Effect of a Traditional Mediterranean Diet on Lipoprotein Oxidation

A Randomized Controlled Trial

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Background: Despite the richness in antioxidants of the Mediterranean diet, to our knowledge, no randomized controlled trials have assessed its effect on in vivo lipoprotein oxidation.

Methods: A total of 372 subjects at high cardiovascular risk (210 women and 162 men; age range, 55-80 years), who were recruited into a large, multicenter, randomized, controlled, parallel-group clinical trial (the Prevención con Dieta Mediterránea [PREDIMED] Study) directed at testing the efficacy of the traditional Mediterranean diet (TMD) on the primary prevention of coronary heart disease, were assigned to a low-fat diet (n=121) or one of 2 TMDs (TMD + virgin olive oil or TMD + nuts). The TMD participants received nutritional education and either free virgin olive oil for all the family (1 L/wk) or free nuts (30 g/d). Diets were ad libitum. Changes in oxidative stress markers were evaluated at 3 months.

Results: After the 3-month interventions, mean (95% confidence intervals) oxidized low-density lipoprotein (LDL) levels decreased in the TMD + virgin olive oil (−10.6 U/L [−14.2 to −6.1]) and TMD + nuts (−7.3 U/L [−11.2 to −3.3]) groups, without changes in the low-fat diet group (−2.9 U/L [−7.3 to 1.5]). Change in oxidized LDL levels in the TMD + virgin olive oil group reached significance vs that of the low-fat group (P=.02). Malondialdehyde changes in mononuclear cells paralleled those of oxidized LDL. No changes in serum glutathione peroxidase activity were observed.

Conclusions: Individuals at high cardiovascular risk who improved their diet toward a TMD pattern showed significant reductions in cellular lipid levels and LDL oxidation. Results provide further evidence to recommend the TMD as a useful tool against risk factors for CHD.

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A DHERENCE TO THE TRADItional Mediterranean diet (TMD) has been associated with a reduction in coronary heart disease (CHD), cancer, and overall mortality.[1-3] This protective effect has been attributed, at least in part, to the richness of this diet in antioxidants.[1,3] Current evidence indicates oxidative damage as a promoter of pathophysiological changes occurring in oxidative stress–associated diseases, such as CHD, cancer, and neurodegenerative disorders and also in aging.[4] Oxidized low-density lipoprotein (oxLDL) level may play a major role in atherosclerosis and cardiovascular disease and is a commonly used marker for oxidative damage.[5,6] An oxLDL predictive value for the onset of mobility disability has also been recently reported.[7] Adherence to a Mediterranean type diet has been shown to be associated with lower plasma oxLDL level in a cross-sectional study[8] and in a linear intervention study in healthy women.[9] However, to our knowledge, no randomized controlled intervention studies have assessed the efficacy of the Mediterranean diet on in vivo LDL oxidation.

Olive oil is the main fat component of the Mediterranean diet.[1] Among olive oils, virgin olive oil (VOO) has the highest antioxidant phenolic content compared with other olive oils such as ordi-

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Table 1. Characteristics at Baseline of the Total Randomized Population and the Analyzed Sample*

Characteristic	Total Randomized Population (n = 772)	Analyzed Sample Evaluated at 3 Months		
		All Participants (n = 372)	Men (n = 162)	Women (n = 210)
Age, y	68.9 ± 6.4	67.5 ± 5.6	66.9 ± 5.8	67.9 ± 5.7
Female sex, %	56.4	59.6		
Smoking status, %				
Never	59.2	62.7	24.8	89.9‡
Former	24.3	23.2	45.4	6.3‡
Current	16.5	14.1	29.8	3.8‡
Educational level, %				
Primary school	72.3	72.9	69.5	84.2‡
High school	16.3	14.1	20.6	9.7
University	11.4	13.0	9.9	6.1
Family history of CHD, %	23.7	24.1	24.6	23.7
BMI	29.8 ± 4.2	30.0 ± 4.3	29.5 ± 4.0	31.0 ± 4.4‡
EEPA leisure time, kcal/d†	168 (62-323)	178 (20-234)	200 (120-336)	142 (42-200)
Diabetes, %	54.6	51.1	52.8	50.0
Hypertension, %	78.3	81.0	79.3	85.5
Cholesterol, mg/dL				
Total	217.9 ± 39.2	215.6 ± 40.8	208.9 ± 41.4	220.8 ± 39.2‡
LDL	143.4 ± 34.5	142.3 ± 36.8	140.2 ± 39.5	144.4 ± 34.5
HDL	45.7 ± 10.0	45.4 ± 9.6	42.2 ± 8.9	47.6 ± 9.5‡
Triglycerides, mg/dL	141.3 ± 64.9	137.0 ± 58.4	134.9 ± 58.2	140.2 ± 58.2
Glucose, mg/dL	120.1 ± 38.9	120.3 ± 38.5	121.6 ± 38.5	119.2 ± 38.6

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CHD, coronary heart disease; EEPA, daily energy expenditure in leisure-time physical activity; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

SI conversion factors: To convert cholesterol, triglycerides, and glucose to millimoles per liter, multiply by 0.0259, 0.0113, and 0.0555, respectively.

