Published by Oxford University Press on behalf of the International Epidemiological Association
© The Author 2010; all rights reserved. Advance Access publication 20 December 2010

International Journal of Epidemiology 2012;**41**:377–385
doi:10.1093/ije/dyq250

COHORT PROFILE

Cohort Profile: Design and methods of the PREDIMED study

Miguel Ángel Martínez-González,[1]*† Dolores Corella,[2,3] Jordi Salas-Salvadó,[3,4] Emilio Ros,[3,5] María Isabel Covas,[3,6] Miquel Fiol,[3,7] Julia Wärnberg,[1,8] Fernando Arós,[9] Valentina Ruíz-Gutiérrez,[10] Rosa María Lamuela-Raventós,[11] Jose Lapetra,[3,12] Miguel Ángel Muñoz,[13] José Alfredo Martínez,[3,14] Guillermo Sáez,[15] Lluis Serra-Majem,[16] Xavier Pintó,[17] María Teresa Mitjavila,[18] Josep Antoni Tur,[19] María del Puy Portillo[20] and Ramón Estruch[3,21†], for the PREDIMED Study Investigators

[1]Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain, [2]Department of Preventive Medicine, University of Valencia, Valencia, Spain, [3]CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III (ISCIII), Spain, [4]Human Nutrition Unit, IISPV, Universitat Rovira i Virgili, Reus, Spain, [5]Lipid Clinic, Department of Endocrinology and Nutrition, Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clínic, Barcelona, Spain, [6]Lipids and Cardiovascular Epidemiology Research Unit, Institut Municipal d'Investigació Mèdica (IMIM), Barcelona, Spain, [7]Institute of Health Sciences (IUNICS), University of Balearic Islands, Palma de Mallorca, Spain, [8]Department of Preventive Medicine, University of Málaga, Málaga, Spain, [9]Department of Cardiology, University Hospital Txagorritxu, Vitoria, Spain, [10]Instituto de la Grasa, Consejo Superior de Investigaciones Científicas, Sevilla, Spain, [11]Nutrition and Food Science Department–XaRTA, INSA, University of Barcelona, Barcelona, Spain, [12]Department of Family Medicine, Primary Care Division of Sevilla, Centro de Salud San Pablo, Sevilla, Spain, [13]Primary Care Division, Catalan Institute of Health, Barcelona, Spain, [14]Department of Nutrition and Food Sciences, Physiology and Toxicology, University of Navarra, Pamplona, Spain, [15]Department of Biochemistry and Molecular Biology, CDB-HGUV, University of Valencia, Valencia, Spain, [16]Department of Clinical Sciences, University of Las Palmas de Gran Canaria, Las Palmas, Spain, [17]Lipids and Vascular Risk Unit, Internal Medicine, Hospital Universitario de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain, [18]Department of Physiology, University of Barcelona, Spain, [19]Department of Fundamental Biology and Health Sciences, University of Balearic Islands, Palma de Mallorca, Spain, [20]Department of Nutrition and Food Science, University of Basque Country, Vitoria, Spain and [21]Department of Internal Medicine, IDIBAPS, Hospital Clinic, University of Barcelona, Barcelona, Spain

*Corresponding author. Department of Preventive Medicine and Public Health, University of Navarra, C/Irunlarrea, 1. 31080-Pamplona (Navarra), Spain. E-mail: mamartinez@unav.es

†These authors contributed equally to this work

Accepted 29 November 2010

How did the PREDIMED study come about?

A call for grants was issued in 2002 by the Spanish Government (Instituto de Salud Carlos III). This call was specifically designed to initiate networking research among Spanish biomedical investigators. During 2002, Ramón Estruch—the leader of our initiative—contacted different Spanish investigators (the rest of us) working in nutrition from different perspectives. We applied together for a grant to start a large randomized trial to test the effectiveness of a Mediterranean diet (MeDiet) on the 'primary' prevention of cardiovascular disease (CVD) and to continue the study as an observational cohort of high-risk participants to be followed-up in the long term. On 6 January 2003 our project was funded.

From January to June 2003, we developed the protocol: logistics, manual of operations, instruments, forms and data entry/management systems. The needed personnel (a minimum of a dietician and a nurse for each of the 11 field centres, FCs) were then hired, trained and certified. Each FC contacted approximately 20 primary care practices (PCPs) to recruit participants. The recruitment of participants started in October 2003. The name PREDIMED (in Spanish: PREvención con DIeta MEDiterránea) was proposed by Dolores Corella. This name is applied to both the cohort study and the networking group.

Despite being an interventional study, the PREDIMED study provides a unique opportunity for conducting the long-term follow-up (after the completion of the trial) of a large observational cohort of high cardiovascular risk subjects in a Mediterranean setting.

