COMPONENTS OF THE MEDITERRANEAN DIET ON CARDIOVASCULAR DISEASE AND MORTALITY IN A POPULATION AT HIGH CARDIOVASCULAR RISK.

Marta Guasch Ferré

Dipòsit Legal: T 1834-2014

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DOCTORAL THESIS

Thesis supervised by Prof. Jordi Salas-Salvadó, and co-supervised by Dr. Mònica Bulló

Unitat de Nutrició Humana
Departament de Bioquímica i Biotecnologia
UNIVERSITAT ROVIRA I VIRGILI
Reus, 2014

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CERTIFICO:

Que aquest treball, titulat "*COMPONENTS OF THE MEDITERRANEAN DIET ON CARDIOVASCULAR DISEASE AND MORTALITY IN A POPULATION AT HIGH CARDIOVASCULAR RISK*", que presenta la Sra. Marta Guasch Ferré per la obtenció del títol de Doctor, ha estat realitzat sota la meva direcció en el Departament de Bioquímica i Biotecnologia d'aquesta Universitat.

Reus, 03 de Setembre de 2014

El Director de la Tesis Doctoral,

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Moltes gràcies a tots i totes.

ABSTRACT

ENGLISH:

Cardiovascular disease (CVD) is one of the main causes of disability and death worldwide. Importantly, in a large extent, CVD are preventable.

The Mediterranean Diet (MedDiet) is recognized as one of the healthier dietary patterns.

To date, strong evidence exists supporting the benefits of the MedDiet for the prevention and management of CVD.

This thesis has been conducted in the framework of the PREDIMED Study, a parallel-group, multicenter, randomized nutrition trial that evaluated the effects of a MedDiet, compared to a low-fat control diet, on the primary prevention of CVD.

The main objective of the present thesis was to asses the associations between the key components and nutrients of the Mediterranean diet (nuts, olive oil and its varieties, and magnesium) and CVD, cause-specific and all-cause mortality on an elderly Mediterranean population at high cardiovascular risk. All of these foods are key components of the MedDiet pattern and are highly consumed in our population.

The results of the present work demonstrate that after 4.8 years of follow-up, the frequency of nut consumption was inversely related to cardiovascular, cancer and total mortality. Olive oil consumption, specifically the extra-virgin variety, was associated with a reduced risk of cardiovascular disease and cardiovascular mortality. It was also observed that dietary magnesium intake was inversely associated with cardiovascular, cancer and total mortality.

In conclusion, the findings of this work support the healthy benefits of the components of the MedDiet on the primary prevention of cardiovascular disease and mortality.

CATALÀ:

Les malalties cardiovasculars (CV) són una de les primeres causes de morbimortalitat mundial. Aquestes malalties, en gran mesura, es podrien prevenir.

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La Dieta Mediterrània ha estat reconeguda com un dels patrons alimentaris més saludables. Fins el moment existeix l'evidència científica que demostra els beneficis de la dieta Mediterrània en la prevenció i el tractament de la malaltia cardiovascular.

La tesi que es presenta s'ha realitzat en el context de l'estudi PREDIMED, un estudi clínic paral·lel, multicèntric i aleatoritzat que ha avaluat l'efecte de la dieta Mediterrània, en comparació a una dieta baixa en greix, en la prevenció primària de la malaltia cardiovascular.

L'objectiu principal d'aquesta tesi ha estat determinar l'efecte dels components i nutrients de la dieta Mediterrània (fruits secs, oli d'oliva i les seves varietats, i magnesi) en la malaltia cardiovascular i la mortalitat (per causa específica i per totes les causes) en una població Mediterrània amb alt risc cardiovascular.

S'ha tingut en compte que tots els aliments que s'han avaluat són components claus del patró de dieta Mediterrània i són consumits en quantitats relativament altes per la nostra població.

Els resultats del present treball, després de fer un seguiment als participants durant una mitjana de 4,8 anys, demostren que consumir fruits secs amb més freqüència està inversament relacionat amb la mortalitat cardiovascular, amb la mortalitat per càncer i amb la mortalitat total. El consum d'oli d'oliva, concretament la varietat extra verge, s'associa a una disminució del risc de malaltia cardiovascular i mortalitat cardiovascular. També s'ha observat que el magnesi dietètic s'associa inversament a la mort cardiovascular, per càncer i mortalitat total.

Podem concloure que els resultats d'aquesta tesi corroboren els efectes beneficiosos dels components de la dieta Mediterrània en la prevenció de malaltia cardiovascular i en la mortalitat.

CASTELLANO:

Las enfermedades cardiovasculares (CV) son una de las primeras causas de morbimortalidad mundial. En gran medida, estas enfermedades se podrían prevenir.

La Dieta Mediterránea ha sido reconocida como uno de los patrones alimentarios más saludables. Hasta el momento, existe evidencia científica que demuestra los beneficios de la dieta Mediterránea en la prevención y el tratamiento de la enfermedad cardiovascular.

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Esta tesis ha sido realizada en el contexto del estudio PREDIMED, un estudio clínico paralelo, multicéntrico y aleatorizado que evalúa el efecto de la dieta mediterránea, en comparación a una dieta baja en grasa, en la prevención primaria de la enfermedad cardiovascular.

El principal objetivo ha sido determinar el efecto de los componentes y nutrientes de la dieta Mediterránea (frutos secos, aceite de oliva y sus variedades, y magnesio) en la enfermedad cardiovascular y la mortalidad (por causa específica y por todas las causas) en una población Mediterránea con alto riesgo cardiovascular.

Todos los alimentos evaluados son componentes claves del patrón de dieta Mediterránea y son consumidos en altas cantidades en nuestra población.

Después de seguir a los participantes durante una media de 4.8 años, los resultados del presente trabajo demuestran que consumir frutos secos con más frecuencia está inversamente relacionado con la mortalidad cardiovascular, mortalidad por cáncer y mortalidad total. Observamos también que el aceite de oliva, concretamente la variedad extra virgen, se asocia a un riesgo reducido de enfermedad cardiovascular y mortalidad cardiovascular. Por otra parte, el magnesio dietético se asocia inversamente a la muerte cardiovascular, por cáncer y mortalidad total.

En conclusión, los resultados corroboran los efectos beneficiosos de los componentes de la dieta Mediterránea en la prevención de enfermedad cardiovascular y muerte.

ABBREVIATIONS

CVD, cardiovascular disease.
LDL, low-density lipoprotein.
CHD, coronary heart disease.
WHO, World Health Organization.
DALYs, disability-adjusted life-years.
HPFS, Health Professionals Follow-up Study.
BMI, body mass index.
USDA, United States Department of Agriculture.
NHS, Nurses Health Study.
EPIC, European Prospective Investigation into Cancer and Nutrition Study.
CI, confidence interval.
ARIC, Atherosclerosis Risk in Communities Study.
CHS, Cardiovascular Health Study.
MESA, Multi-Ethnic Study of Atherosclerosis Study.
PREDIMED, PREvención con DIeta MEDiterránea Study.
EUROASPIRE, Evaluation of secondary prevention of coronary heart disease Study.
OR, odds ratio.
SNP, single nucleotide polymorphism.
MedDiet, Mediterranean Diet.
RCTs, randomized controlled trial.
RR, relative risk.
EVOO, extra-virgin olive oil.
MUFA, monounsaturated fatty acid.
NHANES, National Health and Examination Nutrition Survey.
PUFA, polyunsaturated fatty acids.
HDL, high-density lipoprotein.
GI, glycaemic index.
GL, glycaemic load.
AHA, American Heart Association.
ALA, alpha-linolenic acid.
IOM, Institute of Medicine.
NCEP, National Cholesterol Education Program.
FFQ, food frequency questionnaire.

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1. INTRODUCTION

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1.1. CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is one of the main causes of disability and death worldwide. It is considered as non-communicable, and by definition, is non-infectious and non-transmissible among people. It has been estimated that more than 17 million people died from CVD in 2008. Of them, 3 million died before the age of 60[1]. The percentage of premature deaths from CVD ranges from 4%, in high-income countries, to 42%, in low-income countries[1]. The high prevalence of CVD across the world has become an important health and socioeconomic problem, thus increasing public health cost. Since evidence suggests that most of CVD and its related risk factors are largely preventable by primary prevention, the efforts should be focused on increasing the awareness of its importance. Global and national strategies based on the use of adequate medication to reduce hospitalisation and incremental costs of cardiovascular events and also behavioural risk factors, including changes in lifestyle and diet, could be effective for the primary prevention of CVD.

1.1.1. Definition and physiopathology

CVD, the most frequent cause of worldwide deaths, comprises the diseases of the arterial tree. CVD is often manifested by coronary heart disease, which includes myocardial infarction or angina secondary to ischemic coronary artery disease, cerebrovascular disease (i.e. stroke) and peripheral vascular disease. Other manifestations of CVD are aorta aneurysm or erectile dysfunction[1], which affect other arteries. The physiopathology of atherosclerosis is a progressive process that includes accumulation of lipids, cholesterol, fibrous elements and immune components in the subendothelial layer of the larger arteries. These accumulations lead to a progressive occlusion of the vessel lumen of the arteries; and increasing permeability of the endothelium layer contributes to the accumulation of low-density lipoprotein (LDL) particles in the intima. In the next stage, monocytes can be observed adhering to the surface of the endothelium. Here monocytes proliferate, differentiate into macrophages and take up the

lipoproteins to form subendothelial accumulations of cholesterol-rich macrophages called 'foam cells'[2]. When the foam cells die, they release their lipid-filled content to the necrotic core of the lesion. Some fatty streaks subsequently accumulate in the smooth muscle cells and occlusive fibrous plaques develop[2]. The resulting atheromatous plaques occlude the blood vessel and reduce the elasticity of the artery walls, impeding blood flow. A key challenge is to identify the vulnerable or non-stable plaques because their rupture releases lipid fragments and cellular debris into the vessel lumen, where they are exposed to thrombogenic agents on the endothelial surface, resulting in the formation of a thrombus[3]. The rupture of the atherosclerotic plaque and the formation of a thrombus have important clinical relevance because they are complex pathological processes that develop over many years, ultimately contributing to coronary heart disease (CHD) and cerebrovascular disease with serious consequences.

