

Title: Benefits of the Mediterranean Diet: Insights from the PREDIMED Study

Running title: PREDIMED and the Mediterranean Diet

Miguel A. Martínez-González [1,2,3], Jordi Salas-Salvadó [2,3,4], Ramón Estruch [2,3,5], Dolores Corella D [3,6], Montse Fitó [3,7], Emilio Ros [3,5] for the PREDIMED INVESTIGATORS*

1 Department of Preventive Medicine and Public Health, University of Navarra, IDISNA (Navarra Health Research Institute), Pamplona, Spain.

2 The PREDIMED Research Network (RD 06/0045), Instituto de Salud Carlos III, Madrid, Spain.

3 Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición (CIBERObn), Instituto de Salud Carlos III, Madrid, Spain.

4 Human Nutrition Department, Hospital Universitari Sant Joan, Institut d'Investigació Sanitària Pere Virgili, Universitat Rovira i Virgili, Reus.

5 Institut d'Investigacions Biomèdiques August Pi i Sunyer, Hospital Clinic, University of Barcelona, Barcelona.

6 Department of Preventive Medicine and Public Health, University of Valencia, Valencia.

7 Cardiovascular and Nutrition Research Group, Institut de Recerca Hospital del Mar, Barcelona.

***A complete list of PREDIMED INVESTIGATORS can be found at the end of this manuscript.**

Last names for PubMed indexing: Martinez-Gonzalez, Salas-Salvado, Estruch, Corella, Fito, Ros

Word count: 3053 (excluding title page, abstract and references)

Number of figures: 1

Number of Tables: 3

Correspondence and reprints:

Prof. Miguel Á. Martínez-González, MD, MPH, PhD. Department of Preventive Medicine & Public Health, School of Medicine, University of Navarra, Irunlarrea 1, 31008-Pamplona, Navarra (Spain). E-mail: mamartinez@unav.es. Tel: +34 948 42 56 00 Ext: 806463. Fax: + 34 948 42 57 40.

Funding Disclosure: Supported by the official funding agency for biomedical research of the Spanish government, Instituto de Salud Carlos III (ISCIII), through grants provided to research networks specifically developed for the trial (RTIC G03/140, to Dr. Estruch; RTIC RD 06/0045, to Dr. Martínez-González and through Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBERObn), and by grants from Centro Nacional de Investigaciones Cardiovasculares (CNIC 06/2007), Fondo de Investigación Sanitaria–Fondo Europeo de Desarrollo Regional (PI04-2239, PI 05/2584, CP06/00100, PI07/0240, PI07/1138, PI07/0954, PI 07/0473, PI10/01407, PI10/02658, PI11/01647, P11/02505 and PI13/01090), Ministerio de Ciencia e Innovación (AGL-2009-13906-C02 and AGL2010-22319-C03), Fundación Mapfre 2010, Consejería de Salud de la Junta de Andalucía (PI0105/2007), Public Health Division of the Department of Health of the Autonomous Government of Catalonia, Generalitat Valenciana (ACOMP06109, GVACOMP2010-181, GVACOMP2011-151, ACOMP/2013/165, ACOMP/2013/159, CS2010-AP-111, and CS2011-AP-042), and Regional Government of Navarra (P27/2011).

Role of the Funders: funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Conflicts of interest: MA Martinez-Gonzalez received grants for research from the International Nut Council, Reus, Spain; J. Salas-Salvado has received grants for research from the International Nut Council, Reus, Spain and is a non paid member of its Scientific Advisory Committee; Ramon Estruch no conflicts of interest; D. Corella no conflicts of interest; M. Fito no conflicts of interest and E. Ros has received grants for research from the California Walnut Commission, Sacramento, CA, U.S. and is a non paid member of its Scientific Advisory Committee;

Non standard abbreviations: AF, atrial fibrillation; CHD, coronary heart disease; CV, cardiovascular; CVD, CV disease; DLP, dyslipidemia; EVOO, extra-virgin olive oil; FFQ, food frequency questionnaire; HTN, hypertension; MeDiet, Mediterranean diet; MetS, metabolic syndrome; PAD, peripheral artery disease; PREDIMED (PREvención con DIeta MEDiterránea); RCT, randomized control trial; T2DM, type 2 diabetes mellitus.

ABSTRACT

The PREDIMED (PREvención con DIeta MEDiterránea) multicenter, randomized, primary prevention trial assessed the long-term effects of the Mediterranean diet (MeDiet) on clinical events of cardiovascular disease (CVD). We randomized 7447 men and women at high CVD risk into three diets: MeDiet supplemented with extra-virgin olive oil (EVOO), MeDiet supplemented with nuts, and control diet (advice on a low-fat diet). No energy restriction and no special intervention on physical activity were applied. We observed 288 CVD events (a composite of myocardial infarction, stroke or CVD death) during a median time of 4.8 years; hazard ratios were 0.70 (95% CI, 0.53-0.91) for the MeDiet+EVOO and 0.70 (CI, 0.53-0.94) for the MeDiet+nuts compared to the control group. Respective hazard ratios for incident diabetes (273 cases) among 3541 non-diabetic participants were 0.60 (0.43-0.85) and 0.82 (0.61-1.10) for MeDiet+EVOO and MeDiet+nuts, respectively versus control. Significant improvements in classical and emerging CVD risk factors also supported a favorable effect of both MeDiets on blood pressure, insulin sensitivity, lipid profiles, lipoprotein particles, inflammation, oxidative stress, and carotid atherosclerosis. In nutrigenomic studies beneficial effects of the intervention with MedDiets showed interactions with several genetic variants (TCF7L2, APOA2, MLXIPL, LPL, FTO, M4CR, COX-2, GCKR and SERPINE1) with respect to intermediate and final phenotypes. Thus, the PREDIMED trial provided strong evidence that a vegetable-based MeDiet rich in unsaturated fat and polyphenols can be a sustainable and ideal model for CVD prevention.

Keywords: Mediterranean diet, Dietary intervention, randomized trials, primary prevention, atrial fibrillation, peripheral artery disease, stroke, type-2 diabetes.

Introduction

Cardiovascular (CV) disease (CVD) is the main cause of worldwide premature mortality. Coronary heart disease (CHD) and stroke ranked first and third, respectively, as the leading global causes of disability-adjusted years according to the global burden of disease estimates for 2010¹. Furthermore, the projections of mortality from CVD for 2030 are dismal^{2,3} and underline the need for preventive strategies as a public health priority. In this context, a high-quality diet and a healthy lifestyle at middle age are the most important factors for CVD prevention³⁻⁷. Consequently, the diet-heart hypothesis has been a long-standing tenet in CVD prevention and nutritional epidemiology during the last 50 years⁷⁻⁸. Recently, the relevance of overall high-quality food patterns, rather than the focus on single nutrients and foods, has emerged as a powerful paradigm to address the inherent complexity of dietary exposures and to assess their potential CVD preventive effects. Food patterns can be described as the amounts, proportions, combinations or varieties for the consumption of different foods and beverages and the frequency with which they are usually consumed. This approach allows the assessment of synergistic interactions and cumulative effects among different foods and nutrients, preempts confounding by alternative dietary exposures, avoids some problems of co-linearity between foods or nutrients and thus provides a strong methodological tool in nutritional epidemiology⁹⁻¹⁰. Even though randomized dietary intervention trials are the hallmarks for acquiring knowledge on the effects of diet on CVD, most research in the field of dietary patterns is observational, with some potential for residual confounding and other possible sources of bias. These limitations have been the subject of ample but probably undue criticism^{10,11}.

A weakness of the diet-heart hypothesis is that most of the available experimental research in the field has not used hard clinical outcomes, but only intermediate risk biomarkers. The

existence of multiple pathways leading from diet to CVD speaks against the simplistic approach of giving a high value to changes in any single biomarker. Moreover, the induction period can vary for the different pathways in which diverse biomarkers are involved, thus limiting the possibility of assessing multiple biomarker combinations at any time point. Furthermore, other lesser-known pathways could account for a substantial proportion of clinical CVD events. The most sensible approach, therefore, in order to investigate the diet-heart hypothesis is to use hard clinical CVD events as end-points of randomized controlled trials (RCTs). Most feeding trials, however, are usually short term and rarely include clinical end-points such as CVD events or death¹¹. The PREDIMED trial was designed to overcome both the problem of the single-nutrient approach and the limitations of assessing only intermediate risk markers. Indeed, the PREDIMED randomized trial used an overall food pattern as the intervention and assessed hard CVD events as end-points¹² providing a high level of scientific evidence.