The MeDiet represents the dietary exposure in closest agreement with the Bradford Hill criteria for a potential causal protection against coronary heart

disease (CHD) according to a recent systematic review.[1] This conclusion is mainly supported by observational cohort studies. A recent meta-analysis of these cohorts showed that adherence to the MeDiet was associated with reductions in total mortality and CHD mortality.[2] Subsequently, similar evidences have been collected for 'non-fatal' CVD.[3–5] An increasing body of evidence is supporting also a benefit of the MeDiet against major cancers and neurodegenerative diseases.[6–10]

Our hypothesis was that two traditional MeDiets, one enriched with virgin olive oil (VOO) and another enriched with nuts, both high in total fat and unsaturated fat, would be superior to the usually recommended low-fat diet for the primary prevention of CVD in a high-risk population. This fit well into the paradigm of focusing on dietary patterns instead of isolated foods or nutrients. Overall patterns better represent dietary practices found in free-living populations and provide useful epidemiological information with a high potential for acceptability, palatability and future compliance.

However, no randomized controlled trial has ever been conducted to test the MeDiet in the 'primary prevention' of major chronic diseases. The only available clinical trial supporting a cardioprotective role of the MeDiet is the Lyon Diet Heart Study.[11] It was an important step to support the benefits of the MeDiet, but it included only myocardial infarction survivors (i.e. it was a 'secondary' prevention trial). However, it showed a remarkable 50–70% reduction in CHD event rates and mortality with a 'MeDiet' (enriched with α-linolenic acid, but not with olive oil). These results were criticized because no special consideration was given to olive oil, which is the major source of dietary fat in Mediterranean countries.[12] Another problem was that dietary assessments at baseline and at the end of the study were reported for only 30% of the control group and 50% of the experimental group and no biochemical markers of adherence were obtained. Finally, concerns have been raised regarding the low number of observed endpoints (44 in the control group vs 14 in the treatment group), and the improbable contrast between the large reduction in risk and the lack of changes in most classical risk factors. The PREDIMED study attempts to overcome previous limitations and to provide the best quality of evidence to answer the question of whether the MeDiet, compared with the previously tested model of advice on a low-fat diet, provides relatively higher protection against chronic disease. In the face of the increasing global burden of CVD and cancer, the answer to this question is a major public health priority. The long tradition of adherence to this food pattern in Mediterranean countries, where CHD incidence is low despite high levels of cardiovascular risk factors;[13] the diversity of mechanisms supporting the beneficial effects on cardiovascular health of olive oil[14,15] or nuts;[16] and the higher palatability and acceptance of MeDiets in comparison with low-fat diets[17,18] lend support to our hypothesis.

The results of some observational studies have been subsequently refuted by evidence from clinical trials (i.e. the presumptive cardioprotective effects of postmenopausal hormone therapy[19] or antioxidant supplements[20]). This highlights the need to obtain first-level evidence before considering any global public health strategy. Dietary guidelines can be safely issued when consistency is found between observational and experimental studies.

What does the PREDIMED study cover?

A large cohort has been assembled for long-term follow-up. This cohort includes 7447 high-risk participants. The last participant was recruited on 30 June 2009. Trial closeout will take place by 31 December 2011. Subsequent follow-up will continue as an observational multi-purpose cohort to explore other hypotheses (i.e. the roles of different types of alcoholic beverages in cancer or CVD prevention) and to develop nested case–control analyses for studies of biomarkers and gene–nutrient interactions.

The 'primary aim' of the trial is to assess the effects of two MeDiets on a composite endpoint of cardiovascular death, myocardial infarction and stroke ('primary endpoint') in comparison with a low-fat control diet. 'Secondary endpoints' are death of any cause, incidence of heart failure, diabetes mellitus, dementia or other neurodegenerative disorders and major cancers (colorectal, breast, lung, stomach and prostate). To better understand how dietary changes may modify the risk of clinical events, we also evaluate intermediate outcomes, including changes in blood pressure (BP), weight gain, fasting blood glucose, blood lipids and markers of inflammation.

Who are the participants in the PREDIMED study?

Participants are men (55–80 years old) or women (60–80 years old) who were free of CVD at baseline. Inclusion criteria were to have either type 2 diabetes or $\geqslant 3$ major cardiovascular risk factors, out of the following: current smoking (>1 cig/day during the last month); hypertension (systolic BP $\geqslant 140$ mmHg or diastolic BP $\geqslant 90$ mmHg or antihypertensive medication); LDL cholesterol $\geqslant 160$ mg/dl or lipid-lowering therapy; HDL cholesterol $\leqslant 40$ mg/dl in men or $\leqslant 50$ mg/dl in women; body mass index $\geqslant 25$ kg/m^2; and family history of premature CHD.