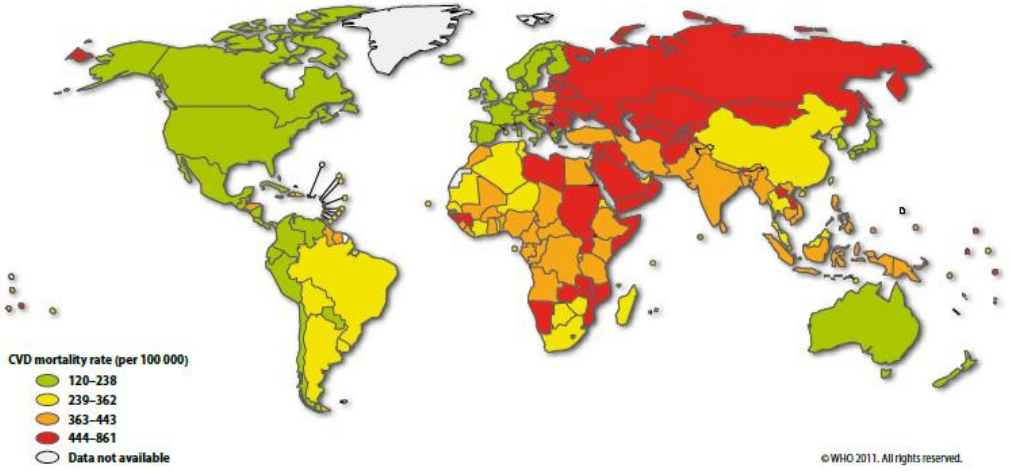
1.1.2. Epidemiology

According to the World Health Organisation (WHO)[1], of the 57 million global deaths that occurred in 2008, 17.3 million (30%) were due to CVD. In recent years, deaths from CVD have declined in high-income countries, but have increased in low- and middle-income countries to account for over 80% of all cardiovascular deaths. It is estimated that in 2030, about 23 million people, 36% more than in 2008, will die from CVD[1] (See **Figure 1**). In 2011, the leading cause of death was CVD (31%) and other non-communicable disease (33%) (See **Figure 2**)**.** Both in men and women, the first cause of cardiovascular death is ischemic heart disease (46% in men and 38% in women), followed by cerebrovascular disease (34% in men and 37% in women)[1] (See **Figure 3**).

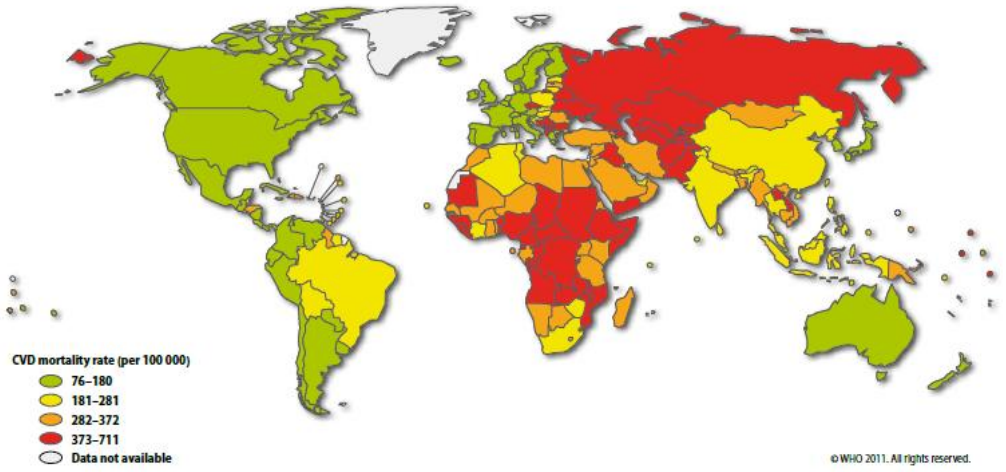
Importantly, 29% of non-communicable disease deaths in low-income countries and 13% in high-income countries occur before the age of 60. According to the WHO, CVD was responsible for the largest proportion (39%) of non-communicable disease deaths in people less than 70 years old[1]. CHD is a major contributor to disability-adjusted life-years (DALYs) loss, which is calculated as the time lost due to premature mortality and time lived with disability. In Europe,

16 million DALYs were lost due to CHD in 2002[4]. In Spain alone, 125,000 deaths per year are attributed to atherosclerosis, 5 million people are hospitalised because of CVD and about 56,000 patients per year are diagnosed with CVD[5].

The main risk factors of CVD are type 2 diabetes, hypertension, dyslipidaemia and abdominal obesity. Other contributors are unhealthy diet, tobacco smoking, physical inactivity and the harmful use of alcohol, which lead to a subsequent weight gain. These features, in combination with an aging population, are triggers that increase the prevalence of CVD, which could be considered the epidemic of the 21st century.



Men



Women

Figure 1. World map showing the global distribution of cardiovascular disease mortality rates (age standardised, per 100 000) (*WHO Report 2011*)[1].

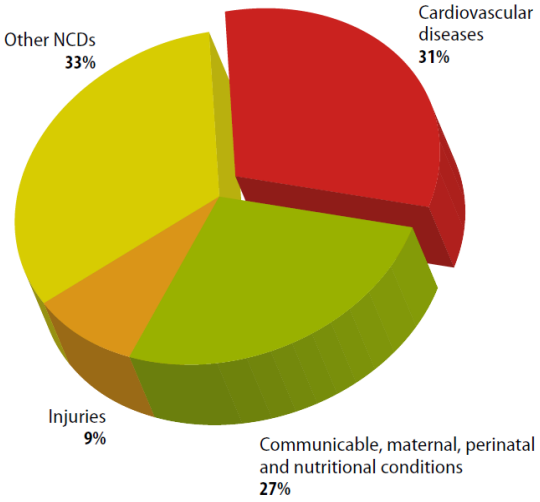
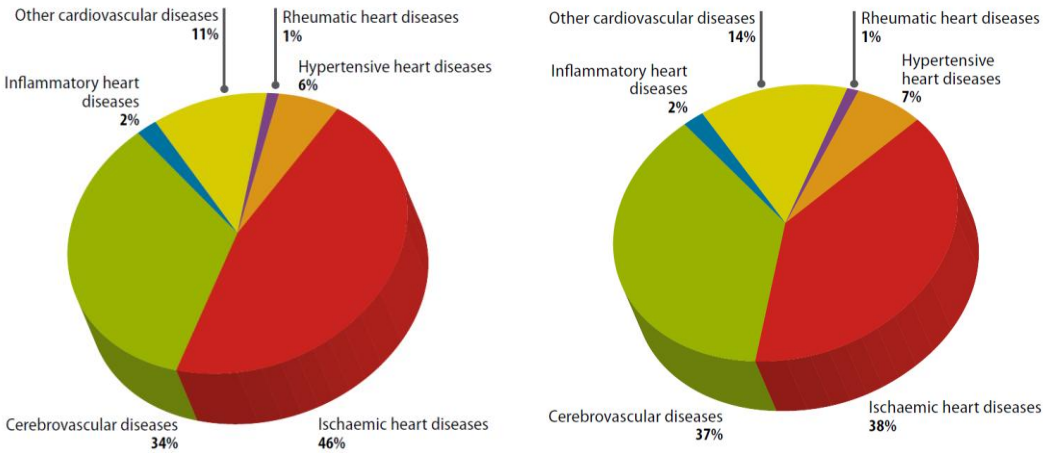


Figure 2. Distribution of major causes of death worldwide (*WHO Report 2011*)[1].



Men **Women**

Figure 3. Distribution of cardiovascular disease deaths due to myocardial infarction, stroke and other types of cardiovascular disease (*WHO Report 2011*)[1].

1.1.3. Cardiovascular risk factors

Cardiovascular risk factors are those that contribute to a higher probability or risk of suffering from CVD, and are related to an increased risk of death from cardiovascular causes. A number of metabolic and behavioural risk factors have been associated with an increased prevalence of CVD and mortality. Non-modifiable risk factors (e.g. age, sex, ethnicity and family history) and modifiable risk factors (e.g. physical activity, tobacco smoking and diet) often coexist and act synergistically to increase the total risk of developing CVD, which can be considered a multifactor disease. Some of the well-identified strong risk factors of CVD are: type 2 diabetes mellitus, hypertension, dyslipidaemia, abdominal obesity and metabolic syndrome. Lifestyle and diet play an important role in the prevention of these risk factors that lead to an increased risk of CVD, its related complications and mortality. It has been demonstrated that reducing diastolic blood pressure by 5 mmHg through pharmacological treatment reduces the risk of stroke by 34% and ischemic heart disease by 21%[6]. A meta-analysis has also shown that reducing LDL cholesterol concentrations by 1 mmol/L was associated with a 26.6% lower risk of CVD, a 28% lower risk of cardiovascular mortality and a 15.6% lower risk of all-cause mortality[7]. In the diabetic population, treatment with lipid-lowering medication also reduces cardiovascular risk[8]. In addition, a systematic review and meta-analysis of 87 studies found that metabolic syndrome is associated with a 2-fold increase in the risk of stroke, CVD and cardiovascular mortality and 1.5-fold increase in the risk of all-cause death[9].

Besides pharmacological treatment to reduce cardiovascular risk factors that lead to hospitalisation, and increased cardiovascular events and its associated costs, a great percentage of CVD and deaths could be prevented by reducing modifiable risk factors. According to the WHO, 9% of deaths are attributed to tobacco smoking, 6% to physical inactivity and 5% to overweight and obesity[1] (See **Figure 4**). Large epidemiologic cohort studies have demonstrated that a healthy lifestyle results in a lower risk of developing CVD and other chronic diseases. In 1986, *Health Professionals' Follow-up Study* (*HPFS*), which included 42,847 men, showed that those who did not smoke, had a body mass index (BMI) < 25 kg/m^2, worked out at least 30 minutes per day, had a moderate alcohol intake (5-30 g/day) and had a healthy diet according to

the United States Department of Agriculture (*USDA*) healthy index, had an 87% lower relative risk of cardiovascular events after 16 years of follow-up. It has also been demonstrated that for those who adopted 2 of these 5 healthy behaviours, the risk of CVD was lowered by 27%[10].

These results were consistent with later results found in the *Nurses' Health Study (NHS)*, including more than 84,000 women, where similar percentages for reduction for CVD risk were observed in the women who had a healthy lifestyle[11]. Large European Studies, such as the *European Prospective Investigation into Cancer and Nutrition* (*EPIC)-Norfolk* cohort, have also demonstrated the beneficial effect of healthy lifestyles on the prevention of CVD. The *EPIC-Norfolk* study followed 20,244 men and women from England over 11 years and evaluated the association of 4 lifestyle factors on mortality. Each of the health behaviours: smoking, being physically inactive, not having a moderate alcohol intake, and a low fruit and vegetable intake as indicated by plasma vitamin C level <50 mmol/l were associated with significantly higher risks of mortality from all causes. After adjusting for potential confounders, the relative risk (95% confidence interval (CI)) of all-cause mortality for those who had one, two, three or four unhealthy factors were 1.39 (1.21-1.60), 1.95 (1.70-2.25), 2.52 (2.13-3.00) and 4.04 (2.95-5.54), respectively (*P* for trend <0.001)[12]. Data from the *Potsdam Study,* which included 23,153 participants followed for 8 years, showed that adherence to four healthy behaviours (no smoking, BMI < 30 kg/m^2, physical activity greater or equal to 3.5 hours per week, and a high consumption of fruits and vegetables, whole grains and a low consumption of red meat) was associated with a 78% lower risk of any of the following chronic disease: diabetes, myocardial infarction, stroke and cancer. Having only one of the healthy behaviours was associated with a reduced risk of chronic disease by 50%[13].

