Scientific Evidence of Interventions Using the Mediterranean Diet: A Systematic Review

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The Mediterranean Diet has been associated with greater longevity and quality of life in epidemiological studies, the majority being observational. The application of evidence-based medicine to the area of public health nutrition involves the necessity of developing clinical trials and systematic reviews to develop sound recommendations. The purpose of this study was to analyze and review the experimental studies on Mediterranean diet and disease prevention. A systematic review was made and a total of 43 articles corresponding to 35 different experimental studies were selected. Results were analyzed for the effects of the Mediterranean diet on lipoproteins, endothelial resistance, diabetes and antioxidative capacity, cardiovascular diseases, arthritis, cancer, body composition, and psychological function. The Mediterranean diet showed favorable effects on lipoprotein levels, endothelium vasodilatation, insulin resistance, metabolic syndrome, antioxidant capacity, myocardial and cardiovascular mortality, and cancer incidence in obese patients and in those with previous myocardial infarction. Results disclose the mechanisms of the Mediterranean diet in disease prevention, particularly in cardiovascular disease secondary prevention, but also emphasize the need to undertake experimental research and systematic reviews in the areas of primary prevention of cardiovascular disease, hypertension, diabetes, obesity, infectious diseases, age-related cognitive impairment, and cancer, among others. Interventions should use food scores or patterns to ascertain adherence to the Mediterranean diet. Further experimental research is needed to corroborate the benefits of the Mediterranean diet and the underlying mechanisms, and in this sense the methodology of the ongoing PREDIMED study is explained.

Key words: Mediterranean diet, prevention, evidence-based nutrition, dietary interventions, clinical trails

doi: 10.1301/nr.2006.feb.S27–S47

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INTRODUCTION

Epidemiological studies[1-3] have observed great geographical differences in the incidence rates of cardiovascular disease. Compared with northern European countries or the United States, there is a low incidence of coronary heart disease (CHD) in countries of southern Europe, such as France, Spain, Greece, and Italy. The Mediterranean food pattern has been the factor most frequently invoked to explain this difference. The term "Mediterranean diet" reflects the dietary patterns characteristics of several countries in the Mediterranean Basin during the early 1960s. The association between greater longevity and reduced mortality and morbidity for CHD has also been observed for certain cancers and other nutrition-related diseases. The common dietary food patterns in these countries have substantiated this concept,[4,5] although the data come mostly from observational studies.

Such patterns were defined in 1993 at the International Conference on the Diets of the Mediterranean, having also been previously defined in other meetings.[4-7] They are comprised of:

- Abundant plant foods (fruits, vegetables, breads, other forms of cereals, beans, nuts, and seeds);
- Minimally processed, seasonally fresh, and locally grown foods;
- Fresh fruits as the typical daily dessert with sweets

based on nuts, olive oil, and concentrated sugars or honey consumed during feast days;
- Olive oil as the principal source of dietary lipids;
- Dairy products (mainly cheese and yogurt) consumed in low to moderate amounts;
- Fewer than four eggs consumed per week;
- Red meat consumed in low frequency and amounts; and
- Wine consumed in low to moderate amounts, generally with meals.

This characteristic definition of the Mediterranean diet and its typical composition is not without ambiguities, which require certain consideration.[8-10]

Evidence-based nutrition is the application of the principles of evidence-based medicine to the area of food and nutrition, in both clinical practice and in the public health.

Usually, in the field of public health nutrition/dietary guidelines/policy development, the application of evidence-based nutrition has several weaknesses, since there are some limitations when analyzing the effect that diet modification has on health:

- The modification of a diet not only requires much collaboration from the patient but also of the environment, with convenient access to products and willingness to buy and cook the food according to the dietary plan. Moreover, measuring dietary adherence entails greater effort from both the participant and the investigator.
- The complexity of dietary modifications makes it difficult to develop a double-blind intervention to analyze its effects on health.
- The enormous diversity of food habits, basal metabolic status, and nutritional objectives and dietary guidelines worldwide are limitations for making comparisons between studies developed in different contexts.

There is very small number of systematic reviews analyzing the effect of the Mediterranean diet on health-related issues, and also the number of randomized, controlled clinical trials is scarce (less than 50). In contrast, the worldwide popularity of the Mediterranean diet as a healthy and recommended diet is evident in the proliferation of media attention (more than 740,000 citations in Google® as of January 2005).

Most of the scientific articles published are observational epidemiological studies (primarily ecological or case control studies and a few cohorts). Almost all the reviews published are non-systematic and reflect an opinion or a collection of self-selected articles rather than an objective analysis of sound evidence.

The objective of this study is to analyze the literature published on the Mediterranean diet and to review all experimental studies analyzing the Mediterranean diet in disease prevention.

METHODS

We searched MEDLINE (National Library of Medicine, Bethesda, MD) for relevant articles about the Mediterranean diet and prevention of certain pathologies published from October 2004 to January 2005. We used the keywords "Mediterranean diet," "health," "cancer," "cardiovascular disease," "bone disease," "prevention," and combinations such as "Mediterranean diet and health," "Mediterranean diet and cancer prevention," "Mediterranean diet and cardiovascular disease," and "Mediterranean diet and bone health." We narrowed the search to clinical trials published in English and limited to those conducted in humans. We focused the search on articles referring to the Mediterranean diet as a whole and excluded studies regarding specific foods of this diet. We also excluded those articles evaluating the effects of an isolated intake of a Mediterranean menu instead of the prolonged effect of such a diet. Additional publications were identified from references provided in original papers.

We found 46 articles that met the inclusion criteria. Regarding the sample size, 22 of the studies had less than 50 subjects, 9 studies included 50 to 100 subjects, 9 studies had a sample of 101 to 500 subjects, 4 studies included a sample having between 500 and 1000 individuals, and 1 study included more than 1000 subjects.

RESULTS

A total of 489 articles studies were selected with the term "Mediterranean diet" and analyzed. The year distribution is shown in Figure 1. After excluding animal research, 416 studies remained, with only 324 having abstracts and, of these, 128 were reviews.

Among the original research articles, 55 were clinical trials and 41 of them were randomized clinical trails.

From the total of 55 clinical trial citations obtained, 43 were selected (12 excluded due to: language, intervention limited to one food and methodological weaknesses, among others), corresponding to 35 different studies. Studies were conducted in Italy, Spain, France, Great Britain, Chile, Sweden, Canada, Australia, United States, Denmark, Finland, and India, and the number of subjects ranged from 11 to 13,000.