Exclusion criteria were the previous history of CVD (i.e. a previous medical diagnosis of CHD, stroke or peripheral arterial disease), any severe chronic illness,

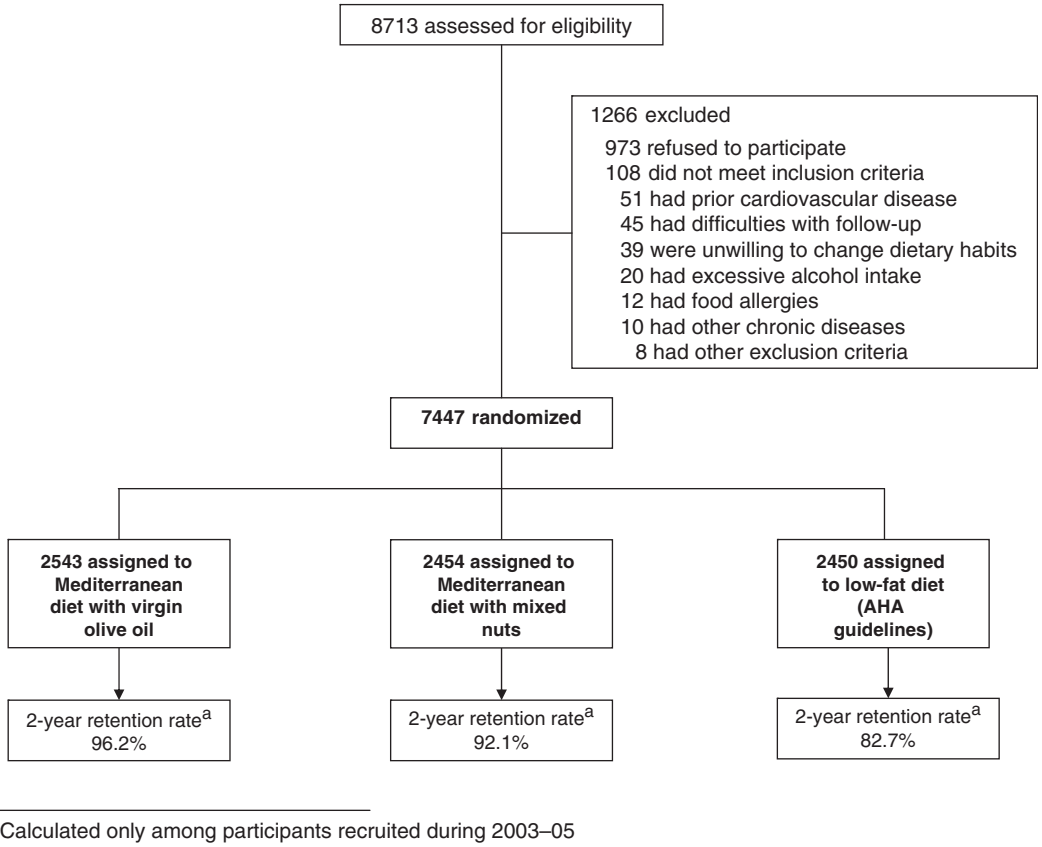

Figure 1 Flow chart of participants in the PREDIMED study

immunodeficiency or human immunodeficiency virus (HIV) positive status, illegal drug or alcohol misuse, history of allergy to olive oil or nuts and low predicted likelihood of changing dietary habits according to the Prochaska and DiClemente stages of change model.[21] Figure 1 provides further details on the selection procedure.

The selection process started by extracting names of potential participants from the records of the PCPs. Most PCPs participating in the study have computer-based records of patients, making the selection relatively simple. The clinical records of these persons were then individually reviewed to exclude those who did not meet eligibility criteria. Potential participants were approached by PCPs by a telephone call or during their clinical visits. If candidates were interested in participating, a face-to-face interview was scheduled. During this interview, the purpose and characteristics of the study were explained, and signed informed consent was obtained from willing participants. A brief explanation of the study, including the possibility that they might receive free allowances of VOO or nuts for the duration of the trial, was given at this first visit. Most (>70%) candidates approached in this way agreed to return for the screening visit.

The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) included similar participants and observed an 8.9% cumulative rate for the primary outcome (fatal CHD + non-fatal myocardial infarction) after 4.9 years of follow-up.[22] Adapting this figure to a 6-year follow-up and including also stroke in the endpoint definition, an 11% absolute risk in the control group could be conservatively assumed in our study. We expect a 25% relative risk reduction in both MeDiet groups. Under these assumptions, the total number of participants (3 equally-sized groups) required was 5631 (1877 per group) for $\beta = 0.2$ and two-tailed $\alpha = 0.05$. We included 7447 subjects to allow for both 10% losses during follow-up and a lower incidence than expected. Figure 1 shows the flow of participants.