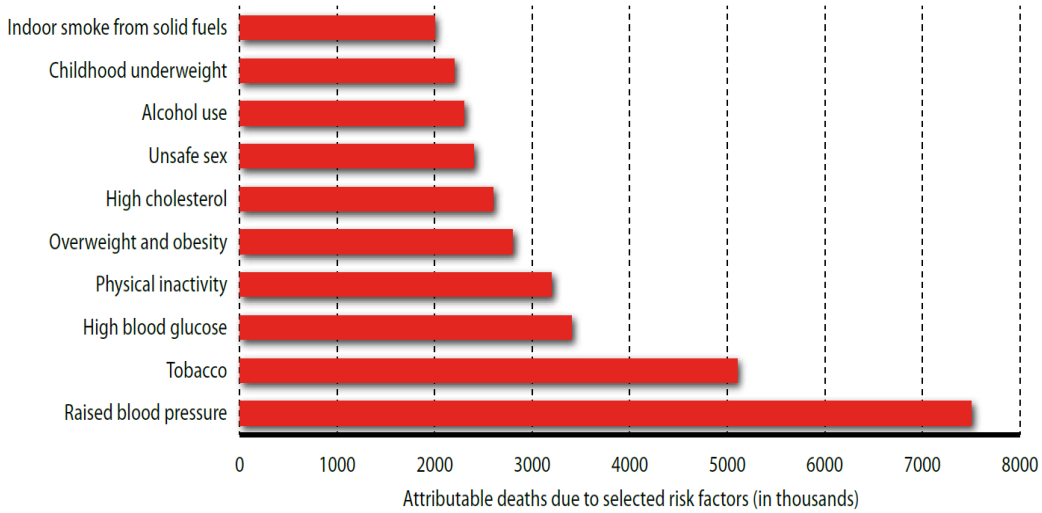


Figure 4. Ranking of 10 selected risk factors of cause of death (*WHO Report 2011*)[1].

1.1.3.1. Non modifiable risk factors

a) Sex and ethnicity

The risk of incident CVD is generally greater in men, albeit the risk in women increases after menopause. This gender difference is due to the protective antioxidant effect of the hormone oestrogen in women during fertile years. Most CVD risk factors, CVD events and CVD-related mortality appear to be significantly higher among blacks than whites.

On the basis of data collected from large prospective studies, such as the *Atherosclerosis Risk in Communities Study* (*ARIC*), the *Cardiovascular Health Study* (*CHS*), and the *Multi-Ethnic Study of Atherosclerosis* (*MESA*), it was determined that CVD events affect blacks earlier in life than whites. However, this association disappeared when the data was adjusted for CVD risk factors. In the *ARIC* study, black men and women aged 45 to 64 at baseline were shown to be more likely than their white counterparts to experience CHD[14].

Coronary artery calcification strongly correlates with the magnitude of coronary atherosclerotic plaque burden and with the development of subsequent coronary events. A family history of premature CHD is a known risk factor for CHD events and may indicate an increased susceptibility to atherosclerosis development and progression. The *MESA* study assessed the association between family history of premature CHD and coronary artery calcification. The *MESA* study results showed that a family history of premature CHD is strongly associated with coronary artery calcification not only in whites and blacks but also in Hispanics and Chinese. In fact, the association of a family history of premature CHD with coronary artery calcification appeared stronger among Chinese Americans than other ethnic groups. These relationships were independent of other risk factors[15].

b) Age

One of the most prominent, but also non-modifiable, risk factor of cardiovascular disease is advancing age. The burden of CVD has been accelerated in recent years by an increase in the ageing population. The increase in life expectancy in developed countries is not always balanced with the quality of life in later years, with CVD becoming increasingly common during advancing age. As a person gets older, the heart undergoes subtle physiologic changes, even in the absence of disease. Years ago, the most widely used risk prediction model, the Framingham equation, estimated 10-year risk of CHD and included age as one of the main risk factors for CHD, among others, such as blood pressure and smoking[16]. Subsequently, age has been included in many newer prediction models as a principal risk factor to predict the incidence of CVD.

More recently, a meta-analysis using data from 18 cohort studies and including more than 250,000 black and white participants showed that the risk of CVD increases with age. However, among participants who were 55 years of age, those with optimal risk-factor profile (e.g. optimal cholesterol, optimal blood pressure concentrations, non-smokers and non-diabetic) have a substantially lower risk of dying from CVD through 80 years of age than participants with more major risk factors (4.7% vs. 29.6% among men and 6.4% vs. 20.5% among women)[17].

c) Genetics and familiar history

It is extensively known that CVD is a well-known multifactorial disease that involves both genetic and environmental factors. CVD could also be associated with a multigenetic predisposition; however, this is still not clearly established.

Family history of premature CHD has been strongly related to a higher prevalence and magnitude of CHD in several studies. In a multiethnic US study with a cohort of 38% White, 28% Black, 22% Hispanic, and 12% Chinese participants, the results showed that family history of premature CHD was significantly associated with coronary artery calcification in all ethnic groups, and the relation was independent of other risk factors and the Framingham Risk Score[15]. The findings of this study were consistent with previous published studies that showed familial clustering of CHD. For example, in the *Framingham Heart Study*, the risk for an adverse cardiovascular event was increased for those individuals having a family history of premature heart disease (Odds Ratio (OR), 1.7 to 2.0)[18]. In addition, early twin studies determined that heritability for coronary artery disease is an established risk factor. Data from 21,004 Swedish twins followed for 26 years showed that at younger ages, death from CHD were higher for monozygotic twins than dizygotic twins in both men and women. This study also observed genetic effects to be greater in younger twins than in older twins[19].

Besides twin studies, linkage analysis, allele sharing methods, association studies, and analysis of large crosses in model organisms are other approaches to genetically dissect CVD and other complex diseases. More than 300 candidate genes for coronary artery disease have been identified by the candidate gene approach, which assesses the association of a particular allele or variant of a gene with a disease. This can be done for a wide range of metabolic pathways involved in lipid metabolism, blood coagulation, blood pressure, inflammation, cell cycle regulation, and others. A common problem with many published association studies is that a positive association observed in one study is often not reproduced in subsequent studies. However, certain genes such as *Angiotensin I converting enzyme* (*ACE*), *Angiotensin II receptor type 1* (*AGTR1*), *Angiotensinogen* (*AGT*), *Methylenetetrahydrofolate reductase* (*MTHFR*), *Nitric oxide synthase 3* (*NOS3*), *Paraoxonase 1* (PON1), *Serpin peptidase inhibitor, clade E, member 1*

(*SERPINE1*) and *Interleukin-6* (*IL6*) that were found to be associated with CHD have been widely replicated across multiple study populations[20]. Using the approach of *Genome Wide Association Scan* (*GWAS*) based on *Single Nucleotide Polymorphism* (SNPs), 13 novel genomic regions at which common genetic variants influence the predisposition for CVD have been identified in a meta-analysis conducted by the Coronary Artery Disease Genome-Wide Replication Meta-Analysis (*CARDIoGRAM*) group and ten previously established loci were replicated[21]. The *International Consortium for Coronary Artery Disease* is another group involved in large-scale meta-analyses of *GWAS* and has identified three additional loci. So far, 32 novel loci have been established from both independent *GWAS* studies and meta-analyses for CHD phenotype[22].

A few years ago, nutrigenetics arose as a way to identify informative genetic variants responsible for gene-diet interactions that differ dramatically among individuals. This provides additional information about the mechanisms associated with the interindividual variability in dietary response that can be used to develop more personalised dietary recommendations[23]. For instance, findings from the *PREDIMED Study* (*PREvención con DIEta MEDiterránea Study*) showed that cardioprotective effects of the Mediterranean Diet (MedDiet) may be magnified in those participants with a genetic predisposition for diabetes[24]. Transcription factor 7-like 2 (*TCF7L2*) gene is the strongest and most widely replicated locus associated with type 2 diabetes but few studies have examined the relation of this gene with CVD. In the *PREDIMED Study*, the TCF7L2-rs7903146 polymorphism was associated with type 2 diabetes and stroke (adjusted HR 1.87 (1.62–2.17) and 2.91 (1.36–6.19), respectively, for TT allele compared with CC alelle). The study determined that for allele homozygosis (TT) participants with a higher risk for diabetes and stroke, a greater adherence to the MedDiet attenuated their genetic risk[24], underscoring the idea that the genetic predisposition towards CVD could be reduced by dietary intervention such as the MedDiet. Other findings from the *PREDIMED* Study have suggested that a variant (*rs3812316*, *C771G*, and *Gln241His*) in the *MLXIPL* (*Max-like protein X interacting protein-like*) gene encoding the carbohydrate response element binding protein were associated with lower plasma triglyceride concentrations and could be enhanced by adherence to the MedDiet[25].

1.1.3.2. Modifiable risk factors

a) Physical activity

The first evidence relating physical activity to CVD dates from the 1953. "*The London Bus Study*" showed that individuals who worked as ticket collectors on double-decker London buses had a lower risk of dying from CVD compared to the drivers, who did not engage in physical activity during work time[26]. Since then, many studies have related physical activity, during both leisure and work, to lower prevalence and incidence of CVD. Recent studies have demonstrated that low to moderate physical activity reduces the risk of cardiovascular events. Thus, physical activity is a staple lifestyle target for the primary and secondary prevention of CVD[27,28]. In the context of CVD prevention, regular physical activity has been observed to contribute to weight loss, glycaemic control, and improved blood pressure, lipid profile and insulin sensitivity[28]. The beneficial effects of physical activity on CVD may be, in part, mediated by these risk factors. In fact, the current scientific societies recommend at least 30 minutes of low to moderate physical activity most days of the week to reduce the risk of cardiovascular.

In addition, physical activity has also been associated with reduced risk of death from CVD. The risk of all-cause mortality is increased by 20-30% in people who are insufficiently physically active compared with those who moderately or intensely exercise at least 30 minutes most days of the week. Data from the WHO has corroborated that approximately 3.2 million deaths each year are attributable to insufficient physical activity[1].

On the other hand, results from prospective observational studies suggest a strong positive relation between sedentary lifestyle, CVD and mortality[29]. A noteworthy finding was that sedentary women who became physically active between baseline and after 6 years of follow-up had 32% and 36% lower all-cause and cardiovascular mortality rates, respectively, compared with women who were sedentary at both visits[29]. In addition, the risk of all-cause mortality in physically unfit or sedentary diabetics is >2 times higher compared with physically fit men and women diabetics regardless of body weight[29].

b) Tobacco smoking

Tobacco smoking is one of the main causes of death worldwide. Even though more than 3,000 people die every day from tobacco-smoking related complications, about 30% of the world's population smokes[1]. Harmful risks to health from tobacco use result not only from direct consumption of tobacco but also from exposure to second-hand smoke[30]. Approximately six million people die every year from smoking and exposure to second hand smoke, accounting for 6% of all female and 12% of all male deaths in the world. By 2030, deaths related to smoking will increase to more than 8 million deaths per year[1]. It is estimated that smoking causes 10% of CVD, and that people who smoke have doubled the probability of suffering from a major cardiovascular event compared with non-smokers[1].

