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Cohort profile: Design and methods of the PREDIMED study

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Cohort profile: Design and methods of the PREDIMED study

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 randomised 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 January 6th, 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, FC) was then hired, trained and certified. Each FC contacted approximately 20 Primary Care Practices (PCP) 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, therefore providing useful epidemiological information with a high potential for acceptability, palatability and future compliance.

However, no randomised 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] That trial, an important step forward the benefits of the Mediterranean diet, included only myocardial infarction survivors (i.e., it was a secondary prevention trial) and showed a remarkable 50-70% reduction in CHD event rates and mortality with a “MeDiet” (enriched with alpha-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 versus 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 a relatively higher protection for the prevention of 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, acceptance and compliance 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 June 30th, 2009. Trial closeout will take place by December 31st, 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 on 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, 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 year-old) or women (60-80 year-old) who were free of CVD at baseline. Inclusion criteria were to have either type 2 diabetes or ≥3 major cardiovascular risk factors, out of the following: current smoking (>1 cig/day during the last month); hypertension (systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg or antihypertensive medication); LDL-cholesterol ≥160 mg/dl or lipid-lowering therapy; HDL-cholesterol ≤40

mg/dl in men or ≤50 mg/dl in women; body mass index ≥25 kg/m^2; and family history of premature CHD.

Exclusion criteria were previous history of CVD (i.e. a previous medical diagnosis of CHD, stroke or peripheral arterial disease), any severe chronic illness, immunodeficiency or human immunodeficiency virus (HIV) positive status, illegal drug or alcohol abuse, 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 a 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 in this first visit. Most (>70 %) candidates approached in this way agreed to return for the screening visit.

The ALLHAT trial included similar participants and observed a 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 end-point 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 required was 5631 (1877 per group) for beta=0.2 and 2-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 randomised 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 nurse staff of the PCP. At baseline, General Practitioners (GP) were not informed of the allocation of participants. This is consistent with CONSORT guidelines for randomised trials to prevent selection biases[23].

Characteristics of participants according to group allocation are shown in Table 1. The Institutional 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 3 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/wk) is provided to the first group and 30 g/d 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 to 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 alpha-linolenic acids). The dietary fiber 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 2 screening visits, participants randomised to each one of the 3 treatment arms have a face-to-face interview with the dietician and a group session (<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 2 MeDiet groups. The focus can be shifted from changing

portion sizes, frequency of intake or cooking methods. We have reported an adequate effectiveness of the intervention after one 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 for this aim.

The PREDIMED group sessions are organized separately for each of the 3 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 supply 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 and alpha-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 (-80ºC). 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.[37-39] 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 GP visits in their PCPs. 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].

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The Spanish Ministry of Health - Instituto de Salud Carlos III (ISCIII) funded the project for the period 2003-2005 (RTIC G03/140). In 2006 a new funding modality was established by ISCIII through the CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), which is providing funding for 7 of the original research groups, while 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.

What are the main strengths and weaknesses?

The strengths are the randomised 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?

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.

Conflict of interest statement

The authors declare that they do not have any conflict of interest.

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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)	Contol (low-fat) (n=2450)
Age (mean, SD)	67 (6)	67 (6)	67 (6)
Sex (% 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 cholesterol (%)	71.6	73.3	71.9
Family history of CHD* (%)	22.7	21.7	22.8
BMI (mean, SD)	30.0 (3.7)	29.7 (3.8)	30.2 (4.0)
Waist circumference (mean, SD)	100 (10)	100 (11)	101 (11)
Adherence to MeDiet** (mean, SD)	8.7 (2.0)	8.7 (2.0)	8.4 (2.1)
Spanish region (% participants)			
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

Mediet: Mediterranean diet; VOO: virgin olive oil; SD: standard deviation; CHD: coronary heart disease

* Definite myocardial infarction or sudden death before 55 years in male first-degree relatives or before 65 years in female first-degree relatives

** 14-point score of adherence to Mediet

Table 2. Short questionnaire to assess adherence to the Mediterranean diet (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 sweet or carbonated beverages do you drink per day?	< 1
8. How much wine do you drink per week?	≥ 3 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
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

Table 3. Measurements in the PREDIMED study.