Study participants were randomized to three equally sized groups. Tables of random allocation were centrally elaborated. The study nurses in charge of the random allocation were independent of the nursing staff of the PCP. At baseline, general practitioners (GPs) were not informed of the allocation of participants. This is consistent with CONSORT guidelines for randomized trials to prevent selection biases.[23]

Characteristics of participants according to group allocation are shown in Table 1. The Institutional

Table 1 Description of participants in the PREDIMED study at baseline according to intervention group

Characteristics at baseline	MeDiet + VOO ($n = 2543$)	MeDiet + nuts ($n = 2454$)	Control (low-fat) ($n = 2450$)
Age (mean years, SD)	67 (6)	67 (6)	67 (6)
Gender (women) (%)	58.7	54.0	59.7
Diabetes (%)	50.2	46.5	48.4
Hypertension (%)	82.1	82.4	83.7
Current smokers (%)	13.9	14.5	13.8
Former smokers (%)	24.3	25.8	23.8
High blood total cholesterol (%)	71.6	73.3	71.9
Family history of CHD[a] (%)	22.7	21.7	22.8
BMI (mean, SD)	30.0 (3.7)	29.7 (3.8)	30.2 (4.0)
Waist circumference (mean cm, SD)	100 (10)	100 (11)	101 (11)
Adherence to MeDiet[b] (mean, SD)	8.7 (2.0)	8.7 (2.0)	8.4 (2.1)
Percentage of participants from each region			
North (Navarra and Basque country)	23.4	22.9	22.2
North-East (Catalonia)	31.3	33.2	31.5
East (Valencia and Balearic Islands)	23.0	24.2	23.1
South (Andalusia and Canary Islands)	22.3	19.7	23.1

SD: standard deviation.
[a]Definite myocardial infarction or sudden death before 55 years in male first-degree relatives or before 65 years in female first-degree relatives.
[b]14-point score of adherence to MeDiet.

Review Board (IRB) of Hospital Clinic (Barcelona, Spain) approved the study protocol on July 2002. This IRB is accredited by the US Department of Health and Human Services (DHHS). Later, the IRBs of all other centres also approved the protocol. The trial is registered (http://www.controlled-trials.com/ISRCTN35739639).

What are the interventions in the PREDIMED study?

Participants were randomly assigned to three interventions: MeDiet with VOO, MeDiet with mixed nuts or control group (low-fat diet). The two groups allocated MeDiets receive intensive education to follow the MeDiet and supplemental foods at no cost. VOO (1 l/week) is provided to the first group and 30 g/day of mixed nuts (15 g walnuts, 7.5 g hazelnuts and 7.5 g almonds) to the second group. In the control group, participants do not receive education on the MeDiet, but are given advice to follow a low-fat diet.

Besides being an excellent source of monounsaturated fat (MUFA), VOO also contains significant amounts of phenolic antioxidants and other phytochemicals (tocopherols, polyphenols) because it is obtained from the first pressing of the ripe fruit (i.e. it is an olive juice). In contrast, these phytochemicals are present to a lower extent in common refined olive oils (ROO).[14] ROO lose polyphenols and other elements in the refining process, although fatty acid composition is similar to that of VOO.[24] When compared with ROO, VOO increases HDL cholesterol, total plasma antioxidant capacity and LDL resistance to oxidation. *In vivo* markers of lipid and LDL oxidation decrease in a dose-dependent manner with the phenolic content of the olive oil.[25] Most nuts are rich in MUFA (mostly oleic acid), whereas walnuts are high in polyunsaturated fatty acids (PUFAs, i.e. linoleic and α-linolenic acids). The dietary fibre content in nuts is also high. Nuts are good sources of arginine, potassium, vitamin E and other bioactive compounds. This may help explain their beneficial health effects.[16] The rationale for the free provision of these food items (VOO and nuts) is that they may contribute to a higher compliance with the overall MeDiet food pattern.

The PREDIMED dieticians are directly responsible for the dietary intervention. After two screening visits, participants randomized to each one of the three treatment arms had a face-to-face interview with the dietician and a group session (less than 20 subjects). A 14-point score of adherence to the MeDiet is a main tool to change dietary habits (Table 2).[26–28] A similar 9-point score is used for the low-fat control group. For total fat intake, the recommendations given to participants in the low-fat diet group are opposite to those given to participants in the two MeDiet groups. The focus can be shifted from changing portion sizes, frequency of intake or cooking

Table 2 Short questionnaire to assess adherence to the MeDiet

Questions	Criteria for 1 point
1. Do you use olive oil as main culinary fat?	Yes
2. How much olive oil do you consume in a given day (including oil used for frying, salads, out-of-house meals, etc.)?	⩾4 tbsp
3. How many vegetable servings do you consume per day? [1 serving: 200 g (consider side dishes as half a serving)]	⩾2 (⩾1 portion raw or as a salad)
4. How many fruit units (including natural fruit juices) do you consume per day?	⩾3
5. How many servings of red meat, hamburger or meat products (ham, sausage, etc.) do you consume per day?	<1
6. How many servings of butter, margarine, or cream do you consume per day? (1 serving: 12 g)	<1
7. How many sweetened and/or carbonated beverages do you drink per day?	< 1
8. How much wine do you drink per week?	⩾7 glasses
9. How many servings of legumes do you consume per week? (1 serving: 150 g)	⩾3
10. How many servings of fish or shellfish do you consume per week? (1 serving 100–150 g of fish or 4–5 units or 200 g of shellfish)	⩾3
11. How many times per week do you consume commercial sweets or pastries (not homemade), such as cakes, cookies, biscuits or custard?	<3
12. How many servings of nuts (including peanuts) do you consume per week? (1 serving 30 g)	⩾1 >=3
13. Do you preferentially consume chicken, turkey or rabbit meat instead of veal, pork, hamburger or sausage?	Yes
14. How many times per week do you consume vegetables, pasta, rice or other dishes seasoned with sofrito (sauce made with tomato and onion, leek or garlic and simmered with olive oil)?	⩾2