*Data are presented as percentage of participants or mean ± SD value unless otherwise indicated.

†Median (interquartile range).

‡$P<.05$ vs men.

nary or pomace olive oil.[10] Nuts, which are also typical Mediterranean foods, are a rich source of nutrients and antioxidant phytochemicals.[11] We designed a large-scale feeding trial in a population at high risk for CHD to assess the effects on cardiovascular outcomes of 2 Mediterranean diets, one supplemented with VOO and the other with mixed nuts, compared with a low-fat diet (the Prevención con Dieta Mediterránea [PREDIMED] Study). We report herein the results of a 3-month intervention on markers of lipid oxidative damage and endogenous antioxidant status in 372 participants recruited into the trial.

METHODS

STUDY DESIGN

The PREDIMED study is a large, parallel-group, multicenter, randomized, controlled, 4-year clinical trial aimed at assessing the effects of the TMD on the primary prevention of cardiovascular disease (http://www.predimed.org).[12] The trial is currently taking place, with an estimated number of 9000 participants at high risk for CHD to be assigned to 3 intervention groups: (1) TMD + VOO; (2) TMD + nuts; and (3) a low-fat diet. We designed the present study to assess the 3-month effects of the dietary interventions on lipid oxidative damage in 372 participants entering the study during the first 6 months of recruitment. The institutional review boards approved the study protocol, and participants signed an informed consent. Before and after 3 months of intervention, biological samples were obtained after an over-

night fast, coded, shipped to central laboratories, and frozen at –80°C until the assay.

PARTICIPANTS

From October 2003 to March 2004, a total of 930 asymptomatic subjects at high risk for CHD, aged 55 to 80 years, were selected in 10 Spanish Primary Care Centers. They fulfilled at least 1 of the 2 following criteria: (1) type 2 diabetes mellitus or (2) 3 or more CHD risk factors (smoking, hypertension, dislipemia, obesity, or family history of CHD). Exclusion criteria included 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 behavior[13] concerning the participant's dietary habits; 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 a screening visit by the physician. Eligible subjects were then randomized to 1 of the 3 diet groups by means of a computer-generated random number sequence.

BASELINE ASSESSMENTS

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[14] (values of 0 or 1 were assigned to each of 14 items); (2) a 137-item food frequency questionnaire[15]; (3) the Minnesota Leisure Time Physical Activity questionnaire[16]; and (4) a 47-item general questionnaire assessing life-style, health conditions, sociodemographic variables, history of illness, and medication use.

INTERVENTION

On the basis of the baseline TMD 14-item questionnaire, each participant was given personalized dietary advice by the dietician during a 30-minute session. Participants allocated to a low-fat diet were advised to reduce all types of fat and were given written recommendations according to American Hospital Association guidelines.[17] The TMD participants received instructions directed to upscale the TMD 14-item score, including (1) the use of olive oil for cooking and dressing; (2) increased consumption of vegetables, nuts, and fish products; (3) consumption of white meat instead of red or processed meat; (4) preparation of home-made sauce by simmering tomato, garlic, onion, and aromatic herbs with olive oil to dress vegetables, pasta, rice, and other dishes; and (5), for alcohol drinkers, to follow a moderate pattern of red wine consumption. No energy restrictions were suggested for the TMD groups. Participants in the TMD groups were given free VOO (15 L for 3 months) or sachets of walnuts, hazelnuts, and almonds (1350 g of walnuts [15 g/d], 675 g of hazelnuts [7.5 g/d], and 675 g of almonds [7.5 g/d], for 3 months). To improve compliance and account for family needs, participants in the corresponding TMD groups were given excess VOO or additional packs of nuts. One week after a participant's inclusion, the dietician delivered a 1-hour group session for each TMD group, with up to 20 participants per session. Each session consisted of an informative talk and written material with elaborated descriptions of typical Mediterranean foods, seasonal shopping lists, meal plans, and recipes. All participants had free and continuous access to their dietitian throughout the study

EVALUATION OF THE INTERVENTION

After the 3-month interventions, all baseline procedures were repeated. Biological assessment of the intervention compliance was performed in two thirds (n=98) of the participants selected at random and matched by age and sex. Tyrosol and hydroxytyrosol levels, the major phenolic compounds present in olive oil,[10] were measured in urine by gas chromatography–mass spectrometry 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 of the TDM + nuts group.