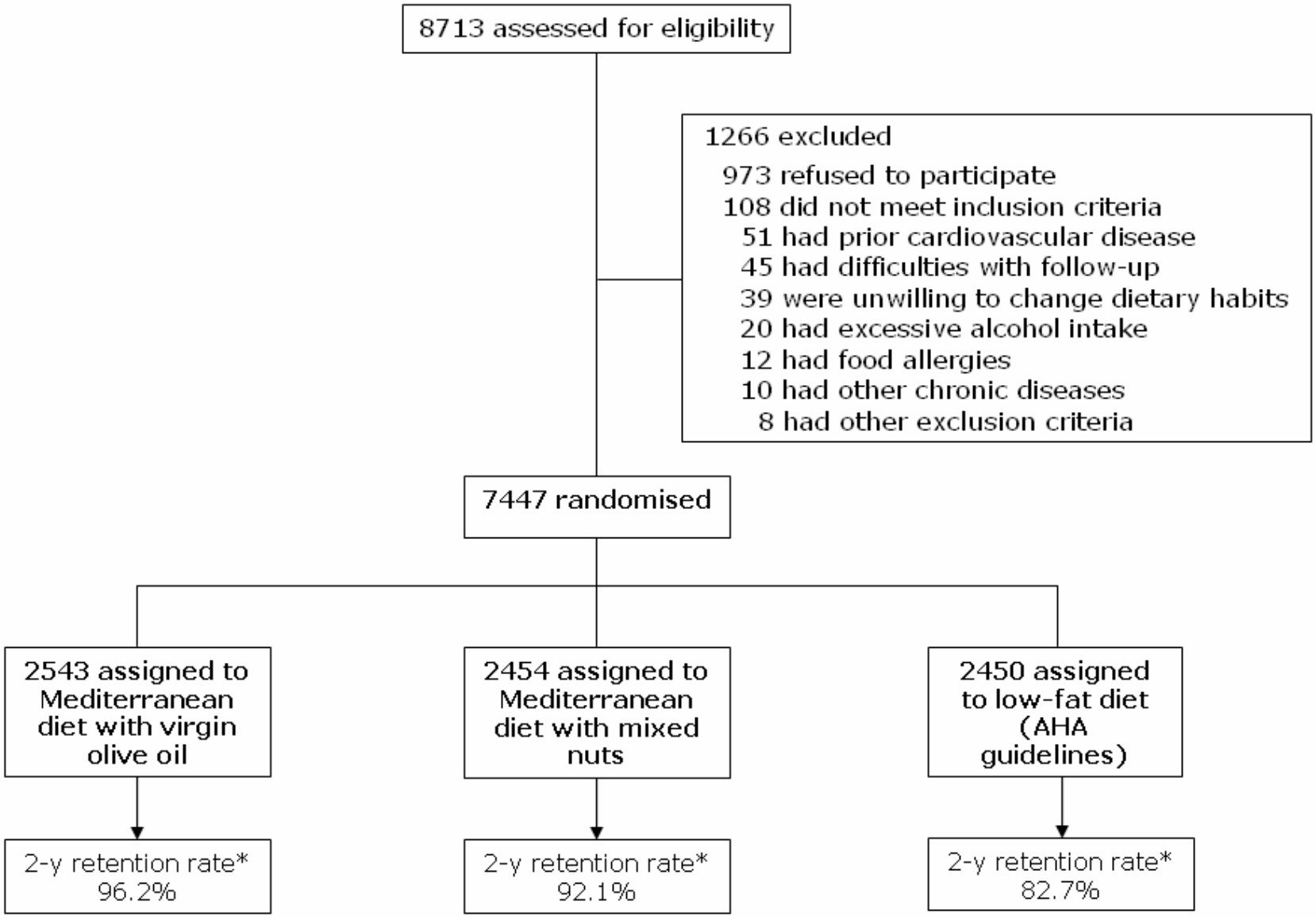
Measurements	Description of measurements		Number of repeated measurements							
	Number of items	Content	Baseline	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7*
Eligibility questionnaire	33	Socio-demographic, inclusion and exclusion criteria, smoking	1							
General questionnaire**	77	Marital status, 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**	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***	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 (-80ºC)	1	2	(o)	3	(o)	4	5	6
Urine sample		16 aliquots (-80ºC)	1	2	(o)	3	(o)	4	5	6
Toenail sample		A clip of each toenail	1					2		
SF36****	36	Quality of life	1	2	3	4	5			

BP: blood pressure; MeDiet: Mediterranean diet; CAGE: 4-item screening test for alcohol dependence; ECG: electrocardiogram;

SF-36: short-form 36, 36-item questionnaire for quality of life.