Scientific evidence of the cardio-metabolic benefits of the Mediterranean diet

The abundant and consistent observational evidence that was available to support the benefits of the Mediterranean diet (MeDiet) and, specifically, of tree nuts and olive oil, on CV health prompted us to choose this traditional dietary model enriched with olive oil or nuts as the intervention¹³⁻⁴⁴. Table 1 summarizes the results of meta-analyses and systematic reviews assessing the effects of MeDiet on different cardiometabolic outcomes.

The MeDiet is defined as the traditional dietary pattern found in the early 1960s in Greece, Southern Italy, Spain and other olive-growing countries of the Mediterranean basin. It is a frugal diet that uses generous amounts of olive oil as main culinary fat and has a high

consumption of plant-derived foods (fruit, vegetables, legumes, nuts and seeds, and whole grain cereals); frequent but moderate intake of wine (especially red wine), usually with meals; moderate consumption of seafood and dairy products (especially yogurt and cheese, but not whole milk, butter or cream), poultry and eggs; and low consumption of sweet desserts, red and processed meats. In comparison with other healthy patterns, such as the DASH diet, the healthy US dietary pattern or the Alternative Healthy Eating Index, the consumption of fruit and fish is usually higher in the MeDiet, while the consumption of dairy products tends to be lower. In healthy vegetarian food patterns, meat and seafood are not consumed, but eggs and dairy are most frequently included. Legumes, nuts/seeds, and processed soy are all higher in a healthy vegetarian food pattern than in the healthy U.S.-style or Mediterranean-style patterns. A considerable scientific advantage of the MeDiet over other healthy dietary patterns was the availability of a previous randomized trial, the Lyon Diet Heart study, conducted in myocardial infarction survivors (i.e., it was a secondary prevention trial). It showed that a MeDiet enriched with alpha-linolenic acid, but not olive oil, provided a strong protection against recurrent CHD⁴⁵.

Our hypothesis when designing the PREDIMED trial was that the MeDiet would be superior to a low-fat diet for primary CVD prevention. This hypothesis had never been tested previously using a RCT design.

Design and methods of the PREDIMED study

The PREDIMED study was a primary prevention trial which tested the long-term effects of the MeDiet on incident CVD in men and women at high CVD risk aged 55-75 y (men) or 60-80 y (women). PREDIMED was a multicenter, nutritional intervention RCT carried out in Spain from 2003 to 2011. The study was funded by the official Spanish agency for scientific research, Instituto de Salud Carlos III¹². A web site (www.predimed.es) and the supplemental

material published together with the final results⁴⁶ provide full details of the study protocol. We selected participants from >200 primary care facilities affiliated with 11 recruiting sites. All participants were at high risk for CVD, but had no history of previous CVD episodes at enrolment. Criteria for recruitment were the presence of either type 2 diabetes mellitus (T2DM) or ≥ 3 risk factors (smoking, overweight or obesity, hypertension/HTN, dyslipidemia/DLP, and family history of early-onset CVD). Participants were randomized into one of three diets: 1) MeDiet supplemented with extra-virgin olive oil (EVOO) ;2) MeDiet supplemented with nuts; and 3) control diet (advice on a low-fat diet).

Full-time registered dietitians delivered the intervention. Throughout the study, participants attended quarterly individual visits and group sessions in which they were instructed to follow the allocated diets. Participants also attended quarterly group sessions where they received written material with information on key Mediterranean foods and seasonal shopping lists, menus and specific recipes for a typical week. This material was discussed in detail with the dietitians. Allotments of EVOO (1 L per week, including a minimum of 50 mL/day for participants and the rest for family needs) or mixed nuts (30 g/day: 15 g walnuts, 7.5 g almonds and 7.5 g hazelnuts plus extra allocations for the family) were supplied at no cost to each participant randomly assigned to the MeDiet groups on a quarterly basis during the group sessions with dietitians. Participants in the control diet group attended similar quarterly sessions with explanations and written material on the low-fat diet and they received non-food gifts in these sessions. The three diets were energy-unrestricted and no intervention on physical activity was conducted.

A validated 14-point MeDiet screener⁴⁷ was used by dietitians as a tool to both assess actual adherence to the MeDiet and enhance future adherence. These 14 items were:

1. Use of olive oil as the main culinary fat

2. Consumption of ≥ 4 tablespoons/d of olive oil (including oil used for frying, salads, out-of-house meals, etc.)
3. Consumption of ≥ 2 servings/d of vegetables
4. Consumption of ≥ 3 servings/d of fruits
5. Consumption of < 1 serving/d of red meat, hamburger or meat products (ham, sausage, etc.)
6. Consumption of < 1 serving/d of butter, margarine, or cream
7. Consumption of < 1 serving/d of sweetened and/or carbonated beverages
8. Consumption of ≥ 1 serving/d of wine
9. Consumption of ≥ 3 servings/week of legumes
10. Consumption of ≥ 3 servings/week of fish or shellfish
11. Consumption of < 3 servings/week of commercial sweets or pastries (not homemade), such as cakes, cookies, biscuits or custard
12. Consumption of ≥ 3 servings/week of nuts (including peanuts)
13. Preferential consumption of chicken, turkey or rabbit meat instead of veal, pork, hamburger or sausage
14. Consumption of ≥ 2 servings/week of sofrito, a sauce made with tomato and onion, leek or garlic and simmered with olive oil.

Validated food frequency questionnaires covering 137 foods were collected yearly by the dietitians. This repeated collection of dietary data allowed us to use the PREDIMED trial as a unique setting for subsequent cohort studies analyzed as a prospective observational follow-up study with repeated measurements of diet, thus improving the quality of our dietary assessment¹⁰.

Fasting blood and spot urine were obtained and serum, plasma and DNA samples were saved. Objective biomarkers of adherence to the supplemental foods (urinary hydroxytyrosol as

marker of EVOO consumption and plasma α -linolenic acid as marker of walnut consumption) were determined in random sub-samples.

The pre-specified primary end-point of the trial was incident CVD (a composite of non-fatal myocardial infarction/MI, non-fatal stroke or CVD death). This composite event occurred in 288 participants during a median follow-up of 4.8 years. The trial was neither powered nor designed to independently assess each of the three components of the combined end-point. Secondary outcomes included total mortality, T2DM, metabolic syndrome (MetS), peripheral arterial disease (PAD), atrial fibrillation (AF), neurodegenerative diseases and major cancers. An event adjudication committee, whose members were blinded to group allocation, was responsible for event ascertainment. All participants provided written informed consent and the protocol was approved by the Institutional Review Boards of all participating centers.

Main results of the PREDIMED trial

We randomized 7447 participants into the three PREDIMED intervention groups. The groups were well-balanced with respect to their baseline characteristics and pharmacologic treatments. Though small, between-group differences in some baseline characteristics were observed, but they were not clinically meaningful. Furthermore, we adjusted all risk estimates for these variables. The mean age of participants was 67 years, 57% were women and the mean body mass index was 30 kg/m². The baseline prevalence of diabetes was nearly 50% and the prevalence of DLP and HTN was higher than 70% and 80%, respectively.

Compliance with the intervention in the two MeDiet groups was adequate⁴⁸. Our tracking of objective biomarkers in random participant subsamples also indicated compliance with the intended dietary intervention. However, the achieved absolute difference in adherence to the MeDiet (according to the 14-item screener) between the intervention group and the control group was modest, amounting to a maintained difference of 2 points out of 14.

There were no between-group differences in physical activity during the study. No diet-related adverse effects occurred.

We assessed the effect of baseline adherence to the 14-point score with respect to the subsequent incidence of the primary CVD end-point during follow-up⁴⁹. As shown in Figure 1, the effect was remarkable. The multivariable-adjusted hazard ratio for participants with a baseline 14-item screener in the 2nd-3rd quintile who scored between 8-9 points was 0.72 (95% confidence interval [CI]: 0.55-0.94), and for those with the highest adherence (two upper quintiles, scoring 10-14 points) it was 0.47 (CI: 0.35-0.65).

The observed rates per 1000 person-years for the primary end point were 8.1, 8.0, and 11.2 in the MeDiet+EVOO, MeDiet+nuts, and control groups, respectively. The unadjusted hazard ratios were 0.70 (CI, 0.53-0.91) for the MeD+EVOO and 0.70 (CI, 0.53-0.94) for the MeDiet+nuts. The relative risk reductions, absolute risk reductions and number needed to treat are shown in Table 2 after multivariable adjustment for sex, age, adiposity variables, and baseline CVD risk factors. No effect on all-cause mortality was apparent. Significant disease risk reductions were also observed for incident T2DM (in the subset of participants initially free of T2DM)^{50,51} and for other CVD outcomes, such as PAD⁵² and AF⁵³. Hence, the PREDIMED study showed with a RCT design for the first time that a MeDiet supplemented with either EVOO or nuts is useful in the primary prevention of CVD, PAD, AF, and T2DM in individuals at high risk.