OUTCOME MEASURES

Anthropometric data were obtained by standardized methods.[12] Serum glucose, cholesterol, and triglyceride levels were measured using standard enzymatic automated methods (Trinder; Bayer Diagnostics, Tarrytown, NY). High-density lipoprotein (HDL) cholesterol was quantified after precipitation with phosphotungstic acid and magnesium chloride, and LDL cholesterol was calculated by the Friedewald formula. Circulating oxLDL level in plasma was measured by enzyme-linked immunosorbent assay using the mAb-4E6 antibody (Mercodia AB, Uppsala, Sweden). Serum glutathione peroxidase activity (GSH-Px) was measured by spectrophotometry (Ransel RS 505, Randox Laboratories, Crumlin, Northern Ireland). Malondialdehyde in mononuclear cells isolated from fresh blood was measured by high-performance liquid chromatography with electrochemical detection in a randomly selected subsample (n=71). Protein concentration in mononuclear cells was measured by the Lowry method.

SAMPLE SIZE AND POWER ANALYSIS

The total sample of 372 participants (>100 in each group) allowed at least 80% power to detect a statistically significant dif-
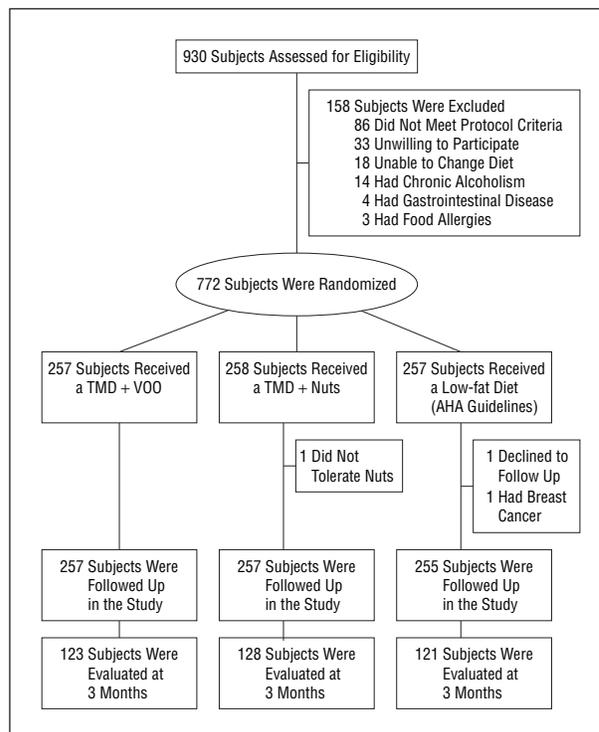

Figure 1. Flow of patients through the trial. AHA indicates American Hospital Association; TMD, traditional Mediterranean diet; VOO, virgin olive oil.

ference among diet groups of 10 oxLDL units, assuming a dropout rate of 15% and a type I error of .05 (2-sided).

STATISTICAL ANALYSES

Normality of continuous variables was assessed by normal probability plots. The unpaired t test was used to assess differences between samples and sex at baseline. One-factor analysis of variance or Kruskal-Wallis test was used to determine differences in basal characteristics among the 3 interventions as appropriate. The χ^2 test or univariate analyses were used to assess the relationship among oxLDL and GSH-Px tertiles at baseline and several possible confounder variables. Comparisons among 3-month changes were carried out by a covariance model, with the baseline value as the covariate. Age, sex, center, and weight and physical activity at baseline were considered as additional covariates. When assessing oxidative stress markers, tobacco consumption was included in the model. An additional model was fitted for oxidative stress markers. In this model, diabetes and LDL/HDL cholesterol ratio (variables in which baseline differences of $P<.10$ among intervention groups and among oxLDL and GSH-Px tertiles were observed) were added as covariates. Statistical significance was defined as $P<.05$.

RESULTS

STUDY POPULATION

Of the 930 eligible participants, 772 met the eligibility criteria and were randomized. The effect of the 3 interventions on oxLDL and the antioxidant enzyme GSH-Px was assessed in a subpopulation of 372 individuals selected at random and with similar characteristics to those of the whole group (**Table 1**). **Figure 1** shows the flow of participants through the study. Differences between

Table 2. Baseline Characteristics of the Study Participants by Groups of Intervention*