Studies were classified into six groups according to their objectives and outcome measures: lipoproteins/ endothelial resistance/ diabetes, cardiovascular disease, arthritis, cancer, body composition, and psychological function.

A first group consisted of different intermediate

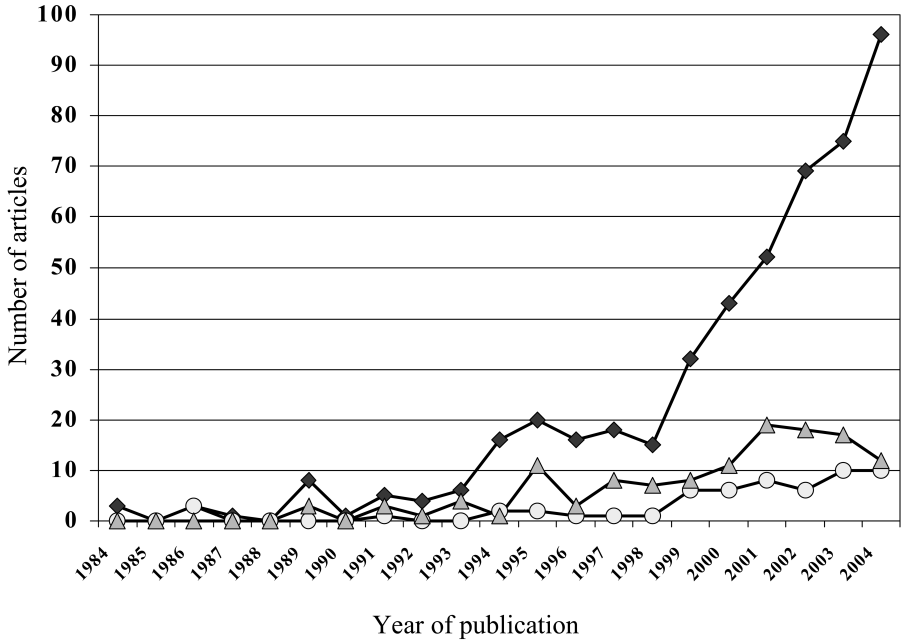

Figure 1. Number and type of articles published about the Mediterranean diet. ◆, Publications; △, reviews; ○, clinical trials.

outcomes such as lipoproteins, glycemic control, endothelial resistance, inflammation markers, and antioxidant capacity. It included 30 articles published from 1982 to 2004, with more than half (18) published from 2001, and only 5 before 1995.[11-40]

A second group measured cardiovascular disease incidence or morbidity with five articles.[41-45] A third group included two articles on arthritis.[46,47] A fourth group focused on cancer with only one article.[48] Three articles on body weight and obesity comprised the fifth group,[49-51] and the last group included two articles on psychological function.[52,53]

All results are summarized in Table 1. Most of the clinical trails in the first group analyzing the effect of Mediterranean diet on lipids found reductions in total cholesterol, low-density lipoprotein (LDL) cholesterol (decrease in small LDL particles number in some), tryglicerides, apoprotein B, and very-low-density lipoprotein (VLDL) cholesterol, and an increase in high-density lipoprotein (HDL) cholesterol. An increase of the total plasma antioxidant capacity was also observed in two studies, but not in another. Endothelium function improved with the Mediterranean diet, and endothelial-dependent vasodilatation was increased by adding nuts to the Mediterranean diet. Insulin resistance and metabolic syndrome were reduced after changing to a Mediterranean diet, but some studies showed no effects on insulin or glucose levels. All of the articles addressing cardiovascular disease and secondary prevention showed an odds ratio for fatal myocardial infarction between 0.25 and 0.7. The single study on arthritis functionality and pain demonstrated benefits, and the sole study on cancer showed a risk reduction of 60% in the Mediterranean diet group. The studies on body weight also showed favorable results with the Mediterranean diet, particularly the study by McManus et al.[51] which in addition to higher weight losses, showed greater compliances to diet therapies. Finally, the Mediterranean diet did not show any alterations in mood in the last group.

DISCUSSION

The aim of this article was not to cast doubts on the level of evidence for Mediterranean diet interventions but to emphasize the weaknesses of research on the Mediterranean diet and to stress the need for further research and systematic reviews. One of the most immediate conclusions obtained from this review is that the scientific evidence for the Mediterranean diet is mostly sustained by observational studies and personal reviews.

For some of the years during the period analyzed, the number of original articles related to the Mediterranean diet was similar to the number of reviews. Additionally, it is remarkable that most of the reviews are non-systematic and at times are very subjective and biased.

An example can be found in an interesting review article of the Mediterranean diet in Greece by Simopoulos.[54] The author cited 114 references, but none included Trichopoulou (author of 53 of the 284 references in the search "diet and Greece") or Kafatos (author of 28 of the 284 references). Another very similar article from the previously mentioned author[55] reviewed the relationship between the Mediterranean diet and cancer in Greece,

Table 1. Clinical Trials on the Mediterranean Diet

Author/Year Publication	Country	Type of Study		Population	Methodology	Outcome	Follow Up	Results
DIABETES/LIPOPROTEINS/ENDOTHELIAL RESISTANCE								
Vincent et al., 2004[11]	Marseille, France	RCT	Primary prevention	212 subjects, M/F with at least 1 CV risk factor	MD or a traditional low-fat/cholesterol diet	BMI, fasting lipids and lipoproteins, apolipoproteins, glucose, insulin and homocysteine	3 months (still on going)	BMI: −5.2% (vs. −4.2%); TC: −7.4% (vs. −4.4%); LDLC: −9.9% (vs. −5.4%); plasma TG: −13.0% (vs. −7.9%); plasma glucose: −3.0% (vs. −3.5%); plasma insulin: −21.3% (vs. −17.5%) ($p < 0.05$ for all)
Esposito et al., 2004[12]	Naples, Italy	RCT, single-blind	Primary prevention	180 subjects with metabolic syndrome (99 M, 81 F)	Control group following a prudent diet and intervention group following a MD	Nutrient intake, Endothelial function score (BP and platelet aggregation response to L-arginine), lipid and glucose parameters, insulin sensitivity and circulating levels of high sensitivity C-reactive protein and interleukins 6, 7 and 18	24 months	↓ serum concentrations of high sensitivity-C-reactive protein ($p = 0.01$), interleukin 6 ($p = 0.04$), interleukin 7 ($p = 0.4$) and interleukin 18 ($p = 0.3$), ↓ insulin resistance ($p < 0.001$). Improved endothelial function score (mean + SD) change, +1.9 (0.6) $p < 0.001$). At 2 years follow up 40 subjects in intervention group still had features of the metabolic syndrome vs. 78 of the control group
Ros et al., 2004[13]	Barcelona, Spain	R-crossover-CT	Primary prevention	21 hyper-cholesterolemic subjects (8 M, 12 F)	4 weeks of a cholesterol lowering MD/4 weeks of a diet similar of energy and fat content where walnuts replaced aprox 32% energy from MUFA	Brachial artery vasomotor function, vascular cell adhesion molecul-1, endothelium independent vasodilation levels of intercellular adhesion molecul-1, C-reactive protein, homocysteine, oxidation biomarkers TC, LDLC	4 weeks	The walnut diet improved endothelium dependent vasodilation and ↓ levels of vascular cell adhesion molecul-1 ($p < 0.005$ for both), ↓ TC and LDLC ($p < 0.05$) respect to the MD. Endothelium independent vasodilation and levels of intercellular adhesion molecul-1, C-reactive protein, homocysteine, and oxidation biomarkers were similar after each diet