INTERHEART study reported that smoking was associated with a 3-fold increased risk of suffering from non-fatal CHD. In addition, the risk relationship was suggested to be dose-dependent, with the risk increasing by 4.6-fold when 20 or more cigarettes per day were smoked[31]. Moreover, data from the *Evaluation of secondary prevention of Coronary Heart Disease Study (EUROASPIRE III)*, which included European patients younger than 70 years of age who had suffered from cardiovascular events, demonstrated that 30% of the participants were smokers when they suffered the coronary event. These data were consistent with previous results from *EUROASPIRE I* and *EUROASPIRE II* studies[32]. Scientific evidence has also suggested smoking cessation provides a number of benefits on CHD and mortality[1]. The risk of myocardial infarction decreases by half after one year of smoking cessation compared with current smokers. Furthermore, after 5 to 15 years of smoking cessation, the risk of stroke and CHD is the same as the risk of those who had never smoked, if no target organ injuries occurred before smoking cessation[33].

c) Diet

Briefly, a considerable body of evidence shows the negative effects of an unhealthy diet on CVD and mortality. High dietary intake of saturated fat, trans fat, cholesterol, salt and low dietary

intake of fruits, vegetables and fish have been positively associated with cardiovascular risk[1]. Obesity is one of the main risk factors of CVD and is strongly related to an unhealthy diet and physical inactivity.

About 1.7 million (2.8%) deaths worldwide are attributed to a low intake of fruits and vegetables[4], and an adequate consumption of fruit and vegetables has been shown to reduce the risk of CVD[4]. Another important dietary factor is salt, which can be used as a determinant of blood pressure levels and cardiovascular risk[34]. In addition, frequent consumption of high-energy foods, such as processed foods that are high in salt, fats and sugars, leads to increased obesity and consequently a higher cardiovascular risk compared with consumption of low-energy foods[1]. A healthy diet can contribute to a healthy body weight, a desirable lipid profile and an optimal blood pressure, thus, reducing the risk of CVD and mortality. Actions promoting a healthy and balanced diet should be the cornerstone of CVD primary and secondary prevention. More detailed data about dietary patterns, foods, nutrients, the components of the MedDiet and its relation with CVD and mortality are presented below.

1.2. DIET, CARDIOVASCULAR DISEASE AND MORTALITY

1.2.1. Dietary patterns, cardiovascular disease and mortality

A growing body of literature has shown that not only the effect of isolated nutrients or foods (e.g. vitamins, minerals, fruits, vegetables and meat) is important, but we should also consider the diet as a whole and evaluate the combination of all dietary components on health. Dietary patterns allow us to assess the synergy of all the nutrients in different foods and how they interact. Certain types of dietary patterns are considered beneficial for health such as "Mediterranean" and "prudent" patterns, but others are considered disruptive for health, such as "Western" patterns, and have been associated with higher risk for chronic diseases[35]. Besides these principal dietary patterns, dietary patterns, such as the DASH-Diet, the Nordic diet or the Vegetarian diet have also been identified. Dietary patterns can influence cardiovascular health

by modifying risk factors such as obesity, dyslipidaemia and hypertension, as well as factors involved in systemic inflammation, insulin sensitivity, oxidative stress, endothelial function and thrombosis.

1.2.1.1. Western pattern

Current scientific evidence has suggested that Western dietary patterns are associated with an increased risk of CVD and mortality. The Western pattern is characterised by a high intake of red and processed meat, refined grains, processed food, sweets, desserts, high-fat dairy products and fried foods.

Epidemiological evidence from a prospective cohort study conducted in the population of the NHS I, which included about 69,017 women aged 38 to 63 years, found that a higher Western-pattern score was associated with a 46% (Relative risk (RR): 1.46; 95% CI, 1.07-1.99) higher risk of total myocardial infarction after multivariate adjustment. Similar results were found for fatal CHD and nonfatal myocardial infarction[36]. In the same cohort of the NHS, it was also observed that a greater adherence to the Western pattern may increase the risk of cardiovascular and total mortality among initially healthy women[37]. These data are in agreement with the prospective cohort HPFS that included men aged 40 to 75 years, where greater adherence to the Western pattern was associated with a 43% (RR: 1.43; 95% CI, 1.01-2.01) increased risk of CHD compared with individuals with the lowest adherence to the Western pattern[38].

Data from the *MESA* study has also reported a positive association between the *a posteriori* "Fats and Processed Meat" dietary pattern and the incidence of CVD[39]. Recently, the associations between Western pattern and the risk of CHD have been analysed in Spanish individuals participating in the *EPIC*-Spain cohort study. The results from this study did not find any significant associations between the "Westernised pattern" and the risk of CHD. However, a MedDiet pattern was inversely associated with the risk of CHD[40]. The authors of the paper postulated that the pattern described as the Westernised pattern is not as unhealthy as the

Western patterns of other regions of the world, namely the United States. The Western dietary pattern has also been associated with an increased risk of cardiovascular and total mortality[37,41,42].

1.2.1.2. Prudent or healthy patterns

On the other hand, prudent or healthy patterns have been associated with beneficial effects on cardiovascular health and other chronic diseases. The prudent pattern is characterised by a high intake of vegetables, fruits, legumes, whole grains, low-fat dairy, fish and poultry. Both the *HPFS*[38] and the *NHS*[36] have reported that greater adherence to what they termed "Prudent pattern" was associated with about a 25% lower risk of CHD. Results of the *MESA* study also showed an inverse association between the Whole Grains and Fruit dietary pattern and the risk of CVD[39]. In addition, the prudent diet was associated with a 28% (RR: 0.72; 95% CI, 0.60–0.87) lower risk of cardiovascular mortality and a 17% (RR: 0.83; 95% CI, 0.76–0.90) lower risk of all-cause mortality when the top quintile was compared with the lowest quintile of adherence in the *NHS*[37].

1.2.1.3. Mediterranean type diet

The much appreciated MedDiet is characterised by high intake of fruits, vegetables, legumes, fish, whole grains, nuts and olive oil; moderate consumption of dairy products and wine, and low intake of red and processed meat and foods that contain high levels of added sugar[43] (See **Figure 5**). The relatively high intake of nuts, olive oil and moderate intake of wine, particularly red wine during meals, makes the MedDiet unique and different from the other prudent diet patterns. The traditional MedDiet has been allied with a reduced risk of cardiovascular risk factors, CVD and mortality[44].

The first scientific evidence, of the health benefits of the MedDiet, probably came from a study in which patterns of food consumption in seven different countries were compared. The ecological study which was conducted several years ago[45] in the Mediterranean regions, revealed that the

dietary habits and the type of fat consumed had healthy compounds that prevent mortality from cardiovascular diseases[45]. As per the *MONICA* study, in Southern European Countries, low rates of CHD and its risk factors were also attributed to the cardio-protective effects of the MedDiet[46].

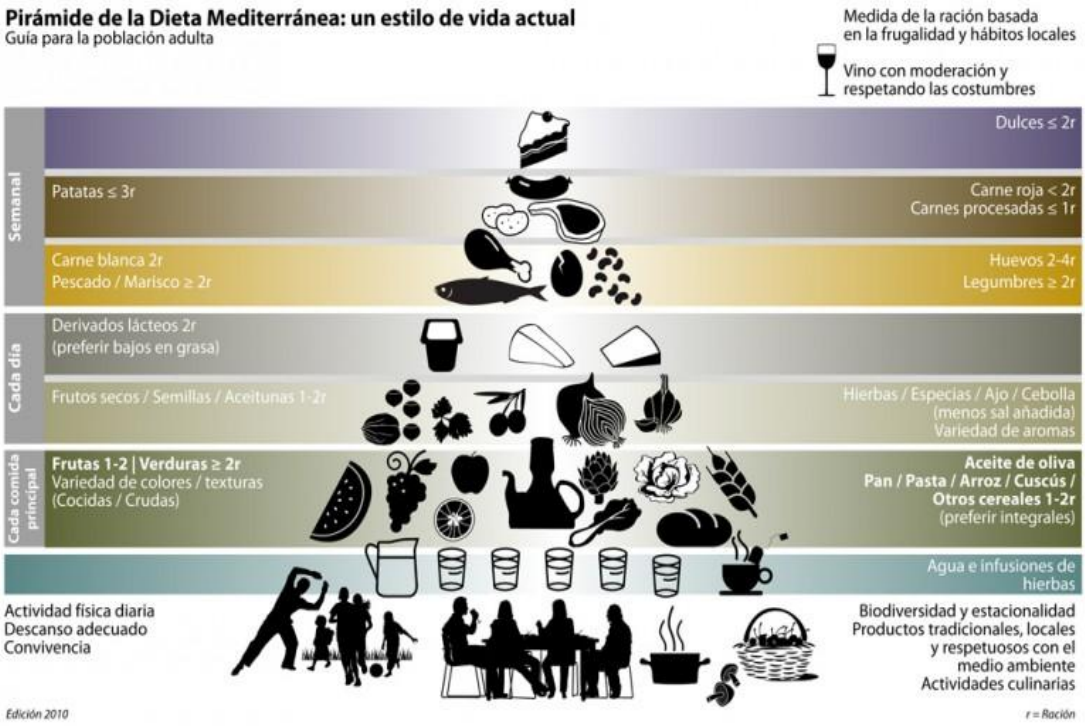


Figure 5. Mediterranean diet pyramid. *Fundación dieta Mediterránea 2010.*

Over the years, a number of significant prospective cohort studies and intervention trials have been conducted, which analysed the relation between MedDiet, CVD and mortality. The foremost finding of such prospective cohort studies suggests associations between MedDiet pattern and a reduced risk of CHD and mortality[44]. **Table 1** shows the data from prospective cohort studies performed to analyse the associations between MedDiet, CVD risk and all-cause

mortality. Most of the studies found a consistent inverse association between MedDiet, CVD and all-cause death, in different populations.

For instance, in the *NHS*, a greater adherence to the MedDiet, as reflected by a higher Alternate MedDiet Score, was found to be associated with 29% (RR: 0.71; 95% CI, 0.62-0.82) lower risk of CHD incidence and 27% (RR:0.87; 95% CI, 0.73-1.02) lower risk of stroke in women[47]. In the *EPIC-Spain* cohort study, MedDiet was associated with 27% (RR: 0.73; 95% CI, 0.57–0.94) lower risk of CHD[40]. *EPIC* also showed that a 2-point increase in a MedDiet score was related to 25% and 8% reduced risk of all-cause mortality in a Greek population[43] and in elderly subjects[48] respectively. Moreover, a meta-analysis comprising seven prospective cohort studies and 2,000,000 subjects, showed that 2-point increase in adherence to the MedDiet, caused significant reduction in overall mortality (by 8%) and CVD incidence and mortality (by 10%)[44].

Table 1. Summary of the evidence from epidemiological cohort studies on the Mediterranean diet and cardiovascular and all-cause mortality.