A beneficial effect of the intervention on MetS status was also observed in the PREDIMED trial^{54,55}. In comparison with the control group, participants randomized to either MeDiet were more likely to show reversion of MetS, with HR 1.35 (CI 1.15–1.58) for the MeDiet+EVOO, and HR 1.28 (CI 1.08–1.51) for the MeDiet+nuts. Similarly, the PREDIMED MeDiet interventions were shown to reduce blood pressure and the risk of HTN^{56,57} and to slow the

progression of subclinical atherosclerosis, as determined by changes in ultrasound-assessed carotid intima-media thickness and plaque^{58,59}.

Number of events hypothetically prevented with the Mediterranean diet

Table 3 shows the number of hard clinical CVD events that could be prevented in a hypothetical cohort of 1,000 persons undergoing the nutritional intervention with the MeDiet used in the PREDIMED trial. These results suggest that even a modest intervention with the MeDiet has the potential to account for a sizable reduction in the number of clinical events in a relatively short period of time.

Observational studies and trials in the context of the PREDIMED trial

Although some authors have suggested that RCTs with hard clinical events as end-points are the only solution to circumventing the problems of measurement error inherent to observational designs in nutritional epidemiology, trials are far from perfect, and also present considerable limitations¹⁰. These problems include the frequent suboptimal compliance, losses to follow-up, and the ethical need to prematurely halt the trial when there is sufficient evidence of benefit, even when the number of observed events is lower than anticipated. In addition, some degree of contamination of the control group with aspects of the intervention intended only to the active intervention arms of the trial is unavoidable. Moreover some exposures or outcomes cannot be assessed with RCTs.

In this context, the symbiosis between properly designed RCTs and large cohort studies with appropriate and careful control of potentially confounding variables, and due precautions to improve dietary measurements, are currently the best possible option to ascertain the health effects of dietary exposures. Adequately designed and tested food frequency questionnaires (FFQs) have been shown to have acceptable validity when

compared to reference measures. In addition, adjustment for total energy intake, usually applying the residuals method along with the use of repeated FFQs in long-term prospective cohort studies, further improves their validity estimates^{9,10}.

The availability of validated FFQs of each participant with yearly repeated measurements is a unique strength of the PREDIMED trial⁶⁰. Furthermore, biomarker analyses have corroborated the validity of our dietary assessment tools⁶¹. Most follow-up studies have collected measurements of dietary intake only at baseline and this is a limitation in nutritional epidemiology because diet may change during follow-up. The PREDIMED study has provided a large body of evidence on the associations between diet and diverse health outcomes taking advantage of the validated FFQs⁶²⁻⁹⁹.

Mechanisms of protection by the Mediterranean diet

CVD protection by the MeDiet can be explained by a beneficial effect on classical and emergent CV risk factors^{56,100-102}. Although the underlying mechanisms of protection against CVD by the MeDiet are not fully understood, the richness of this dietary pattern in antioxidant¹⁰² and anti-inflammatory molecules⁹⁸ is likely to be relevant. On one hand, this can be due to their antioxidant capacity, such as cell redox state modulating enzyme systems. On the other hand, nutrients have the capacity of modulating gene and protein expression and, subsequently, metabolite production. Previous nutrigenomic studies have revealed that the MeDiet has a protective effect on the expression of several proatherogenic genes involved in vascular inflammation, foam cell formation, and thrombosis¹⁰³⁻¹⁰⁴.

Genomics and the Mediterranean diet

We investigated whether the effects of the MedDiet or its components might differ depending on genetic variants. We found several gene-diet interactions in determining both intermediate and CVD phenotypes¹⁰⁵⁻¹⁰⁹. Suffice it to say that we observed that the association of the MC4R rs17782313 or the FTO rs9939609 polymorphisms with T2DM were modulated by the MedDiet¹⁰⁷. When adherence to the MedDiet was low (<9 out of 14 points), carriers of the variant alleles had higher T2DM risk than wild-type subjects. However, when adherence to the MedDiet was high (≥ 9 points), these associations disappeared. These gene-diet interactions remained after adjustment for BMI. Adherence to the MedDiet was found to interact with the TCF7L2-rs7903146 (C>T) polymorphism in relation to fasting glucose, total cholesterol, low-density lipoprotein cholesterol and triglycerides¹⁰⁸. When adherence to the MedDiet was low, participants with the TT genotype had higher fasting glucose concentrations and lipids than CC+CT individuals but when adherence was high, these differences were not apparent. Moreover, TT subjects had a higher stroke incidence in the control group compared with CC, whereas the dietary intervention with MedDiet was associated with reduced stroke incidence in TT homozygotes but not CC homozygotes¹⁰⁸. Both genetic and epigenetic effects on microRNA target site polymorphisms were also analyzed. A gain-of-function microRNA-410 target site polymorphism (rs13702T>C) in the lipoprotein lipase gene, interacted with the MedDiet intervention in the association with triglyceride levels and stroke incidence¹¹⁰. The interplay between genetic and epigenetic factors may contribute to better understand some biological mechanisms underlying CVD progression. Overall these results highlight the relevance of the multi-level omics approaches to a more comprehensive investigation of the mechanisms accounting for the MedDiet protective effects.

Conclusions

The findings from the PREDIMED trial, the Lyon Diet-Heart trial, and many large prospective cohorts are fully consistent. These large observational and experimental studies are also supported by mechanistic investigations aimed to assess classical and emergent CVD risk factors and pathophysiological pathways. Anti-inflammatory effects and reduced oxidative stress are very likely explanations for the protection observed in the PREDIMED trial. Taken together, these research findings converge, demonstrating that the traditional MeDiet offers an affordable, attractive, and easily achievable protection against CVD.

Importantly, these findings suggest that an overall dietary pattern that is rich in high-unsaturated fat from natural vegetable sources is preferable for CV health than a low-fat diet. In addition, the MeDiet has been shown to effectively control the residual risk observed after standard pharmacologic treatment of DLP anomalies and HTN in high-risk individuals. Taking into account the advanced age of many participants in the PREDIMED trial and in some of the available cohorts, it can be concluded that it is never too late to improve the food pattern to improve CV health.

References

1. Murray CJ1, Vos T, Lozano R, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380(9859):2197-223.
2. Mathers CD, Longcar D. Projections of Global Mortality and Burden of Disease from 2002 to 2030. *PLoS Med*. 2006;3(11):e442.
3. Labarthe DR, Dunbar SB. Global cardiovascular health promotion and disease prevention: 2011 and beyond. *Circulation* 2012;125(21):2667-76.
4. Chomistek AK, Chiuve SE, Eliassen AH, Mukamal KJ, Willett WC, Rimm EB. Healthy lifestyle in the primordial prevention of cardiovascular disease among young women. *J Am Coll Cardiol*. 2015;65(1):43-51.
5. Lloyd-Jones DM, Hong Y, Labarthe D, et al. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. *Circulation*. 2010;121(4):586-613.
6. Chiuve SE, Cook NR, Shay CM, Rexrode KM, Albert CM, Manson JE, Willett WC, Rimm EB. Lifestyle-based prediction model for the prevention of CVD: the Healthy Heart Score. *J Am Heart Assoc*. 2014;3(6):e000954.
7. Mozaffarian D, Appel LJ, Van Horn L. Components of a cardioprotective diet: new insights. *Circulation*. 2011;123(24):2870-91.
8. Martinez-Gonzalez MA, Bes-Rastrollo M. Nutrition and cardiovascular disease. In: Rothkopf MM, Nusbaum MJ, Haverstick LP (eds): *Metabolic Medicine and Surgery*. N. York: CRC Press, 2014.
9. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol*. 2002;13(1):3-9.
10. Satija A, Yu E, Willett WC, Hu FB. Understanding nutritional epidemiology and its role in policy. *Adv Nutr*. 2015;6(1):5-18.
11. Smith R. Are some diets "mass murder"? *BMJ*. 2014;349(1):g7654.
12. Martínez-González MÁ, Corella D, Salas-Salvadó J, et al. Cohort profile: design and methods of the PREDIMED study. *Int J Epidemiol*. 2012;41(2):377-85.
13. Maderuelo-Fernandez JA, Recio-Rodríguez JI, Patino-Alonso MC, Pérez-Arechaederra D, Rodriguez-Sanchez E, Gomez-Marcos MA, García-Ortiz L. Effectiveness of interventions applicable to primary health care settings to promote Mediterranean diet or healthy eating adherence in adults: A systematic review. *Prev Med*. 2014 Dec 16. [Epub ahead of print].
14. Widmer RJ, Flammer AJ, Lerman LO, Lerman A. "The Mediterranean Diet, its Components, and Cardiovascular Disease". *Am J Med*. 2014 Oct 15. [Epub ahead of print]
15. Schwingshackl L, Missbach B, König J, Hoffmann G. Adherence to a Mediterranean diet and risk of diabetes: a systematic review and meta-analysis. *Public Health Nutr*. 2014 Aug 22. [Epub ahead of print]
16. Koloverou E, Esposito K, Giugliano D, Panagiotakos D. The effect of Mediterranean diet on the development of type 2 diabetes mellitus: a meta-analysis of 10 prospective studies and 136,846 participants. *Metabolism*. 2014;63(7):903-11.
17. Ros E, Martínez-González MA, Estruch R, Salas-Salvadó J, Fitó M, Martínez JA, Corella D. Mediterranean diet and cardiovascular health: Teachings of the PREDIMED study. *Adv Nutr*. 2014;5(3):330S-6S.