Characteristic	TMD + VOO (n = 123)	TMD + Nuts (n = 128)	Low-Fat Diet (n = 121)	P Value
Age, y	67.6 ± 5.7	67.4 ± 5.6	67.6 ± 6.0	.94
Female sex, %	65.3	51.6	62.4	.08
Smoking status, %				
Never	65.3	62.5	62.4	.32
Former	23.1	20.3	23.7	.30
Current	11.6	17.2	13.9	.34
Family history of CHD, %	23.2	24.6	27.0	.18
Diabetes, %	57.9	43.8	52.5	.10
Hypertension, %	84.3	81.3	83.7	.73
Systolic BP, mm Hg	153 ± 19	153 ± 17	149 ± 15	.33
Diastolic BP, mm Hg	83 ± 10	83 ± 10	82 ± 8	.43
BMI	30.3 ± 4.5	30.0 ± 4.2	30.9 ± 4.0	.27
Waist circumference, cm	100.3 ± 12.9	101.2 ± 10.9	102.4 ± 10.1	.48
EEPA leisure time, kcal/d†	144 (43-260)‡§	200 (93-210)	185 (84-214)	.007
Medications, %				
ACE inhibitors	43.9	45.1	43.4	.54
Diuretics	35.7	39.1	36.2	.59
Lipid-lowering agents	38.9	52.4	52.0	.05
Insulin	5.1	8.4	4.9	.65
Oral hypoglycemic agents	34.7	26.2	39.8	.08
Aspirin or other antiplatelet agents	7.9	18.6	22.1	.008
Disposition to dietary change, %	99.2	98.4	100	.45
Educational level, %				
Primary school	79.9	76.4	81.1	.12
High school	13.4	17.3	12.9	.93
University	6.7	6.3	6.0	.51
Cholesterol, mg/dL				
Total	216.3 ± 40.7	212.6 ± 38.1	218.4 ± 44.1	.57
LDL	145.3 ± 36.3	138.7 ± 34.5	143.2 ± 40.2	.39
HDL	43.7 ± 9.6	46.0 ± 9.3	46.7 ± 10.0	.06
Total-HDL cholesterol ratio	5.2 ± 1.3§	4.7 ± 1.0	4.8 ± 1.0	.02
LDL-HDL cholesterol ratio	3.4 ± 0.9§	3.1 ± 0.9	3.2 ± 1.1	.02
Triglycerides, mg/dL	134.9 ± 57.8	134.5 ± 54.8	142.5 ± 63.4	.54
Glucose, mg/dL	116.3 ± 31.9	117.3 ± 37.2	127.1 ± 44.6	.12
OxLDL, U/L	77.9 ± 28.8	74.4 ± 24.7	73.4 ± 26.0	.39
GSH-Px, U/L	626 ± 144	613 ± 140	613 ± 119	.74

Abbreviations: ACE, angiotensin-converting enzyme; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); BP, blood pressure; CHD, coronary heart disease; EEPA, daily energy expenditure in leisure-time physical activity; GSH-Px, glutathione peroxidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OxLDL, oxidized low-density lipoprotein; TDM, traditional Mediterranean diet; VOO, virgin olive oil.

SI conversion factors: To convert cholesterol, triglycerides, and glucose to millimoles per liter, multiply by 0.0259, 0.0113, and 0.0555, respectively.

*Data are presented as the percentage of participants or mean ± SD unless otherwise specified.

†Median (interquartile range).

‡$P<.05$ vs low-fat diet.

§$P<.05$ vs TDM + nuts.

the sexes are given in Table 1 and among intervention groups in **Table 2**. The lipid cardiovascular risk profile worsened from the low to the high oxLDL tertile ($P<.01$). Glucose, daily energy expenditure in leisure-time physical activity, LDL-HDL cholesterol ratio, and percentage of diabetic participants increased across GSH-Px tertiles ($P<.05$). Systolic blood pressure was lower in participants in the upper GSH-Px tertile ($P=.003$) and tended to be higher in the upper oxLDL tertile ($P=.06$) but not significantly so.

ENERGY BALANCE AND DIETARY ADHERENCE

Adherence to supplemental foods was good. α-Linolenic acid levels increased from baseline in the TDM + nuts group ($P=.04$) and tyrosol and hydroxytyrosol levels increased in the TMD + VOO group ($P<.001$) (**Figure 2**). Physical activity did not change during the intervention periods in any group. A reduction in energy intake was observed in the TMD + VOO and low-fat diet groups (**Table 3**). Participants in the TMD + nuts group increased their intake of total, monounsaturated, and polyunsaturated fat and reduced their saturated fat and carbohydrate intake. In the TMD + VOO group, a decrease in total and saturated fat was observed. Monounsaturated fat intake did not change in this group after the intervention. This fact, together with the lack of change in total olive oil consumption (Table 3) and the increase of tyrosol and hydroxytyrosol levels in urine (Figure 2B and C), indicated that participants in the TMD + VOO group replaced the ordinary olive oil they used to consume by

the VOO provided to them in the frame of the PREDIMED study. Consumption of legumes increased in both TMD groups. Participants in the TMD + nuts group increased their intake of vegetables, fruits, and fish. Consumption of dairy products diminished in all groups and that of meat in both TMD 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.