methods. We have reported an adequate effectiveness of the intervention after 1 year of follow-up.[28] Because unsaturated fats like those contained in olive oil and nuts are still wrongly perceived as fattening, it has been particularly important to allay the fear of an eventual weight gain. Tactful exposition of recent scientific evidence,[18,29–31] together with the fact that body weight did not change after 3 months of MeDiet intervention in the pilot phase of the PREDIMED study,[26] have been instrumental in achieving this aim.

The PREDIMED group sessions are organized separately for each of the three intervention groups. Participants are provided with written material (see: http://www.predimed.org and http://www.predimed.es) including descriptions of seasonal foods, shopping lists, weekly meal plans and cooking recipes. Olive oil and nut industry companies are committed to supplying for free the food supplements used in the study until December 2011. None of the investigators has any commercial interest with these food companies.

How often are cohort volunteers contacted?

Table 3 shows the frequency of contacts with participants. The individual and group visits are repeated every 3 months with the same contents, except that shopping lists and recipes vary with the season of the year. Each visit includes three steps: assessment, intervention and future directions. Once a year, general medical and food frequency questionnaires (FFQ) are obtained, an electrocardiogram (ECG) is performed and blood and urine samples are collected (Table 3).

After the trial formally terminates (December 2011), we will follow our cohort for occurrence of clinical events, ECG and measurement of weight and BP. We will continue to ascertain participants' vital status through yearly personal interviews by PREDIMED personnel, close contact with GPs who care for them and reviews of medical records. On a yearly basis, the Spanish official mortality index (Indice Nacional de Defunciones) is also reviewed.

What variables are measured?

Table 3 shows the variables collected in the PREDIMED study. The yearly administered FFQ provide information about compliance with food and nutrient targets. This FFQ was previously validated in Spain.[32] We have performed a new validation of the FFQ with high-risk persons similar to PREDIMED participants[33] and have confirmed its reproducibility.[34] Biological markers of compliance (plasma oleic

Table 3 Measurements in the PREDIMED study

Measurements	Description of measurements		Number of repeated measurements							
	Number of items	Content	Baseline	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7[a]
Eligibility questionnaire	33	Socio-demographic inclusion and exclusion criteria, smoking	1							
General questionnaire[b]	77	Marital status, job, BP and anthropometry, medical conditions, medications, CAGE	1							
14-item MeDiet questionnaire	14	MeDiet adherence (intervention tool)	1	2–5	6–9	10–13	14–17	18–21	22–25	26–29
Food frequency questionnaire	137	Previously validated[32–34]	1	2	3	4	5	6	7	8
Physical activity questionnaire	67	Validated Minnesota questionnaire[35,36]	1	2	3	4	5	6	7	8
Follow-up questionnaire[b]	75	Risk factors, symptoms and conditions, job, BP, anthropometry, medication		1	2	3	4	5	6	7
Tolerance questionnaire	6	Potential adverse events		1	2	3	4	5	6	7
Abandonment questionnaire[c]	21	Reasons for terminating study		1	2	3	4	5	6	7
ECG			1	2	3	4	5	6	7	8
Blood chemistry	14	Lipids, glucose, renal function, transaminases, blood count and others	1	2	3	4	5	6	7	8
Blood sample		9 tubes (38.5 ml) ~40 aliquots (–80°C)	1	2	(o)	3	(o)	4	5	6
Urine sample		16 aliquots (–80°C)	1	2	(o)	3	(o)	4	5	6
Toenail sample		A clip of each toenail	1					2		
SF36[d]	36	Quality of life	1	2	3	4	5			

CAGE, 4-item screening test for alcohol dependence; SF-36, short-form 36, 36-item questionnaire for quality of life; (o), optional collection.
[a]Only for participants recruited before 2005.
[b]Includes direct measurements of weight, height, waist circumference, BP and ankle-brachial blood pressure index.
[c]Only if applicable.
[d]Only for participants recruited after 2007.

and α-linolenic acid proportions and urinary concentrations of tyrosol and hydroxytyrosol, resveratrol and ethanol) are measured in random subsets of participants from the three arms of the trial.[28] Clinical evaluations are limited to yearly follow-up visits that include the same examinations performed at baseline, with the exception of the general questionnaire, which is substituted by a follow-up questionnaire, and a tolerance/adverse events questionnaire. Although no intervention on physical activity is performed, the Minnesota physical activity questionnaire (validated Spanish version)[35,36] is completed each year.