18. Schwingshackl L, Hoffmann G. Mediterranean dietary pattern, inflammation and endothelial function: a systematic review and meta-analysis of intervention trials. *Nutr Metab Cardiovasc Dis.* 2014;24(9):929-39.
19. Whayne TF Jr. Ischemic heart disease and the Mediterranean diet. *Curr Cardiol Rep.* 2014;16(6):491.
20. Georgoulis M, Kontogianni MD, Yiannakouris N. Mediterranean diet and diabetes: prevention and treatment. *Nutrients.* 2014;6(4):1406-23.
21. Sofi F, Macchi C, Abbate R, Gensini GF, Casini A. Mediterranean diet and health status: an updated meta-analysis and a proposal for a literature-based adherence score. *Public Health Nutr.* 2014;17(12):2769-82.
22. Martinez-Gonzalez MA, Bes-Rastrollo M. Dietary patterns, Mediterranean diet, and cardiovascular disease. *Curr Opin Lipidol.* 2014;25(1):20-6. Erratum in: *Curr Opin Lipidol.* 2014;25(4):326.
23. Esposito K, Giugliano D. Mediterranean diet and type 2 diabetes. *Diabetes Metab Res Rev.* 2014;30(Suppl 1):34-40.
24. Grosso G, Mistretta A, Frigiola A, Gruttadauria S, Biondi A, Basile F, Vitaglione P, D'Orazio N, Galvano F. Mediterranean diet and cardiovascular risk factors: a systematic review. *Crit Rev Food Sci Nutr.* 2014;54(5):593-610.
25. Esposito K, Kastorini CM, Panagiotakos DB, Giugliano D. Mediterranean diet and metabolic syndrome: an updated systematic review. *Rev Endocr Metab Disord.* 2013;14(3):255-63.
26. Rees K, Hartley L, Flowers N, Clarke A, Hooper L, Thorogood M, Stranges S. 'Mediterranean' dietary pattern for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev.* 2013;8:CD009825.
27. Psaltopoulou T, Sergentanis TN, Panagiotakos DB, Sergentanis IN, Kosti R, Scarmeas N. Mediterranean diet, stroke, cognitive impairment, and depression: A meta-analysis. *Ann Neurol.* 2013;74(4):580-91.
28. Nordmann AJ, Suter-Zimmermann K, Bucher HC, Shai I, Tuttle KR, Estruch R, Briel M. Meta-analysis comparing Mediterranean to low-fat diets for modification of cardiovascular risk factors. *Am J Med.* 2011;124(9):841-51.e2.
29. Bulló M, Lamuela-Raventós R, Salas-Salvadó J. Mediterranean diet and oxidation: nuts and olive oil as important sources of fat and antioxidants. *Curr Top Med Chem.* 2011;11(14):1797-810.
30. Kastorini CM, Milionis HJ, Esposito K, Giugliano D, Goudevenos JA, Panagiotakos DB. The effect of Mediterranean diet on metabolic syndrome and its components: a meta-analysis of 50 studies and 534,906 individuals. *J Am Coll Cardiol.* 2011;57(11):1299-313.
31. Esposito K, Kastorini CM, Panagiotakos DB, Giugliano D. Mediterranean diet and weight loss: meta-analysis of randomized controlled trials. *Metab Syndr Relat Disord.* 2011;9(1):1-12.
32. Sofi F, Abbate R, Gensini GF, Casini A. Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. *Am J Clin Nutr.* 2010;92(5):1189-96.
33. Esposito K, Maiorino MI, Ceriello A, Giugliano D. Prevention and control of type 2 diabetes by Mediterranean diet: a systematic review. *Diabetes Res Clin Pract.* 2010;89(2):97-102.
34. Tyrovolas S, Panagiotakos DB. The role of Mediterranean type of diet on the development of cancer and cardiovascular disease, in the elderly: a systematic review. *Maturitas.* 2010;65(2):122-30.

35. Martinez-Gonzalez MA, Bes-Rastrollo M, Serra-Majem L, Lairon D, Estruch R, Trichopoulou A. Mediterranean food pattern and the primary prevention of chronic disease: recent developments. *Nutr Rev.* 2009;67(Suppl 1):S111-6.
36. de Lorgeril M, Salen P. The Mediterranean diet: rationale and evidence for its benefit. *Curr Atheroscler Rep.* 2008;10(6):518-22.
37. Sofi F, Cesari F, Abbate R, Gensini GF, Casini A. Adherence to Mediterranean diet and health status: meta-analysis. *BMJ.* 2008;337:a1344.
38. Buckland G, Bach A, Serra-Majem L. Obesity and the Mediterranean diet: a systematic review of observational and intervention studies. *Obes Rev.* 2008;9(6):582-93.
39. Roman B, Carta L, Martínez-González MA, Serra-Majem L. Effectiveness of the Mediterranean diet in the elderly. *Clin Interv Aging.* 2008;3(1):97-109.
40. Serra-Majem L, Roman B, Estruch R. Scientific evidence of interventions using the Mediterranean diet: a systematic review. *Nutr Rev.* 2006;64(2 Pt 2):S27-47.
41. Martinez-Gonzalez MA, Estruch R. Mediterranean diet, antioxidants and cancer: the need for randomized trials. *Eur J Cancer Prev.* 2004;13(4):327-35.
42. Panagiotakos DB, Pitsavos C, Polychronopoulos E, Chrysohoou C, Zampelas A, Trichopoulou A. Can a Mediterranean diet moderate the development and clinical progression of coronary heart disease? A systematic review. *Med Sci Monit.* 2004;10(8):RA193-8.
43. Trichopoulou A, Vasilopoulou E. Mediterranean diet and longevity. *Br J Nutr.* 2000;84(Suppl 2):S205-9.
44. Trichopoulou A, Martínez-González MA, Tong TY, Forouhi NG, Khandelwal S, Prabhakaran D, Mozaffarian D, de Lorgeril M. Definitions and potential health benefits of the Mediterranean diet: views from experts around the world. *BMC Med.* 2014;12:112.
45. De Lorgeril M, Salen P, Martin JL, Monajud I, et al. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: Final report of the Lyon Diet Heart Study. *Circulation.* 1999;99(6):779-785.
46. Estruch R, Ros E, Salas-Salvadó J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med.* 2013;368(14):1279-90.
47. Schröder H, Fitó M, Estruch R, et al. A short screener is valid for assessing Mediterranean Diet adherence among older Spanish men and women. *J Nutr.* 2011;141(6):1140-5.
48. Zazpe I, Sanchez-Tainta A, Estruch R, et al. A large randomized individual and group intervention conducted by registered dietitians increased the adherence to Mediterranean-type diets: the PREDIMED study. *J Am Diet Assoc.* 2008;108(7):1134-44.
49. Schröder H, Salas-Salvadó J, Martínez-González MA, Fito M, Corella D, Estruch R, Ros E. Baseline Adherence to the Mediterranean Diet and Major Cardiovascular Events: PREDIMED Trial. *JAMA Intern Med.* 2014;174(6):1690-2.
50. Salas-Salvadó J, Bulló M, Estruch R, et al. Prevention of diabetes with Mediterranean diets: A subgroup analysis of a randomized trial. *Ann Intern Med.* 2014;160(1):1-10.
51. Salas-Salvadó J, Bulló M, Babio N, et al. Reduction in the incidence of type 2 diabetes with the Mediterranean diet: results of the PREDIMED-Reus nutrition intervention randomized trial. *Diabetes Care.* 2011;34(1):14-9.