Reference (year)	Population / Region	Cases/Total	Endpoint	Follow-up	Measurement	Results Multivariate HR (95% CI)
Trichopoulou et al, 1995[49]	Elderly/ Greeks	53/182	All-cause death	1-year	1-unit increase in MedDiet score	0.83 (0.69-0.99)
Kouris-Blazos et al, 1999[50]	Elderly/ Greek Australians and Anglo-Celts	38/330	All-cause death	1-year	1-unit increase in MedDiet score	0.83 (0.67-1.02)
De Lorgeril et al, 1999[51]	Adults/ Swiss	3,935/17,861 (1,385 CVD death)	CVD death All-cause death	32-years	1-unit increase in MedDiet score	CVD death: 0.98 (0.92-1.04) All-cause death: 0.96 (0.94-0.98)
Lasheras et al, 2000[52]	Elderly/ Spanish	96/161	All-cause death	9.5-years	1-unit increase in MedDiet score (only at age <80 years)	0.69 (0.43-0.93)
Knoops et al, 2004[53]	Elderly/ 11 European countries	935/2,339 (371 deaths from CVD)	CVD death All-cause death	10-years	At least 4/8 score in MedDiet	CVD death: 0.71 (0.58-0.88). All-cause death: 0.77 (0.68-0.88)
Trichopoulou et al, 2005[54]	Elderly/ 10 European countries	4,047/74,607	All-cause death	7.5-years	2-unit increase in MedDiet score	0.83 (0.75-0.93)
Lagiou et al, 2006[55]	Adult women/ Swedish	572/42,237	All-cause death	12.01-years	2-unit increase in MedDiet score	0.93 (0.83-1.03)

Table 1. Continuation.

Reference (year)	Population / Region	Cases/Total	Endpoint	Follow-up	Measurement	Results Multivariate HR (95% CI)
Mitrou et al, 2007[56]	Adults/ American	27,779 /380,296 (3,451 deaths from CVD)	CVD death All-cause death	10-years	6-9 MedDiet score compared to 0-3.	CVD death in men: 0.78 (0.69-0.87) in women: 0.81 (0.68-0.95) All-cause death in men: 0.91 (0.88-0.94) in women: 0.80 (0.75-0.85)
Brunner et al, 2008[57]	Adults/ British	33/1,311 (Fatal CHD + nonfatal MI) 51/1,318 (all-cause death)	Fatal CHD + nonfatal MI All-cause death	15-years	MedDiet pattern compared to unhealthy dietary pattern	Fatal CHD + nonfatal MI: 0.77 (0.50-1.19) All-cause death: 0.81 (0.57-1.15)
Trichopoulou et al, 2009[58]	Adults/ Greek	1,075/23,349	All-cause death	8.5-years	2-unit increase in MedDiet score	0.86 (0.80-0.93)
Fung et al, 2009[47]	Adult women/ American	794/76,522	CVD death	20-years	Quintile 5 of MedDiet score compared to Quintile 1	Fatal CHD: 0.58 (0.45-0.75) Fatal Stroke: 0.69 (0.44-1.07)
Buckland et al, 2009[59]	Adults/ Spanish	609/41,078	Fatal and non-fatal CHD	10.4-years	1-unit increase in MedDiet score	Men: 0.94 (0.91-0.97) Women: 0.93 (0.87-0.99)

Table 1. Continuation.

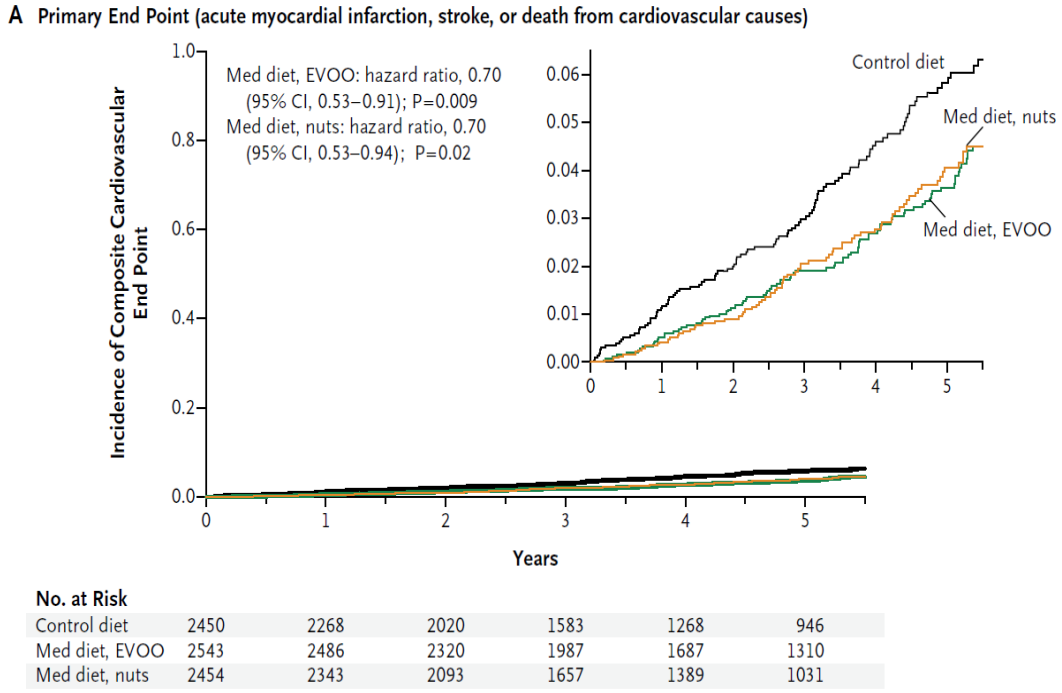
Reference (year)	Population/ Region	Cases/Total	Endpoint	Follow-up	Measurement	Results Multivariate HR (95% CI)
Martínez-González et al, 2011[60]	Middle-aged adults/ Spanish	100/13,609 (incident CVD, 8 fatal)	Fatal and non-fatal CVD and fatal and non-fatal CHD	4.9-years	2-units increase in MedDiet score	Incident CVD: 0.80 (0.62-1.02) Incident CHD: 0.74 (0.55-0.99)
Buckland et al, 2011[61]	Adults/ Spanish	1,855/40,622 (399 from CVD)	CVD death All-cause death	13.4-years	2-units increase in MedDiet score	CVD deaths: 0.88 (0.81-0.95) All-cause death: 0.94 (0.90-0.97)
Gardener et al, 2011[62]	Adults/ American	518/2,568 (incident CVD, 314 CVD deaths)	Incident CVD CVD death	9-years	1-unit increase in MedDiet score	Ischemic stroke: 1.00 (0.90-1.10) MI: 0.94 (0.84-1.05) CVD death: 0.91 (0.85-0.98)
McNaughton et al, 2012[63]	Elderly/ British	654/972	All-cause death	14-years	Quartile 4 of MedDiet score vs quartile	0.78 (0.62-0.98)
Zazpe et al, 2014[64]	Middle-aged adults/ Spanish	148/16,008	All-cause death	6.96-years	Tertile 3 of MedDiet score vs tertile 1	0.53 (0.34-0.84)
Atkins et al, 2014[65]	Elderly men/ British	933/3,328 (327 CVD deaths)	CVD death All-cause death	11.3-years	Quartile 4 of MedDiet score vs quartile 1	CVD death: 0.63 (0.42-0.94) All-cause death: 0.75 (0.60-0.94)

Abbreviations: HR, hazard ratio; CI, confidence interval; MedDiet, Mediterranean diet; CVD, cardiovascular diseases; CHD, coronary heart disease; MI, myocardial infarction.

The intervention studies have also helped to confirm causality on the protective role of the MedDiet on cardiovascular health. The *Lyon Diet Heart Study* was a randomised controlled trial (RCT) of secondary prevention and evaluated the effect of the MedDiet on the risk of suffering from a second myocardial infarction after 46 months of follow-up[66]. It was observed that the individuals who were randomised to the MedDiet group, had 47% reduced risk of myocardial infarction and cardiovascular death, as compared to the control group[66]. In the *GISSI-Prevenzione* secondary prevention trial, patients who had suffered from a myocardial infarction and who were advised to have high intake of fish, fruits, olive oil, raw and cooked vegetables, had 49% reduced risk of death in comparison to those who weren't advised so[67].

Until now, there has been no evidence of the effect of MedDiet on primary prevention of CVD. The *PREDIMED* trial is the largest and unique intervention study which has been designed to evaluate the effect of the MedDiet on primary cardiovascular prevention as compared to a low-fat control diet[68]. So far, the results have demonstrated that the MedDiet supplemented with extra-virgin olive oil (EVOO) or nuts is associated with lower blood pressure, improved lipid profile, reduced concentration of inflammatory markers (such as C-reactive protein), decreased insulin resistance and diminished risk of type 2 diabetes and metabolic syndrome[69–72]. The most important observations of the *PREDIMED* Study suggested that among the persons who are at high risk of cardiovascular disease, an energy-unrestricted MedDiet supplemented with EVOO led to reduced risk of the primary end-point (a composite of myocardial infarction, stroke and cardiovascular death) by 30% (RR: 0.70; 95% CI, 0.54-0.92) and by 28% when the MedDiet was supplemented with nuts (RR: 0.72; 95%, 0.54-0.96). The results were expressed in comparison to the control group, following a low-fat diet[68] (See **Figure 6**).

The epidemiological evidence relating the MedDiet to cancer mortality is still inconclusive. However, a meta-analysis including seven prospective cohort studies suggested that 2-point increase in MedDiet score was associated with 6% reduced risk of death due to cancer (HR: 0.94; 95%, 0.92-0.96)[44].



No. at Risk						
Control diet	2450	2268	2020	1583	1268	946
Med diet, EVOO	2543	2486	2320	1987	1687	1310
Med diet, nuts	2454	2343	2093	1657	1389	1031

Figure 6. Incidence of composite of primary endpoint in the *PREDIMED* trial[68].

1.2.2. Components of the Mediterranean diet, cardiovascular disease and mortality

The beneficial health effects of the MedDiet are primarily attributed to the diet pattern's key foods and characteristic food groups, which contain a number of bioactive compounds, such as a favourable fatty acids, high dietary fibre, antioxidants and phytochemicals[73]. Mediterranean food has strongly contributed to the observable healthy benefits of the MedDiet on cardiovascular health[74].

1.2.2.1. Olive oil

Olive oil has been proposed as one of the key components of the MedDiet which makes it cardio-protective[75]. Olive oil is the main source of vegetable fat and is used abundantly as a culinary fat and for dressing dishes. It is mainly comprised of the mono-unsaturated (MUFA) oleic acid[76,77]. The best quality olive oil, which is rich in taste and colour, and also contains high amount of bioactive compounds, is EVOO variety. Virgin and EVOO varieties are one of the important sources of vitamin E, polyphenols (mainly flavonoids) and other minor bioactive phytochemicals and lipid molecules (such as squalene, tocopherols, etc.)[76]. In the Mediterranean diet pyramid, olive oil is included as an essential food in the main meals.

Experimental studies and clinical trials have shown that olive oil, especially the virgin olive oil, due to its antioxidant capacity, is beneficial in the prevention of CHD and its risk factors[75]. A MedDiet supplemented with olive oil has been observed to improve the lipid profile, and have favourable effects on endothelial dysfunction, hypertension, inflammation, insulin sensitivity and diabetes[69–72,75].