Blood and urine samples are collected at baseline and Years 1, 3, 5 and 6 (or final visit). Tubes for EDTA plasma, citrate plasma, buffy coat and serum are collected and aliquots are kept frozen (–80°C). The Short-Form 36 to assess quality of life is completed by all participants recruited after 2007. Toenails are collected at baseline and the final visit. In two centres (Barcelona-North and Pamplona), carotid intima–media thickness has been measured in subsets of participants.[37,38] Outcomes are ascertained on a yearly basis by a Clinical Events Committee whose members are blinded to the intervention group.

What is the attrition?

A high retention rate is a major methodological requirement in follow-up studies. The attrition rate after 2 years' follow-up for participants recruited before 2006 ($n = 4.381$) was 9.3%. The highest retention rates occurred in the MeDiet with VOO group and the lowest retention rate was observed in the control (low-fat diet) group (Figure 1). The highest retention rate in the two groups allocated to MeDiets can be partly attributed to the free provision of food items (VOO and nuts). However, in the PREDIMED trial, we will eventually be able to obtain a nearly complete follow-up for the main outcomes because participants represent a stable and well-defined population regularly attending their GPs. In addition, a comprehensive search for events is performed yearly through review of the medical records of participants in all the hospitals of the city where the respective FC is located.

What has been found so far?

The pilot study of the PREDIMED trial ($n = 772$) suggested that a MeDiet was a safe strategy to reduce the levels of major cardiovascular risk factors after a 3-month follow-up.[26] Inverse baseline associations with inflammatory markers for cereals, fruits, nuts and VOO were found.[39] In the first 3204 participants, the 14-point score was able to predict the prevalence of diabetes, hypertension and obesity or the joint presence of metabolic conditions.[40] In a 3-month longitudinal study, we found a favourable effect of the MeDiet interventions on LDL oxidation[41] and cellular and serum inflammatory biomarkers related to atherosclerosis.[42] After a 12-month follow-up of the first 1224 participants, the prevalence of metabolic syndrome was reduced in all groups, but it was more marked in the MeDiet groups, especially in the MeDiet + nuts group.[43] Other studies have assessed dietary associations with hypertension[44–46] and gene–nutrient interactions in obesity and weight gain.[47–49] In 2009, more than 30 papers derived from the PREDIMED study have been either published or are accepted for publication in peer-reviewed journals. In 2010, a substantial effect of both MeDiets in the reduction of type 2 diabetes risk after a median follow-up of 4.0 years was reported from a nested analysis conducted in one of the centres.[50]

What are the main strengths and weaknesses?

The strengths of the study are the randomized design, the large sample size, the storing of abundant biological samples, the objective assessment of compliance with biomarkers and the close monitoring of participants. The main weakness is the difficulty to change long-established dietary habits and increase adherence to a low-fat diet in participants allocated the control group.

From a public health perspective, a behavioural intervention coupled with an easy (free) access to representative healthy foods is a realistic test of the effectiveness to be attained with official policies and health promotion activities. The PREDIMED trial attempts to obtain relevant information for public health use, because the nutritional intervention is undertaken in free-living persons who receive information, motivation, support and empowerment to modify their food habits in a real-life context, i.e. they continue to buy their foods and cook their meals. Such an intervention provides a real-life scenario that may be easily applied to public health policies.

Where can I find out more?

A list of original publications and other information can be found at www.predimed.es (or www.predimed.org). Collaboration with national and international studies is welcome and can be proposed to: restruch@clinic.ub.es.

Funding

The Spanish Ministry of Health—Instituto de Salud Carlos III (ISCIII) funded the project for the period 2003–05 (RTIC G03/140). In 2006 a new funding modality was established by ISCIII through the CIBER (Centros de Investigación Biomédica En Red) Fisiopatología de la Obesidad y Nutrición (CIBERobn) which is providing funding for 7 of the original research groups, whereas the other 12 were funded by a new research network (RTIC RD 06/0045). Other official funds from Spanish government agencies have been obtained for subprojects related to intermediate outcomes (lipoproteins, inflammatory markers, vascular imaging, genomic and proteomic studies, etc.). Obviously, the donation by food companies of all the VOO and mixed nuts needed throughout the duration of the study is a substantial contribution. None of these companies (Patrimonio Comunal Olivarero, California Walnut Commission, Borges, La Morella Nuts and Hojiblanca) played or will play any role in the design, collection, analysis or interpretation of the data or in the decision to submit manuscripts for publication.

Conflict of interest: None declared.

References

[1] Mente A, Koning L, Shannon HS, Anand SS. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. *Arch Intern Med* 2009;**169:**659–69.

[2] 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:**1189–96.

[3] Fung TT, Rexrode KM, Mantzoros CS, Manson JE, Willett WC, Hu FB. Mediterranean diet and incidence of and mortality from coronary heart disease and stroke in women. *Circulation* 2009;**119:**1093–100.