52. Martínez-González MÁ, Toledo E, Arós F, et al. Extravirgin olive oil consumption reduces risk of atrial fibrillation: the PREDIMED (Prevención con Dieta Mediterránea) trial. *Circulation*. 2014;130(1):18-26.
53. Ruiz-Canela M, Estruch R, Corella D, Salas-Salvadó J, Martínez-González MA. Mediterranean diet inversely associated with peripheral artery disease: the PREDIMED randomized trial. *JAMA*. 2014;311(4):415-7.
54. Babio N, Toledo E, Estruch R, et al. Mediterranean diets and metabolic syndrome status in the PREDIMED randomized trial. *CMAJ*. 2014;186(17):649-57.
55. Salas-Salvadó J, Fernández-Ballart J, Ros E, et al. Effect of a Mediterranean diet supplemented with nuts on metabolic syndrome status: one-year results of the PREDIMED randomized trial. *Arch Intern Med*. 2008;168(22):2449-58.
56. Toledo E, Hu FB, Estruch R, et al. Effect of the Mediterranean diet on blood pressure in the PREDIMED trial: results from a randomized controlled trial. *BMC Med*. 2013;11:207.
57. Doménech M, Roman P, Lapetra J, et al. Mediterranean diet reduces 24-hour ambulatory blood pressure, blood glucose, and lipids: one-year randomized, clinical trial. *Hypertension*. 2014;64(1):69-76.
58. Murie-Fernandez M, Irimia P, Toledo E, et al. Carotid intima-media thickness changes with Mediterranean diet: a randomized trial (PREDIMED-NAVARRA). *Atherosclerosis*. 2011;219(1):158-62.
59. Sala-Vila A, Romero-Mamani ES, Gilabert R, et al. Changes in ultrasound-assessed carotid intima-media thickness and plaque with a Mediterranean diet: a substudy of the PREDIMED trial. *Arterioscler Thromb Vasc Biol*. 2014;34(2):439-45.
60. Fernández-Ballart JD, Piñol JL, Zazpe I, Corella D, Carrasco P, Toledo E, Perez-Bauer M, Martínez-González MA, Salas-Salvadó J, Martín-Moreno JM. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. *Br J Nutr*. 2010;103(12):1808-16.
61. Zamora-Ros R, Urpí-Sardà M, Lamuela-Raventós RM, Estruch R, Martínez-González MA, Bulló M, Arós F, Cherubini A, Andres-Lacueva C. Resveratrol metabolites in urine as a biomarker of wine intake in free-living subjects: The PREDIMED Study. *Free Radic Biol Med*. 2009;46(12):1562-6.
62. Martínez-González MA, Zazpe I, Razquin C, et al. Empirically-derived food patterns and the risk of total mortality and cardiovascular events in the PREDIMED study. *Clin Nutr*. 2014 Sep 16 [Epub ahead of print].
63. Castro-Quezada I, Sánchez-Villegas A, Martínez-González MA, et al. A high dietary glycemic index increases total mortality in a Mediterranean population at high cardiovascular risk. *PLoS One*. 2014;9(9):e107968.
64. Guasch-Ferré M, Hu FB, Martínez-González MA, et al. Olive oil intake and risk of cardiovascular disease and mortality in the PREDIMED Study. *BMC Med*. 2014;12:78.
65. Tresserra-Rimbau A, Rimm EB, Medina-Remón A, et al. Polyphenol intake and mortality risk: a re-analysis of the PREDIMED trial. *BMC Med*. 2014;12:77.
66. Martínez-González MA, Sánchez-Tainta A, Corella D, et al. A provegetarian food pattern and reduction in total mortality in the Prevención con Dieta Mediterránea (PREDIMED) study. *Am J Clin Nutr*. 2014;100(Supplement 1):320S-328S.
67. Juanola-Falgarona M, Salas-Salvadó J, Martínez-González MÁ, et al. Dietary intake of vitamin K is inversely associated with mortality risk. *J Nutr*. 2014;144(5):743-50.

68. Tresserra-Rimbau A, Rimm EB, Medina-Remón A, et al. Inverse association between habitual polyphenol intake and incidence of cardiovascular events in the PREDIMED study. *Nutr Metab Cardiovasc Dis.* 2014;24(6):639-47.
69. Guasch-Ferré M, Bulló M, Estruch R, et al. Dietary magnesium intake is inversely associated with mortality in adults at high cardiovascular disease risk. *J Nutr.* 2014;144(1):55-60.
70. Fernandez-Cao JC, Arija V, Aranda N, Bullo M, Basora J, Martínez-González MA, Díez-Espino J, Salas-Salvadó J. Heme iron intake and risk of new-onset diabetes in a Mediterranean population at high risk of cardiovascular disease an observational cohort analysis. *BMC Public Health.* 2013;13:1042.
71. Gea A, Beunza JJ, Estruch R, et al. Alcohol intake, wine consumption and the development of depression: the PREDIMED study. *BMC Med.* 2013;11:192.
72. Guasch-Ferré M, Bulló M, Martínez-González MÁ, et al. Frequency of nut consumption and mortality risk in the PREDIMED nutrition intervention trial. *BMC Med.* 2013;11:164.
73. Hu EA, Toledo E, Diez-Espino J, et al. Lifestyles and risk factors associated with adherence to the Mediterranean diet a baseline assessment of the PREDIMED trial. *PLoS One.* 2013;8(4) e60166.
74. Ibarrola-Jurado N, Bulló M, Guasch-Ferré M, et al. Cross-sectional assessment of nut consumption and obesity, metabolic syndrome and other cardiometabolic risk factors the PREDIMED study. *PLoS One.* 2013;8(2):e57367.
75. Tresserra-Rimbau A, Medina-Remón A, Pérez-Jiménez J, et al. Dietary intake and major food sources of polyphenols in a Spanish population at high cardiovascular risk the PREDIMED study. *Nutr Metab Cardiovasc Dis.* 2013;23(10):953-9.
76. Juanola-Falgarona M, Salas-Salvadó J, Estruch R, Portillo MP, Casas R, Miranda J, Martínez-González MA, Bulló M. Association between dietary phyloquinone intake and peripheral metabolic risk markers related to insulin resistance and diabetes in elderly subjects at high cardiovascular risk. *Cardiovasc Diabetol.* 2013;12:7.
77. Bautista-Castaño I, Sánchez-Villegas A, Estruch R, et al. Changes in bread consumption and 4-year changes in adiposity in Spanish subjects at high cardiovascular risk. *Br J Nutr.* 2013;110(2):337-46.
78. Medina-Remón A, Vallverdú-Queralt A, Arranz S, et al. Gazpacho consumption is associated with lower blood pressure and reduced hypertension in a high cardiovascular risk cohort. Cross-sectional study of the PREDIMED trial. *Nutr Metab Cardiovasc Dis.* 2013;23(10):944-52.
79. Díaz-López A, Bulló M, Basora J, et al. Cross-sectional associations between macronutrient intake and chronic kidney disease in a population at high cardiovascular risk. *Clin Nutr.* 2013;32(4):606-12.
80. Valls-Pedret C, Lamuela-Raventós RM, Medina-Remón A, et al. Polyphenol-rich foods in the Mediterranean diet are associated with better cognitive function in elderly subjects at high cardiovascular risk. *J Alzheimer Dis.* 2012;29(4):773-82.
81. Bulló M, Casas R, Portillo MP, et al. Dietary glycemic index/load and peripheral adipokines and inflammatory markers in elderly subjects at high cardiovascular risk. *Nutr Metab Cardiovasc Dis.* 2013;23(5):443-50.
82. Bulló M, Garcia-Aloy M, Martínez-González MA, et al. Association between a healthy lifestyle and general obesity and abdominal obesity in an elderly population at high cardiovascular risk. *Prev Med.* 2011;53(3):155-61.