The observational studies have provided promising evidence which suggests that olive oil intake is inversely associated with CVD, in both the Spanish general population[77] and in a cohort of Italian women[78]. In the Spanish cohort of the *EPIC* study, the total olive oil intake was found to be associated with a decreased risk of CHD, cardiovascular mortality and all-cause mortality[79,80]. It is worth mentioning that the effect of virgin olive oil on CHD was greater than the refined olive oil[79]. A lower risk of mortality was associated with regular consumption of olive oil, in an Italian population after myocardial infarction[67] and also in an elderly population[81].

A recent meta-analysis concluded that epidemiologic studies consistently found an inverse association between olive oil consumption and stroke, but there were inconsistencies between the studies which assessed CHD as the end-point[82]. Besides, although the evidence is weak, it has been reported that olive oil could be beneficial in the prevention of certain cancers[83], such as breast cancer.

1.2.2.2. Nuts

Nuts are one of the important components of the MedDiet and a good source of unsaturated fatty acids, rich in vegetable fat and fibre, minerals (potassium, calcium and magnesium), vitamins (folate, vitamin C and E) and other bioactive compounds (phytosterols and polyphenols)[84,85]. There is strong scientific evidence, which suggests that the intake of nuts is favourable for cardiovascular health, and such effect can be attributed to their unique nutritional composition[86]. The current Spanish guidelines encourage the population to consume 3 to 7 servings/week of nuts (1 serving equals 28 g).

A large body of literature have shown that frequent nut consumption is associated with a reduced load of cardiovascular risk factors, like dyslipidaemia, type 2 diabetes and metabolic syndrome[72,87–89]. A pooled analysis of four large-scale observational studies showed that the subjects in the highest category of nut consumption had a 35% reduced risk of incident CHD[88]. The results have been confirmed with the large-scale *PREDIMED* trial which has found 29% reduction in the risk of a composite of cardiovascular events in those individuals who were randomised to a MedDiet supplemented with nuts[68].

The frequency of nut consumption was also found to be related to lower rates of sudden cardiac death in a large cohort of men[90]. Reports from the *Iowa Women's Health study*[91], a large Dutch cohort[92], and the U.S. *NHS* and *HPFS*[93], suggested that frequent nut consumption is inversely related to total and cause-specific mortality. The more recent meta-analysis which included 18 prospective cohort studies indicates that the consumption of nuts is inversely associated with ischemic heart disease, CVD, and all-cause mortality but not significantly with diabetes and stroke. Such findings support the recommendations to include nuts as part of a healthy dietary pattern for the prevention of chronic diseases[94].

The evidence on cancer mortality is scarce[95], however, the prospective studies suggest inverse associations between intake of nuts and colorectal or endometrial cancer[96–98], especially in women, and also prostate cancer[99]. Moreover, a recent prospective epidemiological study on the

NHS and *HPFS* has shown an inverse association between consumption of nuts and death due to cancer[93].

1.2.2.3. Fruits and vegetables

The MedDiet is characterised by high intake of fruits and vegetables, which provide high amounts of vitamins and minerals (beta-carotene, folate, vitamin C, potassium) and also phytosterols and polyphenols (mainly flavonoids). These protective constituents of fruits and vegetables act through a variety of mechanisms, such as reducing antioxidant stress, improving lipoprotein profile, lowering blood pressure, and increasing insulin sensitivity[100]. It is a common belief that higher consumption of fruits and vegetables is protective against CVD[101]. It is recommended the intake of five servings per day of fruits and vegetables.

In general, observational epidemiological studies report a favourable relation between fruit and vegetable intake and CHD occurrence, although sometimes the findings are inconsistent. A meta-analysis of cohort studies have reported that fruit intake decreased the risk of CHD by 4% and vegetable intake by 7%[100]. Consumption of fruits and vegetables have also been inversely associated with cardiovascular and all-cause death[102,103].

1.2.2.4. Legumes and whole grains

The data regarding association of legumes with CHD is still scarce and most of the research has focused on soy products only. However, the results from the *National Health and Examination Nutrition Survey* (*NHANES)* showed that legume consumption four or more times per week was associated with 22% lower risk of CHD as compared to less than once a week intake of legumes[104]. In fact, the Spanish guidelines support that legumes should be consumed two to four times per week (1 serving equals 60-80 g of raw legumes).

Several studies have found an inverse association between the consumption of whole-grains and risk of CVD[101]. Moreover, a review of the available literature concluded that a relationship

between whole-grain intake and CHD is seen with at least 20% and perhaps 40% reduction in risk for those who eat whole-grain food frequently as compared to those who eat them rarely[105]. Whole grains should be chosen in front of refined grains and the current recommendations suggest that 2 servings/d of whole grains should be consumed.

It may be possible that legumes and whole-grains could be inversely related to mortality, owing to the antioxidant activity of phenolic compounds present in legumes and whole-grains[105], the benefits of fibre, vitamins and minerals, and also the low glycaemic index (GI)[85]. However, there is still a lack of evidence on these associations.

1.2.2.5. Dairy products

Dairy products are good sources of vitamins (retinol, riboflavin and vitamin D) and minerals (particularly calcium) but they also contain high amounts of fat, especially saturated fat. For this reason, there is conflicting evidence on the health benefits of dairy products[106]. However, it is recommended the intake of dairy products between two to four servings/d depending on the age and physiologic situation. Low-fat dairy are recommended, in particular, to the adult population.

Based on the current observational studies, the consumption of milk and dairy products is inversely related to the incidence of CVD. A systematic review concluded that the dairy products, although they contribute to the saturated fat composition of the diet, are not associated with an increased risk of CHD[107]. It has been suggested that dairy products could ameliorate characteristics of the metabolic syndrome, a cluster of risk factors that increase the risk of diabetes and CVD[108]. Dairy products, such as cheese, do not exert the negative effects on blood lipids, as predicted solely due to the content of saturated fat. Calcium and other bioactive components may modify the effects on LDL cholesterol and triglycerides. Yogurt may also contribute to beneficial probiotic effects. The consumption of yogurt, and other dairy products, in observational studies, is associated with loss of body weight in the context of energy

restriction[109], and reduced risk of CVD. The findings are, in part, supported by randomised trials[108].

The association between the dairy products and mortality remains unclear, due to inconsistent findings in the observational studies, which have reported positive, negative or no associations[107]. However, a meta-analysis of 17 cohort studies seems to suggest that the intake of milk is not related to cardiovascular and all-cause death[110]. Therefore, studies, which may involve large cohorts, where regular and comprehensive assessments of dairy food consumption can be made, are required to confirm such associations.

1.2.2.6. Fish and seafood

Fish and seafood are rich in protein of high biologic value and are a major source of poly-unsaturated fatty acids (PUFA), omega-3 and omega-6 fatty acids. An optimal balance between omega-6 and omega-3 fatty acids, the anti-inflammatory effects, and other benefits associated to these fatty acids are believed to be important for preventing CHD risk factors[111]. According to the Mediterranean diet pyramid, fish should be consumed at least two times per week.

A few studies have reported associations between fish and lower risk of CHD[112]. A meta-analysis of observational studies has found that fish consumption versus little to no fish consumption was associated with a reduced relative risk of fatal and non-fatal CHD[104].

1.2.2.7. Wine

One of the main characteristics of the traditional MedDiet is the moderate intake of wine mainly during meals. Wine, particularly the red one, contains high amounts of polyphenolic compounds, flavonoids (quercetin, tannins, and catechin) and other compounds which are thought to be beneficial for cardiovascular health. Wine is included in the Mediterranean diet pyramid and it could be consume in moderate amounts by adults.

The consistent epidemiological data suggest that a light to moderate alcohol consumption is associated with reduced risk of fatal and non-fatal CHD and CVD mortality, independently of the type of alcohol beverage[113]. However, red wine, not because of its alcohol content but mainly due to its abundant content of polyphenolic compounds, is thought to provide additional benefits on lowering CVD risk[114]. An updated meta-analysis on the relationship between wine and cardiovascular events has found that the evidence from 16 prospective and case-cohort studies, confirms a J-shaped association between wine intake and fatal or non-fatal cardiovascular events. The lowest risk was apparent at 21 g/d of alcohol intake (RR = 0.69; 95% CI 0.58-0.82)[114]. Similar results from this meta-analysis showed that wine was associated with less risk of cardiovascular mortality (the maximal protection was 34% at 24 g/d) and also provided protection against the risk of total mortality (25% was the lowest risk at 10 g/d intake of wine)[114].

Heavy or binge alcohol consumption, unquestionably, leads to increased morbidity and mortality. Nevertheless, moderate alcohol consumption, especially of alcoholic beverages rich in polyphenols such as wine, seems to confer cardiovascular protective effects both in patients with documented CVD and even in healthy subjects[115].

1.2.3. Nutrients, cardiovascular disease and mortality

In order to establish dietary patterns and to know the foods that provide benefits to health, it is essential to explore which nutrients play important roles in the target pathologies, in this case CVD and also death. Until now, macronutrients (carbohydrates, proteins and fats) and micronutrients, namely vitamins (vitamin C, E, A, B, D and K) and minerals (magnesium, selenium, sodium, calcium and potassium) have been studied to evaluate their effect on CVD and mortality. A plant-based diet, rich in fruits and vegetables, nuts, whole-grain cereals and olive oil, provides a range of nutrients such as dietary fibre, folate, plant sterols, potassium,

antioxidant vitamins, flavonoids and oleic acid, which has been associated with a low risk of CHD and stroke[101].

1.2.3.1. Macronutrients

Traditionally, the evaluation of the relationship between the intake of macronutrients (carbohydrates, protein and fat) and the risk of CVD has been focused on the total consumption rather than their quality. However, several studies conducted during recent years, have highlighted the relevance of the effect of different types of carbohydrates, proteins and fats, and the replacement of one macronutrient by another on cardiovascular health, along with the evaluation of the effect of total macronutrients[116].

Carbohydrates

The scientific evidence, relating carbohydrates to CVD, is still a bit inconsistent. Even though some evidence suggests that low-carbohydrate diets are associated with improvement in certain risk factors (such as serum triglycerides, High-density lipoprotein (HDL)-levels, glycaemia and weight loss)[117], data from two cohort prospective studies have reported that a low-carbohydrate diet based on animal sources was associated with 23% higher risk of all-cause mortality (RR = 1.23; 95% CI 1.01-1.24, comparing extreme deciles) in both men and women. On the contrary, a vegetable-based low-carbohydrate diet was found to be associated with about 20% lower risk of all-cause and CVD mortality rates[118]. In Europe, a cohort study, comprising more than 40,000 participants who were followed-up for 15 years, indicated that after adjusting for potential confounders, a 2-unit increase in the low carbohydrate-high protein score was significantly associated with 5% high incidence of CVD[119]. In addition, a recent meta-analysis of more than 272,216 individuals revealed that the risk of all-cause mortality, among the individuals who had higher punctuation in a low-carbohydrate score, was significantly elevated: the pooled RR (95% CI) was 1.31 (1.07-1.59). The risks of CVD incidence and mortality were not statistically significant, but showed a tendency to be so[120]. Probably, the source and the type of

carbohydrates consumed and the amount of fibre they contain is more important than the total intake of carbohydrates. High intake of dietary fibre is known to be associated with low risk of CVD as depicted in a recent review and meta-analysis[121].

