr>
</tbody>
</table>

*Data are presented as mean changes (95% confidence interval) or percentages, adjusted for center, age, sex, and baseline values.*
Table 3
Changes in the percentage of individuals at risk of developing myocardial infarction related to ApoB/ApoA-I ratio after interventions.4

<table>
<thead>
<tr>
<th></th>
<th>TMD + VOO (n = 181)</th>
<th>TMD + nuts (n = 193)</th>
<th>Low-fat diet (n = 177)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Post-intervention</td>
<td>Change</td>
<td>Post-intervention</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low risk (ApoB/ApoA-I ratio &lt; 0.7)</td>
<td>36.4%</td>
<td>7.8%</td>
<td>29.8%</td>
</tr>
<tr>
<td>Medium risk (ApoB/ApoA-I ratio 0.7–0.9)</td>
<td>38.9%</td>
<td>0%</td>
<td>35.6%</td>
</tr>
<tr>
<td>High risk (ApoB/ApoA-I ratio &gt; 0.9)</td>
<td>24.7%</td>
<td>−7.8%</td>
<td>34.6%</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low risk (ApoB/ApoA-I ratio &lt; 0.6)</td>
<td>35.7%</td>
<td>16.6%</td>
<td>35%</td>
</tr>
<tr>
<td>Medium Risk (ApoB/ApoA-I ratio 0.6–0.8)</td>
<td>34.7%</td>
<td>−9.6%</td>
<td>40.7%</td>
</tr>
<tr>
<td>High risk (ApoB/ApoA-I ratio &gt; 0.8)</td>
<td>29.6%</td>
<td>−7.0%</td>
<td>24.3%</td>
</tr>
</tbody>
</table>


measures tend to lose predictive power [19,20]. All of this indicates ApoB and ApoB/ApoA-I ratio measurements to be good predictors for CHD risk, particularly in elderly high-cardiovascular risk populations such as that of the PREMED Study. In our study, mean decreases for serum ApoB and the ApoB/ApoA-I ratio after TMD + VOO were 4.4% and 6.2%, respectively, with a mean increase of 3.2% in serum ApoA-I. These changes represent from one third to one half of those observed after statin treatment [14].

In our study, consumption of TMD + VOO was associated with an increase in ApoA-I and a decrease in ApoB and ApoB/ApoA-I ratio. A differential effect of olive oil and its phenolic compounds on mRNA and protein expression of the apolipoproteins could explain, at least in part, the differences observed between TMD groups. Olive oil rich diets have been shown to decrease ApoB levels [16], and virgin olive oil supplementation of 4 g/day, but not that of 2 g/day, has shown to decrease the ApoB/ApoA-I ratio in hypercholesterolemic individuals [7]. Chylomicron remnant particles incubated with virgin olive oil decreased the expression of ApoB mRNA in primary rat hepatocytes [21]. In a study directed to assess the differential modulation by triacylglycerol-rich lipoproteins (TAG), enriched with different oleic-acid rich dietary oils, on hepatic gene expression in rat hepatocytes, oleic acid-rich oils, oils other than rich-polyphenol olive oil, increased the apob mRNA expression [22]. The in vivo expression of plasma ApoA-I, and its hepatic mRNA, was higher in rats fed with olive oil than with other vegetable oils [23]. Recent data show that cacao polyphenols increased ApoA1 protein and its mRNA levels, and decreased ApoB protein and its mRNA levels, in both HepG2 and Caco2 cells [24]. An inhibitory effect on ApoB secretion from hepatic cells by green tea, grapefruit, and wine polyphenols has been observed in experimental models [25,26]. Soy nuts and soy proteins, rich in isoflavones, have been shown to reduce the levels of ApoB and the ApoB/ApoA-I ratio in hypertensive, hyperlipidemic women and in type 2 diabetes individuals, respectively [27,28]. Besides an increase in HDL cholesterol, a concomitant increase in ApoA-I concentrations with decreases in ApoB and ApoB/ApoA-I ratio, have been observed after dietary supplementation with polyphenols in humans [29,30]. The role of dietary polyphenols on apolipoprotein metabolism deserves further investigation. Besides olive oil, the high content of pulses, vegetables, and fresh fruit in the TMD, rich in vegetable protein, soluble fiber, and polyphenols, as well as the decrease in total and saturated fat observed, can account for the benefits observed on the cardiovascular risk lipid profile in the TMD + VOO group.

Our trial has strengths, one of which is to have a randomized, controlled design. The design has the strength of reproducing real-life conditions, such as home-prepared foods. A limitation was that this set of initial participants assigned to the low-fat group did not receive a personalized behavioral intervention to follow the intended low-fat diet. Fat intake was only slightly reduced in this group. Thus, an important part of the differences in outcomes observed might be attributed to the supplemental foods (virgin olive oil and nuts). Another limitation of the study is that participants were older subjects at high risk for CHD, thus the results cannot be extrapolated to younger populations.

In conclusion, a TMD pattern particularly when enriched with virgin olive oil promoted benefits on Apo A-I, ApoB and ApoB/ApoA-I ratio. Our findings suggest this fact to be one of the protective mechanisms by which the Mediterranean diet and virgin olive oil could exert protective effects on CHD development. Data from this study provide further evidence to recommend a TMD rich in virgin olive oil as a useful tool for controlling CHD risk, particularly in individuals at high risk for developing CHD.
Acknowledgments

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Appendix A. Supplementary data


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