83. Buil-Cosiales P, Zazpe I, Toledo E, et al. Fiber intake and all-cause mortality in the Prevención con Dieta Mediterránea (PREDIMED) study. *Am J Clin Nutr* 2014;100(6):1498-507.
83. Schröder H, Fitó M, Estruch R, et al. A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. *J Nutr*. 2011;141(6):1140-5.
84. Babio N, Sorlí M, Bulló M, et al. Association between red meat consumption and metabolic syndrome in a Mediterranean population at high cardiovascular risk cross-sectional and 1-year follow-up assessment. *Nutr Metab Cardiovasc Dis*. 2012;22(3):200-7.
85. Lohse B, Psota T, Estruch R, et al. Eating competence of elderly Spanish adults is associated with a healthy diet and a favorable cardiovascular disease risk profile. *J Nutr*. 2010;140(7):1322-7.
86. Casas-Agustench P, Bulló M, Ros E, Basora J, Salas-Salvadó J. Cross-sectional association of nut intake with adiposity in a Mediterranean population. *Nutr Metab Cardiovasc Dis*. 2011;21(7):518-25.
87. Prieto RM, Fiol M, Perello J, Estruch R, Ros E, Sanchis P, Grases F. Effects of Mediterranean diets with low and high proportions of phytate-rich foods on the urinary phytate excretion. *Eur J Nutr*. 2010;49(6):321-6.
88. Zazpe I, Estruch R, Toledo E, et al. Predictors of adherence to a Mediterranean-type diet in the PREDIMED trial. *Eur J Nutr*. 2010;49(2):91-9.
89. Medina-Remón A, Tresserra-Rimbau A, Pons A, et al. Effects of total dietary polyphenols on plasma nitric oxide and blood pressure in a high cardiovascular risk cohort. The PREDIMED randomized trial. *Nutr Metab Cardiovasc Dis*. 2015;25(1):60-7.
90. Schröder H, de la Torre R, Estruch R, et al. Alcohol consumption is associated with high concentrations of urinary hydroxytyrosol. *Am J Clin Nutr*. 2009;90(5):1329-35.
91. Buil-Cosiales P, Irimia P, Ros E, et al. Dietary fibre intake is inversely associated with carotid intima-media thickness a cross-sectional assessment in the PREDIMED study. *Eur J Clin Nutr*. 2009;63(10):1213-9.
92. Escurriol V, Cofán M, Serra M, et al. Serum sterol responses to increasing plant sterol intake from natural foods in the Mediterranean diet. *Eur J Nutr*. 2009;48(6):373-82.
93. Estruch R, Martínez-González MA, Corella D, et al. Effects of dietary fibre intake on risk factors for cardiovascular disease in subjects at high risk. *J Epidemiol Community Health*. 2009;63(7):582-8.
95. Rodríguez-Rejón AI, Castro-Quezada I, Ruano-Rodríguez C, et al. Effect of a Mediterranean Diet Intervention on Dietary Glycemic Load and Dietary Glycemic Index The PREDIMED Study. *J Nutr Metab*. 2014;2014(1):985373 .
96. Sánchez-Taínta A, Estruch R, Bulló M, et al. Adherence to a Mediterranean-type diet and reduced prevalence of clustered cardiovascular risk factors in a cohort of 3,204 high-risk patients. *Eur J Cardiovasc Prev Rehabil*. 2008;15(5):589-93.
97. Toledo E, Delgado-Rodríguez M, Estruch R, et al. Low-fat dairy products and blood pressure follow-up of 2290 older persons at high cardiovascular risk participating in the PREDIMED study. *Br J Nutr*. 2009;101(1):59-67.
98. Salas-Salvadó J, Garcia-Arellano A, Estruch R, et al. Components of the Mediterranean-type food pattern and serum inflammatory markers among patients at high risk for cardiovascular disease. *Eur J Clin Nutr*. 2008;62(5):651-9.

99. Buil-Cosiales P, Irimia P, Berrade N, Garcia-Arellano A, Riverol M, Murie-Fernández M, Martínez-Vila E, Martínez-González MA, Serrano-Martínez M. Carotid intima-media thickness is inversely associated with olive oil consumption. *Atherosclerosis*. 2008;196(2):742-8.
100. Estruch R, Martínez-González MA, Corella D, et al. Effects of a Mediterranean-style diet on cardiovascular risk factors. *Ann Intern Med*. 2006;145:1-11.
101. Damasceno NRT, Sala-Vila A, Cofán M, et al. Mediterranean diet supplemented with nuts reduces waist circumference and shifts lipoprotein subfractions to a less atherogenic pattern in subjects at high cardiovascular risk. *Atherosclerosis* 2013;230(2):347-353
102. Fitó M, Guxens M, Corella D, et al. Effect of a traditional Mediterranean diet on lipoprotein oxidation: a randomized controlled trial. *Arch Intern Med*. 2007;167(11):1195-203.
103. Konstantinidou V, Covas MI, Muñoz-Aguayo D, et al. In vivo nutrigenomic effects of virgin olive oil polyphenols within the frame of the Mediterranean diet: a randomized controlled trial. *FASEB J*. 2010;24(7):2546-57.
104. Llorente-Cortés V, Estruch R, Mena MP, Ros E, Martínez-González MA, Fitó M, Lamuela-Raventós RM, Badimon L. Effect of Mediterranean diet on the expression of proatherogenic genes in a population at high cardiovascular risk. *Atherosclerosis*. 2010;208(2):442-50
105. Corella D, González JI, Bulló M, et al. Polymorphisms cyclooxygenase-2 -765G>C and interleukin-6 -174G>C are associated with serum inflammation markers in a high cardiovascular risk population and do not modify the response to a Mediterranean diet supplemented with virgin olive oil or nuts. *J Nutr*. 2009;139(1):128-34.
106. Corella D, Ortega-Azorín C, Sorlí JV, et al. Statistical and biological gene-lifestyle interactions of MC4R and FTO with diet and physical activity on obesity: new effects on alcohol consumption. *PLoS One*. 2012;7(12):e52344.
107. Ortega-Azorín C, Sorlí JV, Asensio EM, et al. Associations of the FTO rs9939609 and the MC4R rs17782313 polymorphisms with type 2 diabetes are modulated by diet, being higher when adherence to the Mediterranean diet pattern is low. *Cardiovasc Diabetol*. 2012;11(1):137.
108. Corella D, Carrasco P, Sorlí JV, et al. Mediterranean diet reduces the adverse effect of the TCF7L2-rs7903146 polymorphism on cardiovascular risk factors and stroke incidence: A randomized controlled trial in a high-cardiovascular-risk population. *Diabetes Care*. 2013;36(11):3803-11.
109. Ortega-Azorín C, Sorlí JV, Estruch R, et al. Amino acid change in the carbohydrate response element binding protein is associated with lower triglycerides and myocardial infarction incidence depending on level of adherence to the Mediterranean diet in the PREDIMED trial. *Circ Cardiovasc Genet*. 2014;7(1):49-58.
110. Corella D, Sorlí JV, Estruch R, et al. MicroRNA-410 regulated lipoprotein lipase variant rs13702 is associated with stroke incidence and modulated by diet in the randomized controlled PREDIMED trial. *Am J Clin Nutr*. 2014;100(2):719-731.

Acknowledgements

All authors have read and approved the final manuscript. CIBERObn is an initiative of ISCIII, Spain.

Legends

Figure 1. Association between baseline adherence to the Mediterranean diet and risk of major cardiovascular events (primary end-point) in the PREDIMED study.

Baseline adherence to the Mediterranean diet (14-point PREDIMED score) and incidence of the primary end-point in the PREDIMED trial (a composite of myocardial infarction, stroke or cardiovascular death).

Q1-Q5: quintiles.

Complete list of PREDIMED INVESTIGATORS

University of Navarra and Osasunbidea (Servicio Navarro de Salud), Primary Care Centres, Pamplona, Spain: P. Buil-Cosiales, J. Diez-Espino, E Toledo, A. Garcia-Arellano, M Ruiz-Canela, B. Sanjulian, A. Marti, F.J. Basterra-Gortari, V. Estremera-Urabayen, A Sola-Larraza, F Barcena-Amigo, C Oreja-Arrayago, M. Serrano-Martínez, MJ Lasanta-Saez, L Quintana-Pedraza, C Amezcua-Goñi, P Cia-Lecumberri, F Artal-Moneva, JM Esparza-López, E Figuerido-Garmendia, H Ruiz-Millan, R Oses-Primo, JA Tabar-Sarrias, L Fernandez-Urzainqui, MJ Ariz-Arnedo, T Forcen-Alonso, P Pascual-Pascual, ML Garces, V Extremera, L Garcia-Perez.

University Rovira i Virgili, Reus, Spain: N. Babio, M. Bullo, J. Basora, N. Gonzalez, C. Molina, F. Marquez, P. Martínez, N. Ibarrola, M. Sorli, J. García Roselló, A, Castro, F. Martin, N, Tort, A. Isach, M. Guasch-Ferre, M. Baldrich, J.J. Cabre, G. Mestres, F. Paris, M. Llauradó, R. Pedret, J. Basells, J. Vizcaino, and J. Fernandez-Ballart.