OXIDATIVE STATUS

Oxidized LDL decreased significantly in both TMD groups (**Table 4**). In the unadjusted model, the decrease in oxLDL level reached significance ($P=.04$) in the TMD + VOO group vs that of the low-fat diet group and was not significant in the TMD + nuts group ($P=.09$). Models adjusted by possible confounder variables confirmed the aforementioned results. No changes were observed in GSH-Px values after the interventions (Table 4). In a subsample of 71 individuals, malondialdehyde levels in mononuclear cells decreased in both TMD groups. The mean (95% confidence interval) decreases were –0.20 (–0.31 to –0.77) nmol/mg of protein and –0.24 (–0.30 to –0.17) nmol/mg of protein for TMD + VOO and TMD + nuts groups, respectively ($P<.01$). No changes were observed in the low-fat diet group (0.02 [–0.09 to 0.13] nmol/mg of protein). Changes in malondialdehyde level in TMD groups vs that of the low-fat diet group reached significance ($P=.004$). No sex differences were observed.

CLASSIC CARDIOVASCULAR RISK FACTORS

Body mass index and waist circumference did not change in the 3 interventions. Systolic ($P=.008$) and diastolic ($P=.03$) blood pressures decreased in both TMD groups. In the TMD + nuts group, a reduction in triglyceride level ($P=.04$) and an increase in HDL cholesterol level ($P=.03$) were observed. Total cholesterol level and total-HDL and LDL/HDL cholesterol ratios decreased in the TMD groups more than that in the low-fat diet group ($P<.05$).

COMMENT

The present study is, to our knowledge, the first randomized controlled clinical trial focused on the effect of a Mediterranean type diet on in vivo LDL oxidation. Individuals at high risk of CHD who improved their diet toward a TMD pattern had significant reductions in their in vivo LDL oxidation compared with individuals assigned to a low-fat diet.

Oxidation of the lipids and apoproteins present in LDL leads to a change in the lipoprotein conformation by which LDL is better able to enter the monocyte-macrophage system of the arterial wall and promote the atherosclerotic process.[18] However, although the role of lipid oxidation in atherosclerotic cardiovascular disease has long been recognized,[19] the clinical relevance of lipoprotein oxidation is under debate. The role of the oxidant-
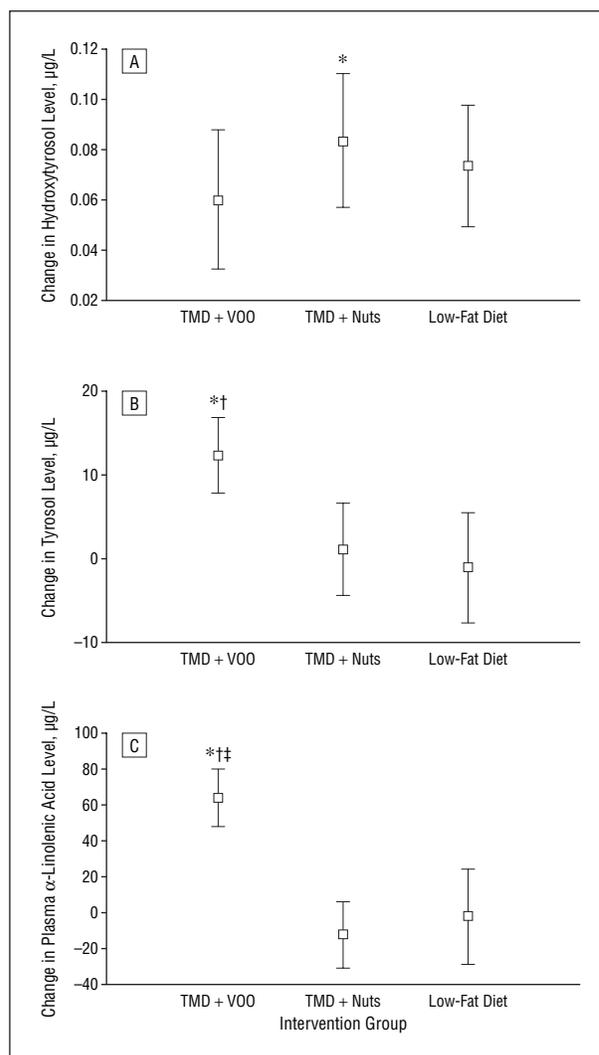