[4] Buckland G, Gonzalez CA, Vilardell M *et al*. Adherence to the Mediterranean diet and risk of coronary heart disease in the Spanish EPIC cohort study. *Am J Epidemiol* 2009; **170:**1518–29.

[5] Martínez-González MA, García-López M, Bes-Rastrollo M *et al*. Mediterranean diet and the incidence of cardiovascular disease: A Spanish cohort. *Nutr Metabol Cardiovasc Dis* 2010; doi:10.1016/j.numecd.2009.10.005 [Epub 20 January 2010].

[6] Benetou V, Trichopoulou A, Orfanos P *et al*. Conformity to traditional Mediterranean diet and cancer incidence: the Greek EPIC cohort. *Br J Cancer* 2008;**99:**191–5.

[7] Buckland G, Agudo A, Luján L *et al*. Adherence to a Mediterranean diet and risk of gastric adenocarcinoma within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort study. *Am J Clin Nutr* 2009;**91:**381–90.

[8] Scarmeas N, Stern Y, Tang MX, Mayeux R, Luchsinger JA. Mediterranean diet and risk for Alzheimer's disease. *Ann Neurol* 2006;**59:**912–21.

[9] Féart C, Samieri C, Rondeau V *et al*. Adherence to a Mediterranean diet, cognitive decline, and risk of dementia. *JAMA* 2009;**302:**638–48, Erratum in: *JAMA* 2009;**302**:2436.

[10] Sánchez-Villegas A, Delgado-Rodríguez M, Alonso A *et al*. Association of the Mediterranean dietary pattern with the incidence of depression: the Seguimiento Universidad de Navarra/University of Navarra follow-up (SUN) cohort. *Arch Gen Psychiatry* 2009;**66:**1090–98.

[11] de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N. 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:**779–85.

[12] Martinez-Gonzalez MA, Sanchez-Villegas A. The emerging role of Mediterranean diets in cardiovascular epidemiology: monounsaturated fats, olive oil, red wine or the whole pattern? *Eur J Epidemiol* 2004;**19:**9–13.

[13] Gabriel R, Alonso M, Segura A *et al*. Prevalence, geographic distribution, and geographic variability of major cardiovascular risk factors in Spain. Pooled analysis of data from population-based epidemiological studies: the ERICE Study. *Rev Esp Cardiol* 2008;**61:**1030–40.

[14] López-Miranda J, Pérez-Jiménez F, Ros E *et al*. Olive oil and health: Summary of the II International Conference on Olive Oil and Health consensus report, Jaén and Córdoba (Spain) (2008). *Nutr Metab Cardiovasc Dis* 2010; **20:**284–94.

[15] Fernández-Jarne E, Martínez-Losa E, Prado-Santamaría M, Brugarolas-Brufau C, Serrano-Martínez M, Martínez-González MA. Risk of first non-fatal myocardial infarction negatively associated with olive oil consumption: a case-control study in Spain. *Int J Epidemiol* 2002;**31:**474–80.

[16] Kris-Etherton PM, Hu F, Ros E, Sabaté J. The role of tree nuts and peanuts in the prevention of coronary heart disease: multiple potential mechanisms. *J Nutr* 2008; **138:**1746S–51S.

[17] McManus K, Antinoro L, Sacks F. A randomized controlled trial of a moderate-fat, low-energy diet compared with a low fat, low-energy diet for weight loss in overweight adults. *Int J Obes Relat Metab Disord* 2001;**25:** 1503–11.

[18] Shai I, Schwarzfuchs D, Henkin Y *et al*. Weight loss with low-carbohydrate, Mediterranean and low-fat diet. *N Engl J Med* 2008;**359:**229–41.

[19] Manson JE, Hsia J, Johnson KC *et al*. Estrogen plus progestin and the risk of coronary heart disease. *N Engl J Med* 2003;**349:**523–34.

[20] Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gludd C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention. Systematic review and meta-analysis. *JAMA* 2007;**297:** 842–57.

[21] Nigg CR, Burbank PM, Padula C *et al*. Stages of change across ten health risk behaviors for older adults. *Gerontologist* 1999;**39:**473–82.

[22] ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group. The Antihypertensive and Lipid-Lowering Treatment Prevent Heart Attack Trial. *JAMA* 2002;**288:**2998–3007.

[23] Altman DG, Schulz KF, Moher D *et al*. The revised CONSORT statement for reporting randomized trials explanation and elaboration. *Ann Intern Med* 2001;**134:** 663–94.

[24] Owen RW, Mier W, Giacosa A, Hull WE, Spiegelhalder B, Bartsch H. Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignans and squalene. *Food Chem Toxicol* 2000;**38:**647–59.

[25] Covas MI, Nyyssönen K, Poulsen HE *et al*. The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. *Ann Intern Med* 2006;**145:**333–41.

[26] Estruch R, Martínez-González MA, Corella D *et al*. Effects of a Mediterranean-style diet on cardiovascular risk factors. A randomized trial. *Ann Intern Med* 2006; **145:**1–11.