Table 1. (Cont'd) Clinical Trials on the Mediterranean Diet

Author/Year Publication	Country	Type of Study		Population	Methodology	Outcome	Follow Up	Results
Ambring et al., 2004[14]	Goteborg, Sweden	R-crossover-CT	Primary prevention	22 healthy subjects (12 M, 10 F)	4 weeks of a Swedish diet, 4 weeks of a MD	Fasting blood lipids, insulin and glucose levels, apo B and LDL particle size. Endothelial dependent and independent vasodilation evaluation and arterial distensibility evaluated by ecocardiography. Fibrinolitic capacity, oxidative stress through urinary F2-isoprostane	4 weeks	↓ TC, LDLC, TG and apoB levels by 17%, 22%, 17% and 16% ($p < 0.05$). No effect on insulin, glucose level, LDL particle size, endothelial function, arterial distensibility, fibrinolitic capacity or oxidative stress
Goulel et al., 2004[15]	Quebec, Canada	Clinical trial	Primary prevention	77 healthy F	12 weeks nutritional intervention with two group sessions, three individual sessions and four 24-h recall	LDL-PPD, cholesterol levels in small (LDLC < 255 Å) and large (LDLC > 260 Å) LDL fractions, plasma lipid and lipoprotein profile	12 weeks	No change on the LDL-PPD, LDL integrated size, and in the LDL distribution among subclasses. No change on LDLC, HDLC, and TG. ↑ LDL-PPD in F in the first tertile of the LDL-PPD distribution at baseline ($p = 0.03$). ↓ of the proportion of LDL% < 255 Å ($p = 0.12$) and ↑ of the proportion of LDL% > 260 Å ($p < 0.05$) in F with a reduced LDL-PPD at baseline. ↓ LDL-PPD and LDL integrated size in F with large LDL particles at baseline (LDL PPD > 260 Å) ($p = 0.007$)
Flynn and Colquhoun, 2004[16]	Australia	Clinical trial	Primary prevention	155 individuals (31 M, 124 F)	3 months on a MD and control group? (non specify)	TC, TG, HDLC, LDLC.	3 months	↓ TG (31.6%), ↑ HDLC (9.6%), no significant changes on TC, LDLC
Urquiaga et al., 2004[17]	Santiago de Chile, Chile	Clinical trial	Primary prevention	21 M	3 months on a MD or western diet. The second month red wine was added to both diets	Plasma fatty acids profile (SFA, MUFA, PUFA, omega-3 fatty acids and omega-6/omega-3 fatty ratio)	3 months	MD group > levels of MUFA, omega-3 fatty acids, < levels of PUFA and omega-6 fatty acids and < omega-6/omega-3 ratio. Wine ↓ MUFA and ↑ PUFA in both dietary groups

Table 1. (Cont'd) Clinical Trials on the Mediterranean Diet

Author/Year Publication	Country	Type of Study		Population	Methodology	Outcome	Follow Up	Results
Bravo-Herrera et al., 2004[18]	Córdoba, Spain	R-crossover-CT	Primary prevention	41 subjects	Three dietary periods; saturated fat enriched diet, low fat and high CHO diet, MD	TC, TG, LDLC, HDLC, expression of a tissue factor in circulating monocytes.	3 months	The MD and CHO diet showed <TC, LDLC, HDLC, and tissue factor expression than the SFA diet
Toobert et al., 2003[19]	Oregon, USA	RCT	Secondary prevention	279 postmenopausal DM2 F	2 groups: usual care (control) and intervention group: an initial 3-days retreat and 6 months of weekly meetings with diet, physical activity and stress management modification	HbA1, lipid profile, plasma fatty acids, BMI, BP, flexibility, quality of life (measured by the Medical Outcomes Study (MOS) Short Form General Health Survey and The Problem Areas in Diabetes (PAID) scale)	6 months	↓ HbAc = 0.4% (p = 0.001), no statistical changes on TC, TG, LDLC, HDLC, ↓ BMI = 0.37 (p = 0.015), improvement of the PAID regimes related distress dimension
Rodriguez Villar et al., 2004[20]	Barcelona, Spain	R-crossover-CT	Secondary prevention	22 subjects (12 M, 10 F) with DM2	6 weeks of a high CHO diet and 6 weeks on a high MUFA diet or vice versa	LDL resistance to oxidation, body weight, glycaemic control, serum lipoproteins	6 weeks	No changes on body weight, glycaemic control, serum concentration of fasting lipids, LDLC and HDLC, apolipoproteins Al and B, and lipoprotein (a). The MD ↓ VLDLC by 35%, VLDL-TG by 16%, and the quotient VLDL-TG to VLDL apolipoprotein B (p = 0.0029) indicating a lesser particle enrichment with TG. No differences were seen on LDL oxidative resistance
Goulet et al., 2003[21]	Quebec, Canada	Clinical trial	Primary prevention	77 F	12 weeks nutritional intervention with two group sessions, three individual sessions and four 24-h recall	Plasma lipid lipoprotein profiles; body weight	12 weeks	↓ TC 2.5% ($p < 0.05$) at week 6 and apoB levels 5.1% ($p < 0.05$) at week 12, no effect on plasma LDLC, HDLC, TG, ↓BMI ($p < 0.01$) at week 12
Sondergaard et al., 2003[22]	Svendborg, Denmark	RCT	Secondary prevention	115 patients (92 M, 39 F) with recent or remote MI or unstable or stable angina pectoris	12 months of statin treatment and MD intervention group or control group	Serum lipids, endothelial function measured with non invasive ultrasound scanning vessel-wall tracking of brachial artery FMD	12 months	↓ TC and LDLC in both groups, ↓ TG levels only in the intervention group ($p < 0.05$) and no changes in HDLC on either group. The intervention group showed an improvement in FMD ($p < 0.01$)