Figure 2. Mean±SD changes in hydroxytyrosol (A), urinary tyrosol (B), and plasma α-linolenic acid (C) after 3-month interventions. *$P<.05$ vs the corresponding baseline. †$P<.05$ vs low-fat diet group. ‡$P<.05$ vs TMD + nuts group. TMD indicates traditional Mediterranean diet; VOO, virgin olive oil.

antioxidant imbalance in atherogenesis has been questioned because, in spite of the consistent results from cohort studies showing an inverse association between customary intakes of dietary antioxidants and CHD development, large intervention trials with antioxidant vitamin supplements have shown no benefit.[20] Recent results from the INTERHEART study, a large intercountry case-control study, support the protective role of dietary antioxidants on CHD risk.[21] A likely explanation for this paradox is that lifetime consumption of the complex mixture of antioxidants in foods is more effective than large doses of a single antioxidant given for a finite period. The latter might deplete the endogenous antioxidant pool, thus turning an antioxidant effect into prooxidant in vivo.[22]

Another subject under debate is the pathophysiological and clinical relevance of the different types of oxLDL biomarkers, their added value in front of classic lipid risk factors, and their response to therapies.[23] There is, however, accumulating evidence showing that circulating

Table 3. Energy, Nutrient, and Food Intake at Baseline and After 3 Months of Intervention*

Variable	TMD + VOO, Mean ± SD	P Value	TDM + Nuts, Mean ± SD	P Value	Low-Fat Diet, Mean ± SD	P Value	P Value†
Energy, kcal/d							
Baseline	2280 ± 585	.02	2491 ± 823	.49	2419 ± 798	<.001	.03‡
Final	2440 ± 705		2437 ± 555		2081 ± 618		
CHO, % energy							
Baseline	44.9 ± 7.3	.78	42.9 ± 6.9	<.001	44.3 ± 7.7	.56	<.001§‖
Final	45.1 ± 7.3		39.4 ± 7.5		44.9 ± 6.4		
Protein, % energy							
Baseline	17.1 ± 3.1	.07	18.2 ± 3.2	.05	17.0 ± 2.9	.06	.12
Final	17.6 ± 2.9		17.7 ± 2.6		17.7 ± 2.8		
Total fat, % energy							
Baseline	36.1 ± 6.4	.05	36.5 ± 7.0	<.001	36.8 ± 7.2	.09	<.001§‖
Final	34.7 ± 6.2		40.9 ± 7.3		35.1 ± 6.0		
SFA, % energy							
Baseline	9.9 ± 2.4	<.001	9.8 ± 2.3	<.001	10.0 ± 2.4	.001	.45
Final	8.6 ± 2.3		8.5 ± 1.9		8.9 ± 2.3		
MUFA, % energy							
Baseline	17.4 ± 4.2	.57	17.7 ± 4.4	<.001	17.1 ± 4.2	.83	<.001§‖
Final	17.1 ± 3.7		19.4 ± 4.0		17.0 ± 4.0		
PUFA, % energy							
Baseline	5.7 ± 2.1	.76	5.6 ± 1.8	<.001	6.4 ± 3.1	.31	<.001§‖
Final	5.8 ± 2.2		9.7 ± 3.9		6.1 ± 2.7		
Olive oil, g/d							
Baseline	33.7 ± 20.6	.44	36.4 ± 20.2	.67	33.6 ± 16.2	.49	.04‡
Final	35.4 ± 14.1		35.6 ± 15.8		30.0 ± 16.1		
Legumes, g/d							
Baseline	21.2 ± 14.3	.01	23.2 ± 20.1	<.001	22.1 ± 12.9	.48	.02‡§
Final	32.0 ± 41.4		35.0 ± 32.9		22.8 ± 19.0		
Vegetables, g/d							
Baseline	475 ± 228	.48	537 ± 292	.06	445 ± 190	.08	.02§‖
Final	481 ± 262		586 ± 251		478 ± 160		
Fruits, g/d							
Baseline	327 ± 165	.32	303 ± 163	.03	337 ± 185	.34	.53
Final	344 ± 156		359 ± 186		359 ± 168		
Dairy product, g/d							
Baseline	416 ± 212	.03	448 ± 260	.007	436 ± 292	.04	.36
Final	379 ± 188		389 ± 249		383 ± 237		
Meat, g/d							
Baseline	144 ± 61	.03	168 ± 54	<.001	153 ± 62	.08	.03§
Final	129 ± 52		128 ± 54		143 ± 64		
Fish, g/d							
Baseline	94 ± 52	.14	123 ± 69	.63	87 ± 44	.79	.007§
Final	100 ± 46		120 ± 55		85 ± 45		
14-Point Mediterranean score							
Baseline	7.9 ± 1.6	<.001	7.9 ± 1.8	<.001	7.8 ± 1.7	.28	<.001‡§
Final	10.5 ± 1.6		10.9 ± 1.6		7.6 ± 1.7		