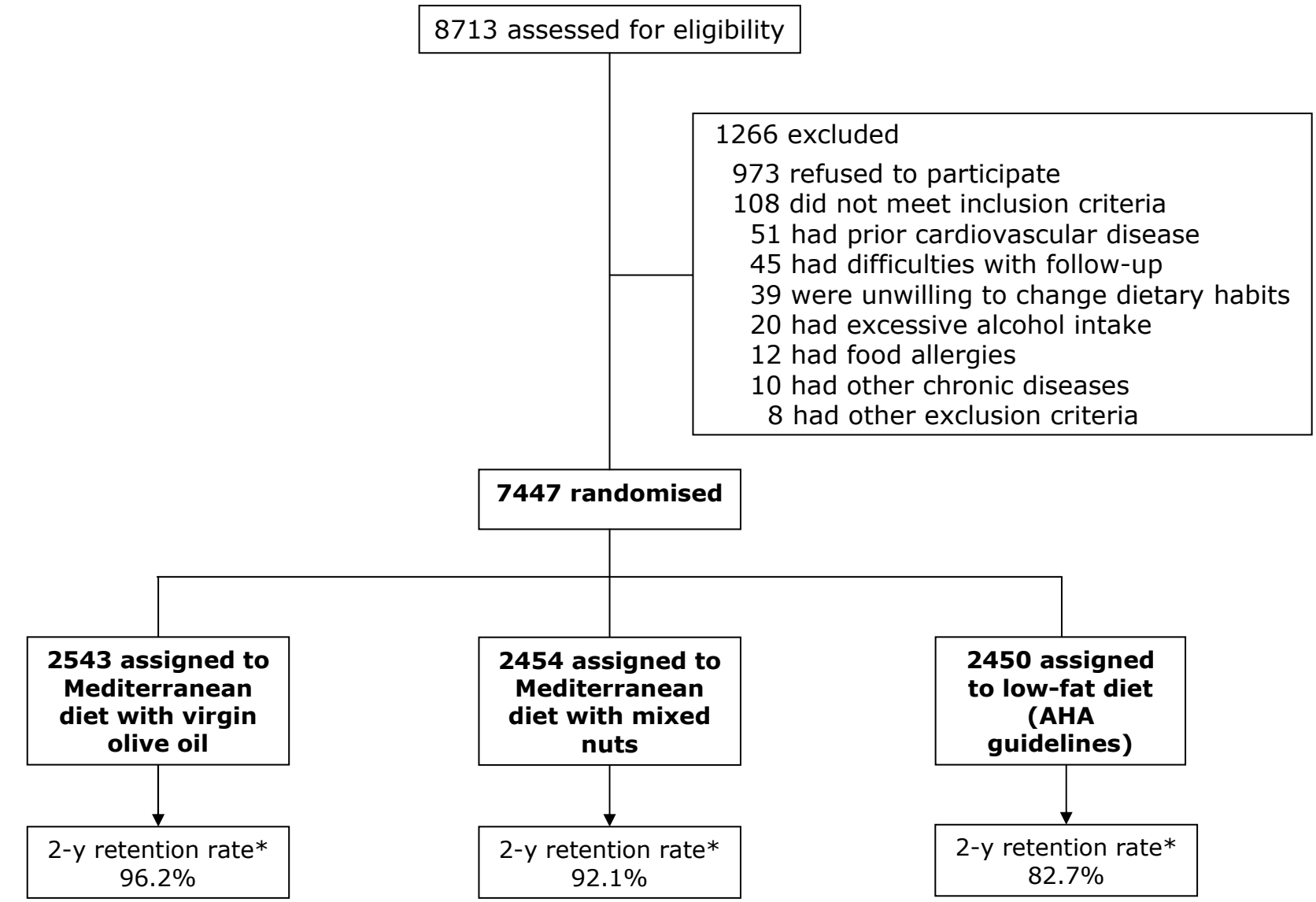
*Only for participants recruited before 2005. **Includes direct measurements of weight, height, waist circumference, BP, and ankle-brachial blood pressure index.

***Only if applicable. ** Only for participants recruited after 2007. (o)- Optional collection.



*Calculated among 4.381 participants recruited during 2003-2005

Figure 1. Flow chart of participants in the PREDIMED study.



*Calculated only among participants recruited during 2003-2005