Table 1. (Cont'd) Clinical Trials on the Mediterranean Diet

Author/Year Publication	Country	Type of Study		Population	Methodology	Outcome	Follow Up	Results
Mezzano et al., 2003[23]	Santiago de Chile, Chile	Clinical trial	Primary prevention	42 healthy M	21 subjects on a MD and 21 subjects on a high-fat diet for 30 days, supplementation with red wine in both groups from day 31 to 60	Primary hemostasis variables (BT, plasma concentrations of vWR: Ag and platelet aggregation and secretion ex vivo)	90 days	The mean BT for the MD group was longer (p = 0.017). The MD produces no changes on vWF:Ag or platelet aggregation. The addition of red wine produced ↑ platelet serotonin secretion after stimulation with collagen and ↑ platelet aggregation at the higher collagen concentration. No changes on BT, plasma vWF:Ag concentration or platelet count
Singh et al., 2002[24]	London, U.K.	RCT, double-blind	Primary prevention	56 healthy subjects (26 M, 30 F)	6 weeks on a MD or vitamin C supplements or placebo	Forearm blood flow (measured by pletismography), endothelium-dependent vasodilatation (measured by bradykinin acetylcholine) and independent vasodilatation (measured with the nitric oxid donor glyceryl trinitrate)	6 weeks	The MD ↑ Bradykinin-dependent vasodilatation (p = 0.011) versus placebo, ↑ Glyceryl trinitrate-dependent relaxation (p = 0.003) versus placebo and ↑ plasma vitamin C levels similar to supplements (p < 0.05)
Perez Jimenez et al., 2001[25]	Cordoba, Spain	R-crossover-CT	Primary prevention	59 young subjects (30 M, 29 F)	28 days of a SFA enriched diet, followed by 28 days of a low fat, high CHO diet or a MD and vice versa	Serum lipid levels, free fatty acids, fasting insulin and glucose, glucose suppression test, in vitro basal glucose-uptake, in vitro insulin-stimulated glucose uptake	28 days	↓ TC (p < 0.001), HDLC (p < 0.001), LDLC (p < 0.001), fasting insulin and free fatty acids (p < 0.001), ↓ mean glucose in steady state plasma glucose in glucose suppression test (p < 000.1), ↓ in vitro basal glucose uptake and insulin stimulated glucose uptake

Table 1. (Cont'd) Clinical Trials on the Mediterranean Diet

Author/Year Publication	Country	Type of Study	Population	Methodology	Outcome	Follow Up	Results
Mezzano et al., 2001[26]	Santiago de Chile, Chile	Clinical trial Primary prevention	42 healthy M	21 subjects on a MD and 21 subjects on a high-fat diet for 30 days, supplementation with red wine in both groups from day 31 to 60	Hemostatic cardiovascular risk factors: Fibrinogen, Factor VIIc, Factor VIIIc, tissue plasminogen activator antigen, plasminogen activator inhibitor antigen, antithrombin III, Protein C and protein S, C-reactive protein	90 days	The MD had lower plasma fibrinogen ($p = 0.03$), factor VIIc ($p = 0.034$) and factor VIIIc ($p = 0.0057$) and higher levels of protein S ($p = 0.013$). Wine produced ↓ plasma fibrinogen ($p = 0.001$) and FVIIc ($p = 0.05$) and ↑ tissue plasminogen activator antigen ($p = 0.01$), plasminogen activator inhibitor antigen ($p = 0.0003$)
Fuentes et al., 2001[27]	Cordoba, Spain	R-crossover-CT Primary prevention	22 hyper-cholesterolemic M	28 days of a SFA enriched diet, followed by 28 days of a low fat, high CHO diet (NCEP-1) or a MD and vice versa.	Serum lipid levels, endothelial function, plasma P-selectin levels	28 days	The NCEP-1 and MD produced ↓ plasma TC ($p = 0.001$), LDLC ($p < 0.001$), and apolipoprotein B level ($p = 0.002$). Measurement of the endothelial function showed no differences in the basal diameters of the brachial artery, or in the glyceryl trinitrate-induced vasodilation. Flow associated vasodilatation of the brachial artery was higher ($p = 0.027$) and P-selectin levels were lower ($p = 0.003$) after the MD and the resistance index after flow-associated vasodilatation and after glyceril trinitrate-induced vasodilatation were lower during the MD

Table 1. (Cont'd) Clinical Trials on the Mediterranean Diet

Author/Year Publication	Country	Type of Study	Population	Methodology	Outcome	Follow Up	Results
Muñoz et al., 2001[28]	Barcelona, Spain	R-crossover-CT Primary prevention	10 hypercholesterolemic M	6 weeks of a cholesterol lowering MD, 6 weeks on a diet with walnut replacing 35% of the energy from MUFA or vice versa	Serum lipid levels (TC, LDLC, HDLC, VLDLC, TG level), apolipoprotein A-I, and B, and LDL association to human hepatoma cells	6 weeks	The walnut diet ↓ TC (4.2%, $p = 0.176$) and LDLC (6.0%, $p = 0.087$). The apolipoprotein B level declined in parallel with LDLC (6.0%). The LDL from the walnut diet ↑ 50% the association rates to the LDL receptor in human hepatoma HepG2 cells ($p < 0.05$). The LDL uptake by HepG2 cells was correlated with alfa-linoleic acid content of the trygliceride plus cholesteryl ester fractions of LDL particles ($r^2 = 0.42$, $p < 0.05$)
Zambon et al., 2000[29]	Barcelona, Spain	R-crossover-CT Primary prevention	49 hypercholesterolemic subjects (28 M, 27 F)	6 weeks of a cholesterol lowering MD, 6 weeks of a diet with walnut replacing 35% of the energy from MUFA	LDL fatty acids, serum lipid levels (TC, LDLC, HDLC, TG level), lipoprotein (a) levels, and LDL resistance to in vitro oxidative stress	6 weeks	The walnut diet caused a bigger ↓ TC, LDLC, and lipoprotein (a) (9%, 11.2%, and 9.1% ($p < 0.001$)) vs. the MD diet which ↓ TC and LDLC by 5% and 5.6%; and lipoprotein (a) by 3.4%. No effects on HDLC, VLDLC, TG, apolipoprot A-I. LDL susceptibility to oxidation was similar in both diets
Madigan et al., 2000[30]	Dublin, Ireland	R-crossover-CT Secondary prevention	11 M DM2	2 weeks on a MUFA rich diet (30 ml olive oil per day) and 2 weeks on a PUFA rich diet (30 ml sunflower oil) and vice versa	Fasting glucose and insulin levels, plasma cholesterol and LDLC, fasting chylomicron and VLDLC, postprandial chylomicron and VLDLC levels	2 weeks	Fasting glucose and insulin were higher on the PUFA diet ($p < 0.01$ and <0.002, respectively). TC and LDLC were higher on the PUFA diet ($p < 0.001$). Plasma TG and HDLC were similar. Fasting chylomicron components apoB48 ($p < 0.05$) and apoB100 ($p < 0.02$) and VLDL