Institute de Recerca Hospital del Mar, Barcelona, Spain: M.I. Covas, S. Tello, J. Vila, R. de la Torre, D. Munoz-Aguayo, R. Elosua, J. Marrugat, and M. Ferrer.

University of Valencia, Valencia, Spain: J.V. Sorli, P. Carrasco, R. Osma, M. Guillen, P. Guillem-Saiz, O. Portoles, V. Pascual, C. Riera, J. Valderrama, A. Serrano, E. Lazaro, A. Sanmartin, A. Girbes, V. Santamaria, C. Sanchez, Z. Pla, and E.

University of Malaga, Malaga, Spain: E Gomez-Gracia, J Fernandez-Crehuet, J. Wärnberg, R. Benitez Pont, M. Bianchi Alba, R. Gomez-Huelgas, J. Martínez-Gonzalez, V. Velasco García, J. de Diego Salas, A. Baca Osorio, J. Gil Zarzosa, J.J. Sanchez Luque, and E. Vargas López.

Hospital Clinic, Institut d'Investigacions Biomediques August Pi i Sunyer, Barcelona, Spain: M. Serra, A. Perez-Heras, R. Sola, E. Ortega, C. Vinas, R. Casas, L. de Santamaria, S. Romero, J.M. Baena, M. García, M. Oller, J. Amat, I. Duaso, Y.

García, C. Iglesias, C. Simon, Ll. Quinzavos, Ll. Parra, M. Liroz, J. Benavent, J. Clos, I. Pla, M. Amoros, M.T. Bonet, M.T. Martin, M.S. Sanchez, J. Altirruba, E. Manzano, A. Altes, M. Cofan, C. Valls-Pedret, A. Sala-Vila, and M. Domenech.

University Hospital of Alava, Vitoria, Spain: F. Arós, I. Salaverria, T. del Hierro, J. Algorta, S. Francisco, A. Alonso, J. San Vicente, A. Casi, E. Sanz, I. Felipe, and A. Loma-Osorio.

Institute of Health Sciences IUNICS, University of Balearic Islands, and Hospital Son Espases, Palma de Mallorca, Spain: M Fiol, D Romaguera, M. García -Valdúeza, M. Monino, A. Proenza, R. Prieto, G. Frontera, M. Ginard, F. Fiol, A. Jover, D. Romaguera and J. García.

Department of Family Medicine, Primary Care Division of Sevilla, Sevilla, Spain: J. Lapetra, M. Leal, E. Martínez, M. Ortega-Calvo, P. Roman, F. Jose García, P. Iglesias, Y. Corchado, E. Mayoral, L. Mellado, L. Miró, JM. Lozano and C. Lama.

University of Las Palmas de Gran Canaria, Las Palmas, Spain: L. Serra-Majem, A. Sanchez-Villegas, J. Alvarez-Perez, E. Diez Benitez, I. Bautista Castaño, I. Maldonado Diaz, M.J. Fernández-Rodríguez, F. Sarmiento de la Fe, C. Simon García , I. Falcon Sanabria, B. Macias Gutierrez, and A.J. Santana Santana.

Hospital Universitario de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain: X. Pinto, E. de la Cruz, A. Galera, Y. Soler, F. Trias, I. Sarasa, E. Padres, and E. Corbella.

School of Pharmacy, University of Barcelona, Barcelona, Spain: R.M. Lamuela-Raventos, M.C. López- Sabater, A.I. Castellote-Bargallo, A. Medina-Reimon, and A. Tresserra-Rimbau.

Primary Care Division, Catalan Institute of Health, Barcelona, Spain: C. Cabezas, E. Vinyoles, M.A. Rovira, L. García , G. Flores, J.M. Verdu, P. Baby, A. Ramos, L.

Mengual, P. Roura, M.C. Yuste, A. Guarner, A. Rovira, M.I. Santamaria, M. Mata, C. de Juan, and A. Brau.

Other investigators of the PREDIMED network: J.A. Tur (University of Balearic Islands), V. Ruiz-Gutierrez (Instituto de la Grasa, Sevilla), M.P. Portillo (University of Basque Country) and G. Saez (University of Valencia). All in Spain.

Table 1. Scientific evidence on the Mediterranean diet.
Systematic reviews assessing the association between adherence to the Mediterranean diet and cardio-metabolic outcomes.

<u>Systematic review</u>	N (Studies)	Exposure	Outcome	Effects of increased adherence to MeDiet*	Comment
Esposito 2011 ³¹	16	MeDiet (randomized trials)	Weight loss	-1.75 kg (- 2.86; -0.64)	Greater weight loss with energy restriction and longer follow-up
Buckland 2008 ³⁸	21	MeDiet (randomized trials)	Weight loss	Beneficial (13 studies) No evidence (8 studies)	Qualitative systematic review
Nordmann 2011 ²⁸	6	MeDiet (randomized trials)	Risk factors	Beneficial	Reductions in BMI, BP, glucose and CRP
Grosso 2014 ²⁴	58	MeDiet	Risk factors	Beneficial	Qualitative systematic review
Schwingshackl 2014 ¹⁸	17	MeDiet	Flow- mediated dilatation	WMD 1.86%(0.23- 3.48)	Adiponectin levels also increased
Schwingshackl 2014 ¹⁸	17	MeDiet	High- sensitivity CRP	WMD= -1 mg/l(-1.5;- 0.5)	Similar favourable changes in IL-6 and ICAM-1
Kastorini 2011 ³⁰	50	MeDiet (randomized trials)	Metabolic syndrome	RR = 0.50 (0.29-0.85)**	Significant beneficial effects were found for each of the metabolic syndrome criteria
Esposito 2013 ²⁵	14	MeDiet	Metabolic	Beneficial	Qualitative systematic

			syndrome		review
Schwingshackl2014 ¹⁵	9	MeDiet	Type-2 diabetes	RR = 0.81 (0.73-0.90)	Quantitative meta-analysis: long-term studies were fairly homogenous ($I^2=0\%$) and showed a stronger risk reduction RR = 0.75 (0.68-0.83)
Koloverou 2014 ¹⁶	17	MeDiet	Type-2 diabetes	RR = 0.77 (0.66-0.89)	Quantitative meta-analysis: subgroups by region, health status, and degree of confounding control rendered similar results.
Esposito 2010 ³³	9	MeDiet	Type 2 diabetes and glycemic control	Beneficial	Qualitative systematic review
Esposito 2014 ²³	5	MeDiet (observational)	Type-2 diabetes	Beneficial	Qualitative systematic review
Esposito 2014 ²³	5	MeDiet (randomized trials)	Glycemic control	Beneficial	Qualitative systematic review
Georgoulis 2014 ²⁰	17	MeDiet	Type-2 diabetes and other outcomes	Beneficial	Qualitative systematic review
Roman 2008 ³⁹	20	MeDiet	CVD and risk factors	Beneficial	Qualitative systematic review
Widmer2014 ¹⁴	Not stated	MeDiet and its components	CVD	RR = 0.95 (0.83-0.97)	Qualitative systematic review: favourably compared with pharmacologic

					interventions
Ros2014 ¹⁷	Not stated	MeDiet	CVD	Beneficial	Qualitative systematic review: the results of the PREDIMED trial are presented in the context of their consistency with observational results.
Whayne 2014 ¹⁹	Not stated	MeDiet	CVD	Beneficial	Qualitative systematic review
Sofi2008 ³⁷	4	MeDiet (+2/9 points)	CVD	RR = 0.91 (0.87-0.95)	This meta-analysis was subsequently updated
Sofi 2010 ³²	8	MeDiet (+2/9 points)	CVD	RR = 0.90 (0.87-0.93)	Quantitative meta-analysis: I ² =35%
Sofi2014 ²¹	20	MeDiet (+2/9 points)	CVD	RR = 0.90 (0.87-0.92)	Quantitative meta-analysis: I ² =38%
Martínez-González 2014 ²²	2	MeDiet (randomized trials)	CVD	RR = 0.62 (0.45-0.85)	Quantitative meta-analysis: I ² =55%
Martínez-González 2014 ²²	16	MeDiet (observational, +2/9 points)	CVD	RR = 0.90 (0.86-0.94)	The heterogeneity disappeared after removing 3 studies assessing only fatal cases
Martinez-Gonzalez 2009 ³⁵	5	MeDiet	CVD	Beneficial	Qualitative systematic review
de Lorgeril 2008 ³⁶	Not stated	MeDiet	CVD	Beneficial	Qualitative systematic review
Rees 2013 ²⁶	11	MeDiet (?) -only trials	CVD	No evidence	The selection of trials apparently had little connection with the concept of MeDiet.