The other factors, that have been widely studied in relation to the carbohydrates of the diet, are the glycaemic index (GI) and the glycaemic load (GL). GI has been defined as the incremental area under the blood glucose response curve of a 50 g carbohydrate portion of a test food, and is expressed as percent of the response to the same amount of carbohydrate from a standard food taken by the same subject[122]. A low-GI and energy-restricted diet containing moderate amounts of carbohydrates, may be more effective than a high-GI and a low-fat diet, in reducing body weight and controlling the metabolic risk factors[123]. There is evidence from epidemiological cohort studies which suggests that high dietary GI and GL increase the risk of CHD[124]. A report from the *NHS* found that after adjustment for potential confounders, dietary GL was directly associated with the risk of CHD (HR: 1.98, 95% CI, 1.41-2.77 for the highest quintile of GL compared to the reference quintile)[125]. The results from the *ARIC* study indicated that every 30-units increase in GL was associated with 14% (HR: 1.14; 95% CI, 1.02-1.26) increased risk of incident CHD in white non-diabetic individuals[126]. Among the Dutch and the Italian women, who consumed modest GL diets, high dietary GL and GI increase the risk of CVD, particularly in overweight women[127,128]. On the contrary, in a large prospective cohort study (*EPIC-MORGEN*), it was observed that the high dietary GL and carbohydrate intake from high-GI foods, increases the overall risk of CVD in men but not in women[129]. The two recent meta-analyses concluded that the high dietary GL and GI significantly increase the risk of CHD in women but not in men, the unfavourable effects being more pronounced in overweight or obese participants[124,130]. Results from another meta-analysis support that there is a linear dose-response relationship between the GL and the CHD risk[131]. Despite the findings that the dietary GI is slightly associated with the risk of CHD, the results of the meta-analysis were insignificant for stroke and stroke-related death[131].

Protein

Generally, it is believed that the vegetable proteins protect from CVD whereas animal proteins increase the risk[132]. The scientific data relating the protein consumption and health outcomes was reviewed recently, in both healthy adults[133] and elderly population[134]. The evidence was found to range from suggestive to inconclusive for protein intake and mortality and morbidity. In contrast, the intake of vegetable proteins was associated with decreased risk of CVD in several studies. The potential adverse effects of protein intake exceeding 20-23% of total energy, remain to be investigated[133,134]. The results from the recent *PREDIMED* Study, demonstrated a U-shape relationship between protein consumption and both body weight and mortality (*Unpublished results*). In particular, the total protein intake was found to be associated with significant increase in risk of all-cause mortality in a Mediterranean population which is at high cardiovascular risk. These data are in line with the earlier studies, which indicated that a high protein intake leads to increased risk of CVD and mortality[118,135]. However, the evidence is still indecisive because some of the studies have found no associations[136,137] or suggested protection due to high protein intake[119,138,139]. Probably, the recommendations should focus on the type of proteins consumed, giving preference to the vegetal proteins and restricting the animal proteins[118,132].

Fats

Dietary fatty acids play significant roles in the cause and prevention of CVD and mortality. Briefly, the type of fat, rather than the total, or the ratio or balance between the saturated and certain unsaturated fats, may be the determinant. CVD risk could be reduced by decreasing the saturated fatty acids and replacing them by a combination of PUFA and MUFA. **Table 2** summarises the data available on the effect of dietary fats and major fat food sources on CVD and mortality.

The trans-fatty acids, from partially hydrogenated vegetable oils, are well-known for their adverse effects, and should be significantly decreased from the dietary patterns for humans.

Both the n-6 and n-3 PUFA are essential and are linked to the benefits against the CVD risk. The intake of MUFA has been related to beneficial health effects, however, its relation with CVD is still unclear[140]. Lately, attention has been given to the effects of saturated fatty acids on cardiovascular health. Substantial accruing evidence from meta-analyses of prospective cohort studies and RCTs indicates that the effects of consumption of saturated fatty acids on CVD risk vary depending upon the replaced nutrient. Therefore, the American Heart Association (AHA) recommends to limit the intake of saturated fatty acids[140]. Recently, the research in the field, has questioned if there are really positive significant associations between saturated fat intake and CVD, as traditionally speculated. Most of the meta-analyses failed to show associations between the intake of saturated fat and risk of CHD, stroke or mortality. However, the studies were unable to consider the effects of replacing nutrients alone and it may be a bias in self-reporting the intake of saturated fat in dietary questionnaires[141–144]. For the moment, the available data is insufficient to conclude that the dietary saturated fat is associated with an increased risk of CVD, stroke and mortality.

On the other hand, PUFA in place of saturated fat, was found to be associated with reduced risk of CHD and death in a pooled analysis of 11 prospective cohort studies, however, no association was seen for replacement with MUFA[145]. Another recent pooled analysis, of 8 RCTs, concluded that the CHD risk is reduced by 10% for every 5% intake of energy from PUFA replacing saturated fatty acid[146]. Regarding the intake of the trans-fatty acids, there is strong evidence from meta-analyses supporting a highly significant association between the trans-fat and CVD morbidity and mortality[141,142,147]. This may be explained, chiefly, by the adverse effects of the trans-fat on HDL and LDL cholesterol levels, particularly the industrial hydrogenated trans-fat[148,149]. The AHA recommends limiting the use of trans-fat to maximum extent. Owing to the detrimental effects of the trans-fat, there is no convincing reason to continue their use in food.

The long-chain PUFA, most often, includes n-6 PUFA (linolenic and arachidonic acid) and n-3 PUFA (alpha-linolenic acid (ALA), eicosapentaenoic and docosahexanoic acid). It has been suggested that PUFA improves blood lipids, blood pressure, inflammation and vascular function. The AHA concluded that consumption of at least 5-10% energy intake from n-6 PUFA reduces

the risk of CHD as compared to its lower intake[150]. In addition, for every 5% energy substitution of mainly n-6 PUFA, in place of saturated fat, a reduction of 10% in the CHD risk was observed[146]. The n-6 PUFA has also been inversely associated with sudden cardiac death[151]. A meta-analysis of 20 RCTs suggested that the n-3 PUFA supplementation protected against the cardiovascular death, but had no significant effect on CVD and total mortality[152]. Another meta-analysis among the individuals with high CVD risk from 21 clinical trials, reported a 10% decrease in risk of cardiac death and 9% reduction in risk of CVD events and a trend towards low total mortality[153]. Two RCTs have not found any significant association for major cardiovascular events, cardiovascular death and death from any cause[154,155]. Thus, the evidence on n-3 PUFA is still inconclusive, but it seems that the protective effect of fish on CVD could be attributed to its n-3 PUFA content[140]. Moreover, the ALA exposure was found to be associated with moderately low risk of CVD in healthy adults[156] . Even though MUFA (oleic acid is the major dietary MUFA) is reported to have various benefits against the cardiovascular risk factors, e.g. high HDL-cholesterol, low blood pressure and improved inflammatory status, there is no clear evidence available which indicates reduction in the risk of CVD and mortality by MUFA consumption[140]. Nonetheless, due to lack of any reported detrimental effects of MUFA-rich diets and due to the beneficial effects of MUFA against other cardiovascular risk factors, future studies may show a reduction in the risk of CVD and mortality.

Table 2. Summary of the effects of dietary fat and major fat food sources on cardiovascular disease and mortality.

Dietary factor	Endpoints	Effect	Scientific evidence
Dietary fat			
Saturated fat	Incident CVD	Possible increased risk but not demonstrated	Meta-analysis of cohorts[116,141,142] Meta-analysis of RCTs[157]
	Total and CVD mortality	Possible increased risk but not demonstrated	Meta-analysis of cohorts[116] Meta-analysis of RCTs[157]
Trans fat	Incident CVD	Convincing, significantly associated with increased risk	Meta-analysis of cohorts[142,147,149] and cohorts and RCTs[141]
	Total and CVD mortality	Possible increased risk but not demonstrated for total mortality Convincing, significantly associated with increased risk of CVD death	Meta-analysis of cohorts and RCTs[141] and RCTs[157]
PUFA in place of saturated fat	Incident CVD	Probable, significantly associated with reduced risk	Pooled analysis of cohorts[145] and meta-analysis of cohorts and RCTs[146,157]
	Total and CVD mortality	Possible decreased risk but not demonstrated for total mortality Probable, significantly associated with reduced risk of CVD death	Pooled analysis of cohorts[145] and meta-analysis of RCTs[157]

Table 2. Continuation.

Dietary factor	Endpoints	Effect	Scientific evidence
Dietary fat			
MUFA in place of saturated fat	Incident CVD	Possible decreased risk but not demonstrated	Meta-analysis of cohorts and RCTs[142,145,157]
	Total and CVD mortality	Possible decreased risk but not demonstrated	Pooled analysis of cohorts[145] and meta-analysis of RCTs[157]
Seafood omega-3	Incident CVD	Probable, significantly associated with reduced risk	Meta-analysis of RCTs[152,153,158,159]
	Total and CVD mortality	Possible decreased risk but not demonstrated for total mortality Probable for significantly reduced CVD death	Meta-analysis of RCTs[152,153,158,159]
Plant food omega-3	Incident CVD	Possible decreased risk but not demonstrated	Meta-analysis of cohorts [156]
	Total and CVD mortality	Possible decreased risk but not demonstrated	Meta-analysis of cohorts [156]

Table 2. Continuation.

Dietary factor	Endpoints	Effect	Scientific evidence
Major fat food sources			
Processed meat	Incident CVD	Probable, significantly associated with increased risk	Meta-analysis of cohorts[160] and 3 cohorts [161,162]
	Total and CVD mortality	Probable, significantly associated with increased risk	3 cohorts[161,162]
Fish and seafood	Incident CVD	Possible decreased risk but not demonstrated	Meta-analysis of cohorts and RCTs[163]
	Total and CVD mortality	Possible decreased risk but not demonstrated	Meta-analysis of cohort studies[164,165]
Dairy products	Incident CVD	Possible decreased risk but not demonstrated	Meta-analysis of cohort studies[107]
	Total and CVD mortality	Possible decreased risk but not demonstrated	Meta-analysis of cohort studies[110]
Eggs	Incident CVD	Possibly no effect	Meta-analysis of cohort studies[166,167]
	Total and CVD mortality	Possibly no effect	Meta-analysis of cohort studies[166,167]

Table 2. Continuation.

Dietary factor	Endpoints	Effect	Scientific evidence
Major fat food sources			
Olive oil	Incident CVD	Probable, significantly associated with reduced risk	Meta-analysis of cohort studies[168] and 1 RCT[68]
	Total and CVD mortality	Possible decreased risk but not demonstrated	Meta-analysis of cohort studies[168] and 3 cohorts[67,78,79]
Nuts	Incident CVD	Convincing, significantly associated with decreased risk	Meta-analysis of cohort studies[94,169] and 1 RCT[68]
	Total and CVD mortality	Convincing, significantly associated with reduced risk	Meta-analysis of cohort studies [94,169] and 3 cohorts[93]

Adapted from Michas et al. 2014[140]. Abbreviations: CVD, cardiovascular diseases; RCTs, randomized controlled trials. PUFA, polyunsaturated fats, MUFA, monounsaturated fats. Summary of the evidence for convincing, probable, possible or insufficient effects of dietary fats and major fat food sources on cardiovascular disease, cardiovascular mortality and total mortality. Best available evidence for each diet-disease relationship was obtained from high-quality published meta-analyses of prospective cohorts or randomized trials (RCTs), as well as individual RCTs or prospective cohorts.