Table 1. (Cont'd) Clinical Trials on the Mediterranean Diet

Author/Year Publication	Country	Type of Study	Population	Methodology	Outcome	Follow Up	Results
							phospholipid levels ($p <$ 0.02) were higher on the PUFA diet. Postprandial Chylomicron apoB48 and apoB100 ($p < 0.05$), VLDL apo B48 and B100 ($p < 0.05$), and VLDL phospholipids ($p < 0.01$) were higher on the PUFA diet. The PUFA diet produced an increased in the phospholipids ($p <$ 0.01) and total fatty acids ($p < 0.05$) LDL fasting composition
Ryan et al., 2000[31]	Dublin, Ireland	clinical trial	secondary prevention	11 M DM2	2 months on a PUFA rich diet and 2 months on a MUFA rich diet (MD)	Insulin mediated glucose transport, fatty acid composition of the adipocyte membranes and endothelium-dependent and independent FMD.	2 months	<Fasting plasma insulin and < fasting insulin/glucose ratio ($p < 0.02$ and $p <$ 0.002) with the MD diet. The MD produced a > quantity of oleic acid ($p <$ 0.0001) and < quantity of linoleic acid ($p < 0.0001$) and stearic acid ($p < 0.01$) in the adipocyte membrane. ↑ mean insulin-mediated glucose transport with the MD (0.29 ± 0.14 and 0.56 ± 0.17 nmol/10^5 cells/3 min, at 1 ng/ml and 5 ng/ml insulin with the linoleic acid, and 0.53 ± 0.18 and 0.79 ± 0.28 mmol/10^3 cells/3 min, at 1 ng/ml and 5 ng/ml insulin with the oleic acid) ($p < 0.0001$). ↑ endothelium-dependent FMD in the reactive hyperaemia phase ($p <$ 0.0001) and ↑ in the glyceryl trinitrate-induced

Table 1. (Cont'd) Clinical Trials on the Mediterranean Diet

Author/Year Publication	Country	Type of Study	Population	Methodology	Outcome	Follow Up	Results
							FMD ($p < 0.05$) in the MD. Correlation between the ratio of adipocyte membrane oleic/linoleic acid and insulin-mediated glucose transport at 1 μg/ml insulin ($p < 0.001$) and at 5 ng/ml insulin ($p < 0.05$) and this ratio with the endothelium-dependent FMD ($p < 0.001$)
Barbagallo C et al., 1999[32]	Palermo, Italy	Clinical trial	Secondary prevention	78 renal transplant recipients (51 M, 27 F)	24 weeks of usual diet and 10–12 weeks of MD	Plasma lipid levels and changes in lipid-related cardiovascular risk classes	10–12 weeks	↓ 6.5% TG ($p < 0.02$), ↓ 10.4% LDLC ($p < 0.0001$), ↓ 10.0 LDLC/HDLC ($p < 0.001$), ↓ TC and LDLC in patients in "desirable LDLC" (6.7% and 4.0%, $p < 0.05$), in "borderline high-risk LDLC" (9.4% and 8.7%, $p < 0.001$) and in "high-risk LDLC" (16.4% and 19.7%, $p < 0.0001$). ↓ LDLC/HDLC in patients in "borderline high-risk LDLC" (6.8%, $p < 0.05$) and in "high-risk LDLC" (21.1%, $p < 0.0001$). ↓ TG in patients in "desirable LDLC" (12.3%, $p < 0.01$)
Leighton et al., 1999[33]	Chile	Clinical trial	Primary prevention	21 M	3 months on a MD or western diet. The second month red wine was added to both diets	Plasma vitamin C and E, total plasma antioxidant capacity, oxidative DNA damage in blood leukocyte DNA, endothelial function (flow mediated vascular reactivity)	3 months	The high fat diet ↓ vitamin C levels, and ↑ oxidative DNA damage. The MD ↑ total plasma antioxidant capacity (28%). Wine supplementation produced ↑ plasma vit C (13.5%) and total antioxidant reactivity and a ↓ vitamin E (26%) and oxidative DNA damage in the MD group and a ↓ vitamin E

Table 1. (Cont'd) Clinical Trials on the Mediterranean Diet

Author/Year Publication	Country	Type of Study		Population	Methodology	Outcome	Follow Up	Results
								(15%) and oxidative DNA damage, ↑ total antioxidant reactivity. The endothelial function was suppressed by the high fat diet and was normal after wine supplementation
Pérez-Jiménez et al., 1999[34]	Córdoba, Spain	Clinical Trial	Primary Prevention	25 M	28 days on a low fat NCEP-I-diet, or a MUFA-diet (MD) or a SFA-rich diet	Serum lipid levels (TC, LDLC, HDLC, TG level), apolipoprotein A-I, and B and conjugated diene formation after incubation of LDL particles with Cu. Endothelial products (von Willebrand Factor, E-selectin, Thrombomodulin and Tissue Factor Pathway inhibitor (TFPI)) levels and plasminogen activator inhibitor type I (PAI-1) activity	28 days	The MD diet ↓ von Willebrand Factor, PAI-1, TFPI plasma levels and ↑ lag time of conjugated diene formation
Baroni et al., 1999[35]	Italy	Clinical trial	Secondary prevention	Hyper-cholesterolemic patients	MUFA enriched diet vs. a PUFA enriched diet	LDL fatty acid composition, LDL susceptibility to oxidation		The olive oil diet ↑ MUFA (11%) and ↓ PUFA (10%) concentrations on LDL composition ($p < 0.05$). The MUFA-enriched diet ↓ PUFA/MUFA ratio and the unsaturation index. The oleate-enriched LDL was more resistant to oxidative modifications.
Simoni et al., 1995[36]	Italy	Clinical trial	Secondary prevention	15 hyper-cholesterolemic with ↑ Lp(a) patients	2 months on a Gemfibrozil (600 mg) treatment combined with MD	TC, Lipoprotein (a) values	2 months	↓ Lipoprotein(a) 36.5 to 8.4 mg/dl ($p < 0.0002$) and TC 254.5 to 208.0 mg/dl ($p < 0.0001$)