Panagiotakos 2004 ⁴²	6	MeDiet	CHD	8% to 45% relative risk reduction	Qualitative systematic review
Psaltoupoulou2013 27	22	MeDiet	Stroke	RR = 0.71 (0.57-0.89)	Quantitative meta-analysis: meta-regression suggested stronger protection among males.
Tyrovolas 2010 ³⁴	9	MeDiet	CVD and cancer	Beneficial	Qualitative systematic review
Martinez-Gonzalez 2004 ⁴¹	14	Feeding randomized trials	CVD and cancer	Unknown	The authors support the case for the urgent need of a trial such as PREDIMED
Sofi 2008 ³⁷	8	MeDiet (+2/9 points)	all-cause mortality	RR = 0.91 (0.89-0.94)	This meta-analysis was subsequently updated
Sofi2010 ³²	9	MeDiet (+2/9 points)	all-cause mortality	RR = 0.92 (0.90-0.94)	Quantitative meta-analysis: I ² =33%
Sofi2014 ²¹	18	MeDiet (+2/9 points)	all-cause mortality	RR = 0.92 (0.91-0.93)	Quantitative meta-analysis: I ² =47%
Trichopoulou 2000 ⁴³	3	MeDiet	All-cause mortality	Longer survival	Qualitative systematic review
Serra-Majem2006 40	35	MeDiet	A variety of outcomes	Beneficial	Qualitative systematic review
Trichopoulou2014 44	Not stated	MeDiet	A variety of effects	Beneficial	Opinion of experts around the world
Maderuelo- Fernández 2014 ¹³	14	Interventions to promote MeDiet	Adherence to MeDiet	Beneficial	Qualitative systematic review: hard end-points were not assessed

MeDiet: Mediterranean diet

(+2/9 points): effects associated with increasing 2 points in a 0 to 9 score of adherence to the MeDiet.

CVD: cardiovascular disease

CHD: coronary heart disease

RR: Relative Risk (95% Confidence Intervals)

WMD: weighted mean difference

CRP: C-reactive protein

IL-6: interleukin 6

ICAM-1: intercellular adhesion molecule

BMI: body mass index

BP: blood pressure

I^2 : index to quantify heterogeneity in meta-analyses, please check Higgins et al. BMJ 2003;327:557–60.

*Risk ratios in meta-analyses of epidemiologic studies, usually adjusted for multiple confounders, compared the highest versus the lowest category of adherence to the MeDiet. Outcome changes describe the mean changes for the MeDiet versus comparator diets in meta-analyses of RCTs; only statistically significant changes are shown. Values between brackets are 95% confidence intervals.

**An apparent erratum was corrected. The authors presented the log of hazard ratio (95% CI) as -0.69 (-2.16 to -1.16), but this is impossible, the correct upper limit should probably be -0.16 (as we have assumed).

Table 2. Relative Risk Reduction, Absolute Risk Reduction and Number Needed to Treat associated with the PREDIMED primary prevention intervention for several hard clinical events (assuming median follow-up = 4.8 years).

Clinical event	Mediterranean diet supplemented with Extra-virgin olive oil			Mediterranean diet supplemented with mixed nuts		
	Relative Risk	Absolute Risk	Number needed	Relative risk	Absolute Risk	Number needed
	Reduction	Reduction	to treat	Reduction	Reduction	to treat
Primary CVD end-point	30% (8.0% ; 46%)	1.34% (0.36% ; 2.05%)	75 (49-281)	28% (4.0% ; 46%)	1.25% (0.18% ; 2.05%)	80 (49-562)
Type 2 diabetes	40% (15% ; 57%)	3.52% (1.32% ; 5.02%)	28 (20-76)	18% (-10% ; 39%)	1.59% (-0.88% ; 3.44%)	-
Peripheral artery disease	64% (35% ; 79%)	1.18% (0.64% ; 1.45%)	85 (69-155)	46% (8% ; 68%)	0.85% (0.15% ; 1.25%)	118 (80-679)
Atrial fibrillation	38% (12% ; 55%)	1.54% (0.48% ; 2.22%)	65 (45-206)	10% (-23% ; 34%)	0.40% (-0.93% ; 1.37%)	-

Fully adjusted estimates for the hazard ratios from Cox regression models were used to compute the Relative Risks (RR).

The Relative Risk Reduction (RRR) was computed as $RRR = (1-RR)\%$.

The Absolute Risk Reduction (ARR) was computed taking into account the baseline incidence of events in the control group (I_0) after a median follow-up of 4.8 years and applying the estimates for the Relative Risks, i.e. $ARR = I_0 (1-RR)$.

Table 3. Number of expected prevented cases with the PREDIMED primary prevention intervention for several hard clinical events (median follow-up: 4.8 years) in a hypothetical cohort of 1,000 subjects. Both Mediterranean diet groups were merged together.

<u>Clinical event</u>	Number of prevented cases (95% CI) per 1,000 hypothetical participants receiving the PREDIMED MeDiet-intervention
Primary CVD end-point	13 (4 to 20)
Type 2 diabetes	26 (7 to 41)
Peripheral artery disease	10 (6 to 13)
Atrial fibrillation	11 (2 to 18)

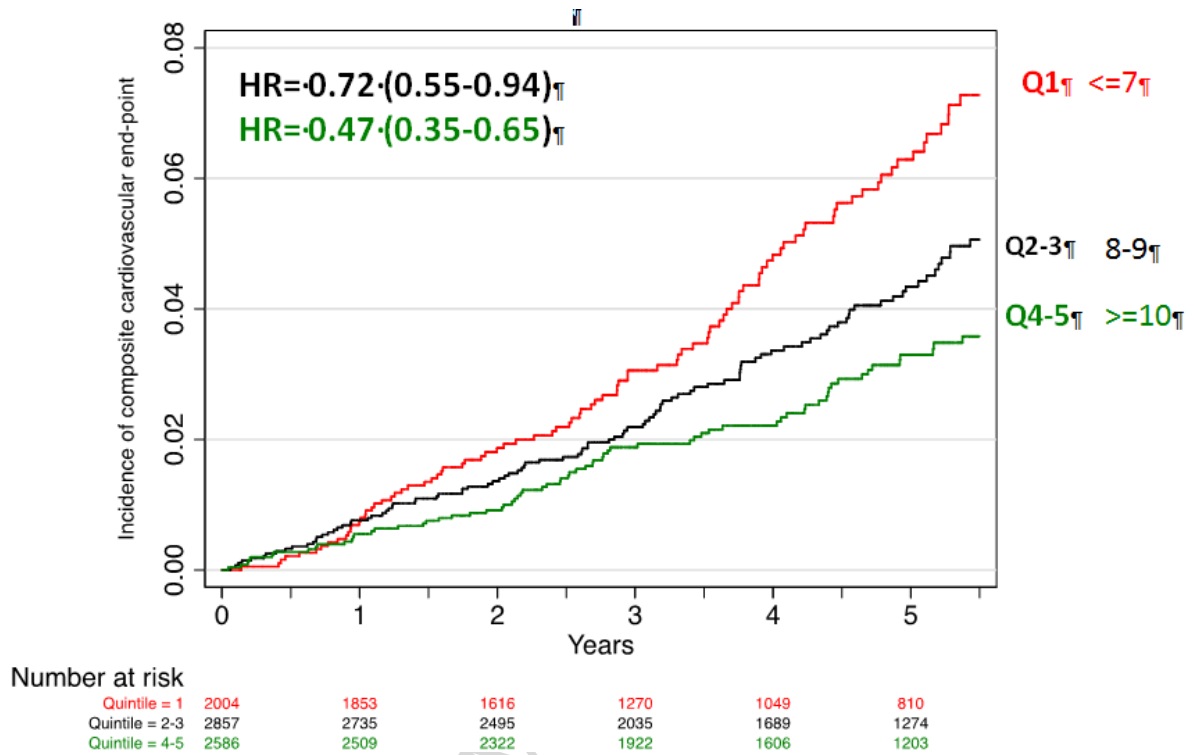


Figure 1. Baseline adherence to the Mediterranean diet (14-point PREDIMED score) and incidence of the primary end-point in the PREDIMED trial (a composite of myocardial infarction, stroke or cardiovascular death). Q1-Q5: quintiles.