Abbreviations: CHO, carbohydrate; MUFA, monounsaturated fatty acids; NS, nonsignificant (*P* ≥ .05); PUFA, polyunsaturated fatty acid; SFA, saturated fatty acids; VOO, virgin olive oil.
*Intakes were based on the analysis of the 137-item questionnaire.[15]
†*P* value for differences among diets.
‡Between TDM + VOO and low-fat diet.
§Between TMD + nuts and low-fat diet.
‖Between TMD + VOO and TMD + nuts.

oxLDL levels were predictors for acute CHD, both in patients with CHD and in the general population,[5,6] and were a prognostic marker for subclinical atherosclerosis.[24] When lipoprotein oxidation was measured with antibodies directed against oxidized phospholipids (OxPLs), which predominantly bind to lipoprotein(a), plasma OxPL level decreased, but the OxPL–apolipoprotein B ratio increased after a lipid-lowering diet and atherosclerosis regression.[19] This phenomenon was also observed after statin treatments.[19] These facts support the hypothesis that lipoprotein(a) binding of OxPL is an innate immune mechanism to clear proinflammatory OxPL from atherosclerotic sites.[19] Antibodies directed against malondialdehyde-lysine epitopes on LDL, such as those used in the present study, do not bind lipoprotein(a). With the use of this method, it has been found that oxLDL level decreased after a lipid-lowering diet, such as the Mediterranean diet.[8,9] Oxidized LDL level, as measured by these

Table 4. Crude and Adjusted 3-Month Changes in OxLDL and GSH-Px Levels After Interventions

Model*	TMD + VOO, Mean (95% CI)	TMD + Nuts, Mean (95% CI)	Low-Fat Diet, Mean (95% CI)	P Value†
Model 1				
OxLDL, U/L	−10.1 (−15 to −5.1)	−7.5 (−12 to −2.6)	−2.6 (−8.0 to 2.9)	.04‡
GSH-Px, U/L	−16.4 (−44.6 to 11.8)	−10.4 (−35.9 to 15.1)	−20.1 (−50.6 to 10.4)	.35
Model 2				
OxLDL, U/L	−9.4 (−13.5 to −5.3)	−8.0 (−12 to −4.0)	−3.2 (−7.6 to 1.3)	.04‡
GSH-Px, U/L	−16.0 (−38.6 to 6.6)	−10.4 (−30.6 to 9.9)	−23.8 (−48 to 0.52)	.44
Model 3				
OxLDL, U/L	−10.6 (−14.2 to −6.1)	−7.3 (−11.2 to −3.3)	−2.9 (−7.3 to 1.5)	.02‡
GSH-Px, U/L	−11.6 (−34.3 to 11.2)	−11.3 (−33.5 to 6.87)	−23.3 (−47.4 to 0.83)	.46

Abbreviations: CI, confidence interval; GSH-Px, glutathione peroxidase; NS, nonsignificant ($P \geq .05$); OxLDL, oxidized low-density lipoprotein; TMD, traditional Mediterranean diet; VOO, virgin olive oil.

*Model 1, unadjusted; model 2, adjusted for sex, age, center, basal weight and physical activity, tobacco consumption, and baseline values; and model 3, adjusted for variables of model 2 plus diabetes and low-density lipoprotein–high-density lipoprotein cholesterol ratio.

†P value for between-group differences.

‡Significant differences between TDM + VOO and low-fat diet.

antibodies, correlates directly with LDL cholesterol level and inversely with HDL cholesterol level.[25] Given that our results were adjusted by these lipoproteins, we can assume an independent effect of the TMD intervention on circulating oxLDL level. In our study, the mean decrease in oxLDL level was −10.6 U/L and −7.3 U/L after TMD + VOO and TMD + nuts, respectively. In a recent study,[6] the mean difference in circulating oxLDL values between patients with CHD and healthy controls, measured by the same antibody and method used in the present study, was 17 U/L. However, the current state-of-the-art knowledge does not allow an estimation of the attributable CHD risk associated with a 1-U/L change in oxLDL level, and further rigorous prospective studies are needed. Longer follow-up of the whole PREDIMED trial will eventually provide the information.