Table 1. (Cont'd) Clinical Trials on the Mediterranean Diet

Author/Year Publication	Country	Type of Study		Population	Methodology	Outcome	Follow Up	Results
Salen et al., 1994[37]	France	Clinical trial	Secondary prevention	41 hyper-cholesterolemic heart transplant M	18 months of MD	Platelet-aggregation, fasting plasma lipids	18 months	↓ platelet aggregation in response to trombine (p = 0.02). Inverse correlation between linoleic acid intake and platelet aggregation (r = −0.44, p = 0.03). ↓ TC and LDLC (p = 0.005 and p = 0.04 respectively)
Moreno Vazquez et al., 1994[38]	Badajoz, Spain	Clinical trial	Primary prevention	90 pilots	A. Uncontrolled diet and exercise programme, B. MD and uncontrolled exercise, C. MD and controlled exercise programme	TC, TG, HDLC, TC/HDLC ratio, and anxiety levels		<TC and TG in MD groups
Ferro-Luzzi et al., 1984[39]	Italy	Clinical trial	Primary prevention	48 M/F	Shift from a MD to a MD high in saturated fats and cholesterol	TC, LDLC, HDLC, apoprotein B	42 days	In M ↑TC 214 ± 30 to 245 ± 33 mg/dl and ↑LDLC 19%, in F ↑HDL (19%) and TC (16%). ↑Apoprotein B in both sexes
Ehnholm et al., 1982[40]	North Karelia, Finland	Clinical trial	Primary prevention	54 individuals	MD	TC, LDLC, apoprotein B, HDLC, apoprotein A-I and A-II	6 weeks	↓TC 263 ± 8 to 201 ± 5 mg/dl in M and 239 ± 8 to 188 ± 8 mg/dl in F (p < 0.0001). ↓LDLC and apoprotein B. ↓HDLC 54 ± 2 to 44 ± 2 mg/dl in M and 56 ± 3 to 47 ± 2 mg/dl in F (p < 0.0001), ↓ Apoprotein A-I
CARDIOVASCULAR								
Barzi et al., 2003[41]	Italy	Clinical trial	Secondary prevention	11323 M/F surviving a MI	Subjects received advice to increase their consumption of fish, fruit, raw and cooked vegetables and olive oil	Association of food intakes (fish, fruit, raw and cooked vegetables and olive oil), a combined dietary score and risk of death	6.5 years	Compared with people in the worst dietary score quarter, odds ratio for people in best score was 0.51 (95% CI 0.44–0.59). ↑ consumption of each food was associated with ↓ risk of death.

Table 1. (Cont'd) Clinical Trials on the Mediterranean Diet

Author/Year Publication	Country	Type of Study		Population	Methodology	Outcome	Follow Up	Results
Singh et al., 2002[42]	Moradabad, India	RCT, single blind	Secondary prevention	1000 subjects with major risk factors or previous heart attack	499 individuals on a indo-MD and 501 controls on a NCEP diet for 2 years	Non-fatal MI, fatal MI, sudden cardiac death; total cardiac endpoints	2 years	adjusted rate ratios: non fatal MI: 0.47 (0.28–0.79), fatal MI: 0.67 (0.31–1.42), sudden cardiac death: 0.33 (0.13–0.86) total cardiac end points: 0.48 (0.33–0.71)
de Lorgeril et al., 1999[43]	Lyon, France	RCT, single blind	Secondary prevention	423 subjects surviving a myocardial infarction	Randomisation to a MD group or control group.	CO1, cardiac death, non-fatal heart attack, CO2, 1 + unstable angina, stroke, heart failure, pulmonary or peripheral embolism, CO3 1 + 2 + events requiring hospitalisation	46 months	The MD showed a ↓ CO1 ($p = 0.0001$) ↓ CO2 ($p = 0.0001$) and ↓ CO3 ($p = 0.0002$)
de Lorgeril et al., 1996[44]	Lyon, France	RCT, single blind	Secondary prevention	605 subjects surviving a MI	Randomisation to a MD group or control group.	Major primary end points (CV death, non fatal MI, Non-CV deaths), major secondary end points (per procedural infarction, unstable angina, nonfatal heart failure, stroke, pulmonary and peripheral embolism), minor secondary end points (stable angina, elective vascular revascularization, post angioplasty restenosis)	27 months	Primary + major secondary end points: risk ratio 0.24 (95% CI 0.13 to 0.44, $p < 0.0001$), major primary and secondary end points + minor end points: risk ratio 0.63 (95% CI 0.46 to 0.87, $p < 0.005$)
de Lorgeril et al., 1994[45]	Lyon, France	RCT, single blind	Secondary prevention	605 subjects surviving a MI	Randomisation to a MD group or control group.	Primary end points (deaths from CV causes and non-fatal acute MI) and subsidiary end points (non cardiac deaths and unstable angina, post infarct recurrent angina, heart failure, stroke, pulmonary and peripheral embolism and venous trombophlebitis)	27 months	Risk ratio for: Cardiovascular deaths 0.24 (95% CI 0.07–0.85, $p < 0.02$), total major primary end points: 0.27 (95% CI 0.12–0.59, $p < 0.001$), overall mortality: 0.30 (95% CI 0.11–0.82 $p < 0.02$)