[27] Martinez-Gonzalez MA, Fernandez-Jarne E, Serrano-Martinez M, Wright M, Gomez-Gracia E. Development of a short dietary intake questionnaire for the quantitative estimation of adherence to a cardioprotective Mediterranean diet. *Eur J Clin Nutr* 2004;**58:**1550–52.

[28] Zazpe I, Sanchez-Tainta A, Estruch R *et al*. A large randomized individual and group intervention conducted by dieticians increased the adherence to Mediterranean-type diets: The PREDIMED study. *J Am Diet Assoc* 2008;**108:** 1134–44.

[29] Sacks FM, Bray GA, Carey VJ *et al*. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *N Engl J Med* 2009;**360:**859–73.

[30] Bes-Rastrollo M, Sánchez-Villegas A, de la Fuente C, de Irala J, Martinez JA, Martínez-González MA. Olive oil consumption and weight change: the SUN prospective cohort study. *Lipids* 2006;**41:**249–56.

[31] Bes-Rastrollo M, Wedick NM, Martinez-Gonzalez MA, Li TY, Sampson L, Hu FB. Prospective study of nut consumption, long-term weight change, and obesity risk in women. *Am J Clin Nutr* 2009;**89:**1913–19.

[32] Martin-Moreno JM, Boyle P, Gorgojo L *et al*. Development and validation of a food frequency questionnaire in Spain. *Int J Epidemiol* 1993;**22:**512–19.

[33] Fernández-Ballart JD, Piñol JL, Zazpe I *et al*. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. *Br J Nutr* 2009;**103:**1808–16.

[34] de la Fuente-Arrillaga C, Vázquez Z, Bes-Rastrollo M, Sampson L, Martínez-González MA. Reproducibility of a Food Frequency Questionnaire (FFQ) validated in Spain. *Public Health Nutr* 2009;**13:**1364–72.

[35] Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish men. The MARATHOM Investigators. *Am J Epidemiol* 1994;**139:** 1197–209.

[36] Elosua R, Garcia M, Aguilar A, Molina L, Covas MI, Marrugat J. Validation of the Minnesota Leisure Time Physical Activity Questionnaire In Spanish Women. Investigators of the MARATDOM Group. *Med Sci Sports Exerc* 2000;**32:**1431–37.

[37] Buil-Cosiales P, Irimia P, Berrade N *et al*. Carotid intima-media thickness is inversely associated with olive oil consumption. *Atherosclerosis* 2008;**196:**742–48.

[38] 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:**1213–19.

[39] Salas-Salvado 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:**651–59.

[40] Sanchez-Tainta A, Estruch R, Bullo M *et al*. Adherence to a Mediterranean-type diet and reduced prevalence of clustered cardiovascular risk factors in a cohort of 3204 high-risk patients. *Eur J Cardiov Prev Rehab* 2008;**15:** 589–93.

[41] 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:** 1195–203.

[42] Mena MP, Sacanella E, Vazquez-Agell M *et al*. Inhibition of circulating immune cell activation: a molecular anti-inflammatory effect of the Mediterranean diet. *Am J Clin Nutr* 2009;**89:**248–56.

[43] Salas-Salvadó J, Fernández-Ballart J, Ros E *et al*. Effect of a Mediterranean diet supplemented with nuts on metabolic syndrome status. *Arch Intern Med* 2008;**168:**2449–58.

[44] Guxens M, Fitó M, Martínez-González MA *et al*. Hypertensive status and lipoprotein oxidation in an elderly population at high cardiovascular risk. *Am J Hypertens* 2009;**22:**68–73.

[45] 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:**59–67.

[46] Barceló F, Perona JS, Prades J *et al*. Mediterranean-style diet effect on the structural properties of the erythrocyte cell membrane of hypertensive patients: the Prevencion con Dieta Mediterranea Study. *Hypertension* 2009;**54:** 1143–50.

[47] Razquin C, Martinez JA, Martinez-Gonzalez MA, Bes-Rastrollo M, Fernández-Crehuet J, Marti A. A 3-year intervention with a Mediterranean diet modified the association between the rs9939609 gene variant in FTO and body weight changes. *Int J Obes* 2010;**34:**266–72.

[48] Razquin C, Martinez JA, Martinez-Gonzalez MA, Mitjavila MT, Estruch R, Marti A. A 3 years follow-up of a Mediterranean diet rich in virgin olive oil is associated with high plasma antioxidant capacity and reduced body weight gain. *Eur J Clin Nutr* 2009;**63:**1387–93.

[49] Razquin C, Martinez JA, Martinez-Gonzalez MA, Corella D, Santos JM, Marti A. The Mediterranean diet protects against waist circumference enlargement in 12Ala carriers for the PPARgamma gene: 2 years' follow-up of 774 subjects at high cardiovascular risk. *Br J Nutr* 2009;**102:**672–79.

[50] 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* 2010; doi:10.2337/dc10-1288 [Epub 13 October 2010].