Recent results from the EUROLIVE study showed that olive oil rich in polyphenols, such as VOO, reduces the oxidative lipid damage more than other types of olive oils.[26] Besides olive oil, the high content of vegetables, fresh fruits, and nuts in the TMD, together with a moderate consumption of wine, guarantees a high intake of antioxidant vitamins and polyphenols. In agreement with previous results,[3,27] following the TMD improved the classic cardiovascular risk lipid profile and blood pressure levels. A synergistic relationship exists between oxidative damage and inflammation, and both of these are related to endothelial dysfunction. The decrease in oxLDL level promoted by the TMD concurs with the reductions in inflammatory markers previously reported in the PREDIMED study[12] as well as in diabetic patients.[27] Also, a major cause for endothelial dysfunction in essential hypertension is a decreased availability of nitric oxide. Oxidative stress, through superoxide anion production, decreases nitric oxide availability, and an inhibition of the nitric oxide synthase expression by oxLDL has been reported.[28] Thus, the reduction in the degree of LDL oxidation may contribute, at least in part, to the decrease in blood pressure observed in this study.

Our trial has several strengths, like its design, which is able to provide first-level scientific evidence[29] and work in real-life conditions, such as with home-prepared foods. Our study also has limitations. The first was to ensure participants' compliance by means of dietary instructions. Adherence to the supplemental foods, however, was good, as observed in the changes of the compliance biomarkers. Another limitation was that participants assigned to the low-fat diet 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 (VOO and nuts). Also, a 3-month period provides no information about the sustainability or long-term effects of the diets on cardiovascular risk factors. However, 2 weeks is the common time frame established for fat-rich diets to reach equilibrium in the plasma lipid profile; longer intervention periods do not modify the lipid concentration.[30]

In summary, a TMD pattern promoted benefits on classic and novel risk factors for CHD. Our findings suggest a decrease in the oxidative damage to LDL to be one of the protective mechanisms by which the Mediterranean diet could exert protective effects on CHD development. Data from this study provide further evidence to recommend the TMD as a useful tool against atherosclerosis development, particularly in individuals at high risk for developing CHD.

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Correction

Error in Figure. In the Original Investigation by Fitó et al titled "Effect of a Traditional Mediterranean Diet on Lipoprotein Oxidation: A Randomized Controlled Trial" published in the June 11, 2007, issue of the ARCHIVES (2007;167[11]:1195-1203), an error occurred in **Figure 2** wherein the y-axis labels in parts A and C were mistakenly transposed. A corrected figure and legend appears below.
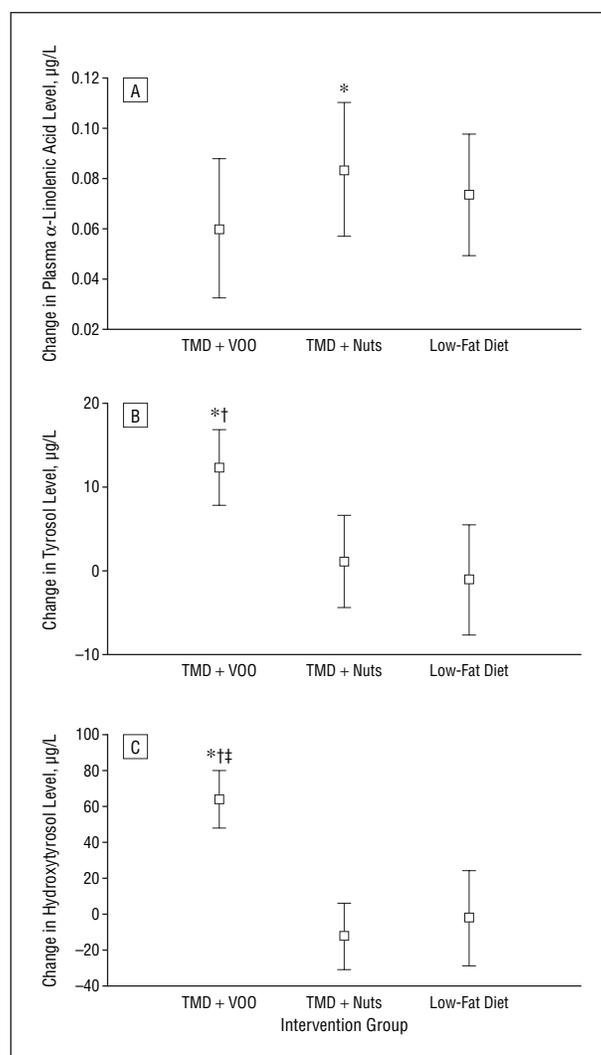

Figure 2. Mean±SD changes in plasma α-linolenic acid (A), urinary tyrosol (B), and hydroxytyrosol (C) after 3-month interventions. $*P < .05$ vs the corresponding baseline. $†P < .05$ vs low-fat diet group. $‡P < .05$ vs TMD + nuts group. TMD indicates traditional Mediterranean diet; VOO, virgin olive oil.