Table 1. (Cont'd) Clinical Trials on the Mediterranean Diet

Author/Year Publication	Country	Type of Study		Population	Methodology	Outcome	Follow Up	Results
ARTHRITIS								
Sköldstam et al., 2003[46]	Sweden	clinical trial	Secondary prevention	51 rheumathoid arthritis patients (10 M, 41 F)	12 weeks on either MD or control diet	Disease Activity Index (DAS28), physical function index (HAQ), health survey of quality of life (SF36), daily consumption of NSAID	12 weeks	↓ DAS28 = 0.56 ($p < 0.001$), ↓ HAQ = 0.15 ($p < 0.02$), swollen joint count ($p = 0.001$), improvement in pain VAS ($p = 0.006$) and in two dimensions of SF-36 Health Survey ($p < 0.02$). NSAID use unaffected.
Hagfors et al., 2003[47]	Sweden	RCT	Secondary prevention	51 rheumatoid arthritis patients (10 M, 41 F)	3 months on either MD or control diet	Antioxidant intake, plasma levels of retinol, antioxidants (α and γ tocopherol, β-carotene, lycopene, vitamin C and uric acid), and urinary Malondialdehyde	3 months	The MD showed > intake of vitamin E ($p = 0.007$) and selenium ($p = 0.004$) and a < intake of retinol ($p = 0.049$) excluding under and over reporters. No changes in urine Malondialdehyde or plasma levels of antioxidants.
CANCER								
de Lorgeril et al., 1998[48]	Lyon, France	RCT	Secondary prevention	605 subjects surviving a MI	MD group or control group	Occurrence of malignant or non-malignant tumor	4 years	↓ risk in MD compared with control subjects: 61% ($p = 0.05$) for cancers and 56% ($p = 0.01$) for the combination of deaths and cancer. The MD group showed > levels of vitamin C and E ($p < 0.05$) and omega-3 fatty acids ($p < 0.001$), and < levels of omega-6 fatty acids measured 2 months after randomisation
BODY COMPOSITION								
Flynn et al., 2004[49]	Australia	Clinical trial	Primary prevention	41 individuals	41 individuals followed for 15 months after completing a 3 months MD	Change in body weight	3 months	24 individuals maintained the weight loss (8.18% of weight lost) and 17 individuals regained the weight lost.

Table 1. (Cont'd) Clinical Trials on the Mediterranean Diet

Author/Year Publication	Country	Type of Study		Population	Methodology	Outcome	Follow Up	Results
Fernandez de la Puebla et al., 2003[50]	Córdoba, Spain	Clinical trial	Secondary prevention	34 hyper-cholesterolemic M who consumed a diet rich in saturated fat	Every 17 subjects underwent two dietary periods of 28 days: MD/carbohydrate rich diet	Body composition, plasma lipoproteins, fatty acids in cholesterol esters	28 days	Decreased in % fat when changing from saturated fat to Mediterranean Diet ($p < 0.05$) or CHO rich diet ($p < 0.05$). Lean mass increased when changing from sat diet to CHO diet ($p < 0.05$).
McManus et al., 2001[51]	Boston, USA	RCT	Primary prevention	101 overweight (10 M, 91 F)	MD versus low fat diet	Change in body weight	18 months	↓4.1 Kg body weight, ↓1.6 Kg/m2 BMI, ↓ 6.9 cm waist circumference ($p < 0.001$). 54% participants in the MD group continued after 18 months for 20% in the control group.
PSYCHOLOGICAL FUNCTION								
Hyyppä et al., 2003[52]	Turku, Finland	R-crossover-CT	Secondary prevention	120 untreated hyper-cholesterolemic M	MD versus simvastatin treatment	Mood changes measured through a psychological distress scale (Brief Symptom Inventory), an anger scale (State-Trait Anger Inventory), and two questionnaires to measure aggression based on the Strauss Scale of Aggression, Steroid Hormone levels	12 weeks	The MD produced no mood changes nor changes in steroid hormones. The MD ↓TC by 7.7%
Wardle et al., 2000[53]	London, United Kingdom	RCT	Secondary prevention	176 hyper-cholesterolemic subjects	12 weeks of a low fat diet, or MD or control group	TC, LDLC, HDLC, TG, social functioning, mood and cognitive function	12 weeks	↓TC 10% ($p < 0.001$), ↓LDLC 8.3% ($p < 0.05$), no changes in mood and aggression. Worse response to one of the four cognitive function tests (sustained-attention task in the intervention groups ($p < 0.001$))

RCT = randomised clinical trial, CV = cardiovascular, MD = Mediterranean Diet, BMI = Body Mass Index, TC = Total Cholesterol, LDLC = LDL Cholesterol, TG = Tryglicerides, BP = Blood Pressure, MUFA = Monounsaturated Fat, LDL-PPD = LDL peak particle diameter, HDLC = HDL Cholesterol, SFA = Saturated fatty Acids, PUFA = Polyunsaturated Fat, CHO = carbohydrate, DM = Diabetes Mellitus, MI = myocardial infarction, FMD = flow mediated vasodilatation, BT = cutaneous bleeding time, vWF: Ag = von Willebrand factor antigen, vWF = von Willebrand Factor, CI = confidence interval, NSAID = non-steroidal anti-inflammatory drugs, VAS = visual analog scale.

with more than 150 references, and more than 20% of them from the author himself, yet not one reference from Trichopoulou (author of 27 of the 70 articles related to "diet and Cancer and Greece").

Other examples in the area of obesity and the Mediterranean diet[56-58] put into evidence the lack of consensus and objectivity that leads to reduced credibility of the research done in Mediterranean countries.

Mediterranean countries have been considered a difficult place to conduct reliable research (experimental studies and large-scale cohort studies), not only due to the traditional subjectivism and lack of cooperation among researchers, but also because of the lack of commitment from the government and other institutions. Additionally, in the past, low priority was given to research careers, particularly in the area of nutrition.[59] Fortunately, there has been rapid progress in recent years, and the number of original articles addressing the Mediterranean diet have been increasing exponentially since 1999 (Figure 1). The rise of institutions and initiatives dedicated to the Mediterranean diet, such as the Foundation for Advancement of Mediterranean diet, which was founded in 1996, may have contributed to this.

Most of the trials analyzed had a limited number of participants (24 of 43 articles included less than 60 participants in the sample), but the most important limitation is the different methodology used to define the intervention. Some authors characterized the Mediterranean diet just as a monounsaturated fatty acid-rich or -enriched diet; others by additional supplementation with walnuts or wine, but only a few defined a score or pattern of the Mediterranean diet. This is probably one of the major weaknesses of these experimental studies. Changing a group of persons to a particular dietary profile is hard to achieve and particularly difficult to maintain and guarantee compliance.

This review shows that the results of the following studies are of special importance: the Lyon Diet Heart Study,[43] the Indo Mediterranean Heart Study,[42] the GISSI Prevention Trial for Secondary Prevention,[41] the study by Esposito et al.[12] on metabolic syndrome, and also three ongoing trials on primary prevention: the Mediet Project[60] in Italy, the Medi-RIVAGE Study[11] in France, and the PREDIMED study in Spain.

However, most of the small clinical studies analyzed in this review contributed greatly to explaining the mechanisms of how the Mediterranean diet itself or some of its components improve certain biological variables and affect disease outcomes.

Recent findings from two large European cohort studies[61,62] have suggested that a high degree of adherence to the Mediterranean diet is associated with a reduction in both total and coronary mortality. In addition, modified Mediterranean diets were associated with remarkable reductions in CHD event rates and cardiovascular mortality in two secondary prevention trials carried out in France (Lyon Diet Heart Study)[43] and India (Indo-Mediterranean diet Heart study).[42] However, no randomized, controlled trial has been conducted to assess to what extent a Mediterranean diet is superior to the usually recommended low-fat diet in the primary prevention of cardiovascular disease and other chronic diseases. Only two small clinical trials are currently being undertaken: the Mediet Project[60] in Italy and the Medi-RIVAGE Study[11] in France. The only large-scale ongoing clinical trial is running in Spain, the PREDIMED Study, which is the most comprehensive and ambitious.

The Mediet Project[60] is a randomized clinical trial being undertaken to investigate the potential impact of the traditional Mediterranean diet on the risk of developing breast cancer in a sample of 115 women. The study is currently ongoing to verify the association of changes in serum and urine hormone levels and breast cancer risk in the intervention group, who attended a weekly cooking course for one year.

The Medi RIVAGE study[11] (Mediterranean diet, Cardiovascular Risks and Gene Polymorphisms) is a randomized clinical trial developed in France conducted in a sample of 212 males and females with at least one cardiovascular risk factor. The study has two main goals. The first one is the prevention of cardiovascular diseases by evaluating the effect of two diets (a Mediterranean-type diet and a low-fat, low-cholesterol diet) on arteriosclerosis risk factors. The second goal is to implement extensive biological investigation in relation to the dietary intervention, with a special interest on fasting and postprandial examinations of lipid parameters and lipoproteins, as well as some genetic polymorphisms that influence lipoprotein metabolism and homeostasis. The study is still ongoing. The data at 3 months of follow-up show that in subjects at risk, changing to a Mediterranean-type diet improves blood biochemical parameters.

The PREDIMED Study (PREDIMED meaning PREvención con DIeta MEDiterránea) was initiated in October 2003 with the recruitment of participants for this primary prevention trial. This parallel group, multi-center, randomized study was designed in 2002 and funded by a grant from the official biomedical research agency of the Spanish government, the Spanish Ministry of Health. The PREDIMED Study is the first large-scale, long-term clinical trial that enrolls high-risk patients to follow a Mediterranean diet supplemented with extra virgin olive oil or nuts for primary cardiovascular disease prevention. The US Food and Drug Administration (FDA) has very recently approved a health claim for olive oil as a putative cardio-protective food.[63] However,

in this era of evidence-based medicine, definite medical advice and treatment should be supported by the results of randomized clinical trials with clinical events as primary outcomes. The results of the PREDIMED Study could provide the firm evidence required to issue dietary guidelines for sound clinical practice.

The primary outcome to be evaluated in this trial is a composite end point of cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke. As secondary outcomes, death by any cause and incidence of angina leading to a revascularization procedure, heart failure, diabetes mellitus, dementia, and cancer were included. Finally, other outcomes such as changes in blood pressure, body weight, adiposity measures, blood sugar, lipid profile, markers of inflammation, and other intermediate markers of cardiovascular risk will also be measured.

A sample size of 9000 with randomization to three equally sized groups (two intervention groups and one control group, with 3000 patients each) will provide sufficient statistical power to evaluate the effect of the Mediterranean diet on the primary outcome. Participants are free-living high-risk persons age 55 to 80 years for men and 60 to 80 years for women with no history of cardiovascular disease, who fulfill at least one of the two following criteria: 1) type 2 diabetes, 2) three or more of these risk factors: current smoker, hypertension, LDL cholesterol $\geq$160 mg/dL, HDL cholesterol $\leq$ 40 mg/dL, BMI $\geq$ 25 kg/m^2, or a family history of premature CHD.

The participants included as controls receive recommendations to follow a low-fat diet according to the American Heart Association guidelines. The two intervention group assignments are designated by allotment of either olive oil (15 liters=1 liter/week for 15 weeks) or packets of walnuts, hazelnuts, and almonds (1350 g walnuts = 15 g/d, 675 g hazelnuts = 7.5 g/d, and 675 g almonds = 7.5 g/d for 90 days), together with instructions about their use and conservation. In the intervention groups, personalized advice regarding dietary changes with the aim of achieving an ideal Mediterranean diet is given. A leaflet with written information about the main food components and cooking habits of the Mediterranean diet is provided, together with recommendations on the desired frequency of intake of specific foods. A group session with up to 20 participants, with separate sessions for each of the two Mediterranean diet groups, is scheduled every 3 months and consists of informative talks and the provision of written material with elaborate descriptions of typical Mediterranean foods and shopping lists, meal plans, and recipes adapted to the season of the year. Each session includes three steps: assessment, intervention, and future directions.

Major measurements and data collection activities also take place at baseline and each subsequent year. The baseline visit includes: 1) a general questionnaire; 2) a food-frequency questionnaire with 137 foods plus information on vitamin supplements and alcohol consumption (adapted from the Nurses' Health Study questionnaire and validated in Spain); 3) the Minnesota physical activity questionnaire (validated Spanish version); 4) measurement of weight, height, waist circumference, blood pressure, and ankle-brachial blood pressure index; 5) collection of fasting blood samples and preparation of serum, plasma, and buffy-coat aliquots; 6) collection of urine samples and toenail specimens; and 7) a 47-item general questionnaire with information about risk factors and medication use. The same assessment is performed in the yearly visits, except that the initial questionnaire is substituted by a follow-up questionnaire, which includes new medical diagnoses and medication. Since the information from the food-frequency questionnaire provides only a subjective assessment of compliance, biological markers (plasma fatty acids and urinary tyrosol and hydroxytyrosol)[64,65] are measured in a random subset (10%) of participants from the three arms of the trial to objectively evaluate intervention compliance.

Participants initially recruited will be followed for up to 5 years, and those entering later will be followed for at least 4 years. Consequently, we expect a median follow-up above 4 years. Primary and secondary outcomes will be detected by the primary care physicians of each participant and confirmed by a clinical events subcommittee. It is our hope that the results of the PREDIMED trial will provide strong evidence to establish dietary guidelines to enforce sound clinical practice and public health policy within the Mediterranean Basin.

Mediterranean diet recommendations need to be evidence based, which requires the development of clinical and observational epidemiology in Mediterranean countries. Also, objective systematic (non-personalized) reviews need to address different areas of the relationship between Mediterranean diet and health.[66] Otherwise, the promotion of the Mediterranean diet will always have shortcomings and thus continue to be viewed with certain misgivings.

ACKNOWLEDGMENTS

The authors would like to thank Lourdes Ribas and Joy Ngo for their assistance with the preparation and editing of the document.

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