1.2.3.2. Vitamins and antioxidants

A few observational studies have suggested an inverse association between vitamins and CVD. However, several large intervention studies have found little or no effect. A meta-analysis on antioxidant supplementation has linked high dose use of vitamin to a modest increase in mortality[170,171]. Most studies have focused on vitamin supplementation rather than the intake of vitamins from foods.

Vitamin C

The data on vitamin C from meta-analysis of prospective cohort studies have indicated a relative lower risk of CVD incidence, in those reported having higher intake of vitamin C supplements[172]. The evidence suggest that ascorbic acid alone did not improve the risk for a composite of cardiovascular events or its components; but there was a decrease in ischemic stroke for the combination of vitamin C and E[173]. This is consistent with meta-analysis of clinical trials where vitamin C showed no effect on the occurrence of CVD[173]. It is likely that if vitamin C reduces CVD, it will be most beneficial for populations with very low dietary intake.

Vitamin E

Findings from epidemiological studies have reported that vitamin E, mainly for being a potent antioxidant, is associated with lower risk of developing CHD[170]. Data from trials are less supportive showing no benefits except for very specific populations. Results from an RCT have provided evidence that high-dose of vitamin E among participants at high cardiovascular risk does not lower the incidence of CVD[174,175]. On the other hand, evidence from the *Women's Health Study have* shown that vitamin E intake was associated with 24% reduction in the risk of cardiovascular mortality (RR 0.76; 95% CI, 0.59-0.98); however, a non-significant reduction in major cardiovascular events was found[176]. The effects of vitamin E from observational and intervention studies are mixed. Data from subgroup analyses from large primary and secondary

prevention trials among women suggest that benefit may be achieved at moderate doses over a long period of time[170].

Vitamin A

The large-scale primary and secondary prevention trials of beta-carotene and vitamin A have failed to show benefits in CVD[170,177]. It could be concluded that there remains insufficient evidence for vitamin A, C, or E, singly or in combination, preventing cardiovascular events in a general population at risk.

B Vitamins

Briefly, the benefits on CVD of greater folate intake (or other B vitamins) are inconclusive, if there are benefits, they are likely to be modest and will mainly be observable in patients without prior CVD[170]. For instance, in a recent meta-analysis of 12 homocysteine lowering trials, the authors concluded that in secondary prevention the strength of the benefit of homocysteine lowering the risk of CVD may not be as strong as previously believed[178]. Current evidence supports the association between elevated plasma homocysteine concentration and CVD, but it does not support supplementation with folate or vitamins B6 or B12 for reducing cardiovascular risk[177].

Vitamin D

Evidence from observational studies has associated lower serum 25-hydroxyvitamin D with higher incidence of CVD. Contrary, clinical trials have not demonstrated a reduction in cardiovascular events with vitamin D supplementation[177]. A recent meta-analysis of observational and RCTs, comparing bottom versus top thirds of baseline circulating 25-hydroxyvitamin D, has shown that those in the bottom had increased risk of death from CVD (pooled HR: 1.35, 95% CI 1.13-1.61), death from cancer (pooled HR: 1.30, 95% CI: 1.07-1.59)

and all-cause mortality (pooled HR: 1.35, 95% CI 1.22-1.49). So, the authors concluded that evidence suggests inverse associations of circulating 25-hydroxyvitamin D with risk of death and also that supplementation with vitamin D3 significantly reduces overall mortality[179]. Another recent review of systematic reviews and meta-analysis suggested that there may be an inverse associations between vitamin D deficiency and the risk of CVD[180].

Vitamin K

Recent results from the *PREDIMED* Study have reported that dietary intake of vitamin K has been related to a reduced risk of cardiovascular and all-cause mortality in a Mediterranean population at high cardiovascular risk[181]. Previous evidence seems to suggest that vitamin K intake is protective against the incidence of CHD or all-cause mortality[182–184], but further studies are required to corroborate these associations.

1.2.3.3. Minerals (magnesium, selenium, sodium, calcium, potassium)

Magnesium

Some existing evidence has suggested that high dietary magnesium intake (major sources of magnesium are vegetables, fruits, legumes, nuts and whole grains) plays a protective role in cardiovascular risk factors and the incidence of CVD. Indeed, a number of meta-analyses on this issue have been conducted. A meta-analysis of 532,979 participants from 19 studies has shown that the pooled RR of total CVD was 15% and 33% lower in the highest category of dietary magnesium and serum magnesium, respectively, than in the reference category[185]. Similarly, in another meta-analysis of seven prospective studies with 241,378 participants, a protection against ischemic stroke was found[186]. The most recent meta-analysis has shown significant associations between circulating magnesium and CVD events. Dietary magnesium was associated with 22% lower risk of ischemic heart disease and showed an inverse significant association between dietary magnesium and fatal ischemic heart disease up to a threshold of

250 mg/d compared with higher intakes[187]. There is also some evidence suggesting an inverse relationship between magnesium intake and cardiovascular mortality[188,189]. Up to the date, prospective studies on cancer mortality have not found associations between dietary magnesium intake and mortality[190,191], further studies are needed to confirm if there is a relation between dietary magnesium and cancer death.

Selenium

Results from a recent meta-analysis of clinical trials showed that there were no statistically significant effects of selenium supplementation on all-cause mortality, CVD mortality, non-fatal CVD events or all cardiovascular events. The authors concluded that the available evidence does not support the use of selenium supplements in the primary prevention of CVD[192].

Sodium

Two recent meta-analyses of RCTs have concluded that higher sodium intake is associated with a higher risk of incident stroke and CVD by nearly 30%. This supports that most people will likely benefit from reducing sodium intake[34,193]. Findings from observational studies have shown a J-shaped relation between sodium intake and risk of CVD[194,195]. Recently, the Institute of Medicine (IOM) explicitly concluded that studies on health outcomes are inconsistent in quality and insufficient in quantity to determine that sodium intake below specific amounts may increase or decrease the risk of heart disease, stroke or all-cause mortality[196]. However, the results of *PREDIMED* Study indicated that, in the context of a MedDiet, reducing sodium intake was associated with a reduced risk of all-cause death, and increased sodium intake was positively associated with CVD and CVD mortality (*Unpublished results*).

Calcium

Lately, calcium intake in relation with CVD received growing attention. The results of the several prospective studies that examined the association between dietary or supplemental calcium and CVD incidence and mortality in both middle-aged and older adults were found to be inconclusive[197]. The relation between blood concentrations of calcium and CVD has also been found to be inconsistent. Secondary analyses of clinical trials have suggested that supplementation with calcium has no established effect on CVD. Large-scale randomised trials on calcium intake as the primary endpoint would be helpful to draw consistent conclusions on this topic[197]. Moreover, supplementation of calcium with or without vitamin D may even modestly increase the risk of CVD, especially myocardial infarction as shown in a reanalysis of *Women's Health Initiative* and its inclusion in a meta-analysis with eight other studies[198].

Potassium

Current body of evidence suggests a moderate association between potassium intake and blood pressure reduction in adults, resulting in benefits in the prevention of the risk of stroke and CHD[199]. Dietary potassium has been inversely associated with the risk of non-fatal and fatal strokes[200,201]. A meta-analysis including 11 prospective studies of potassium intake showed a reduction of 21% with increasing potassium intake and also a trend towards a lower risk of CVD[202].

Even though few other studies regarding other minerals, such as zinc, chromium, or mineral supplementation, and its relation with cardiovascular health have been conducted, there is still a lack of consistent evidence. Further studies need to be conducted to evaluate and confirm these associations.

2. RATIONALE AND JUSTIFICATION

2. RATIONALE AND JUSTIFICATION

CVD is one of the most common chronic diseases worldwide, affecting an extensively increasing number of people, both in developing and developed countries. The mortality rate from this disease remains the topmost cause of death in many countries. A sharp rise in its prevalence has been observed in recent years in parallel to the obesity epidemics. It is estimated that it will continue to increase overwhelmingly in the upcoming years. Moreover, it has become an important public health problem because it is one of the main causes of morbidity and death. Public health services are overstretched by increasing population, and by human and financial burden to deal with heart disease, stroke, diabetes and CVD.

Importantly, in a large extent, CVD are preventable. An increasing body of literature in recent years have found that several modifiable risk factors play important roles in the occurrence of CVD. Thus, the reduction of adverse behavioural risk factors, such as tobacco smoking, physical inactivity and unhealthy diet, is the cornerstone for the prevention of CVD in combination with an adequate pharmacologic treatment to prevent hard cardiovascular events.

Epidemiological and clinical trials have observed inverse associations between healthy diet and CVD, and related outcomes and death. Recently, dietary patterns rather than individual nutrients and food have been identified to be associated with several health outcomes. The MedDiet is recognised as one of the healthiest dietary patterns worldwide. To date, strong evidence exists supporting the benefits of the MedDiet for the prevention and management of CVD. A common characteristic that makes MedDiet unique is the use of olive oil for dressing and cooking and also the high intake of nuts. Among others, olive oil and nuts are key components of the MedDiet that provide high amounts of vegetal fat and many bioactive compounds to the diet. The intake of magnesium is relatively high in Mediterranean population since it is present in some of the characteristic food of the MedDiet, such as in fruits, vegetables, legumes and nuts.

For promoting and recommending MedDiet, it is important to have solid and strong scientific evidence that could explain the benefits and their mechanisms. Even though there is accruing data on the beneficial effect of the MedDiet on many health outcomes, further investigation is

required to confirm the associations and to explore new research areas. Previous epidemiological studies have suggested benefits of the MedDiet for the prevention of CVD and death in healthy populations and also for secondary prevention of CVD. However, the effect of the key components of the MedDiet—olive oil and nuts—on the primary prevention of CVD and mortality remains to be elucidated. The primary prevention of CVD is essential to reduce future morbidity and mortality and to decrease the cost of the treatment.

Therefore, this work has been conducted in the framework of the *PREDIMED* Study, a parallel-group, multicentre randomised trial evaluating the efficacy of a MedDiet compared to a low-fat control diet on the primary prevention of CVD. It has been investigated in depth, as an observational cohort study, the effect of nuts, olive oil and magnesium on the risk of CVD, cause-specific and all-cause mortality on an elderly Mediterranean population at high cardiovascular risk. All of these are key components of the MedDiet pattern and are highly consumed in our population. Moreover, our population is a target population for the primary prevention, since they usually attend Primary Care Clinics.

3. HYPOTHESIS

3. HYPOTHESIS

We hypothesized that the key components and nutrients of the Mediterranean Diet, olive oil, nuts and magnesium, are associated with a decreased risk of cardiovascular disease, cause-specific and overall mortality in Mediterranean individuals at high cardiovascular risk from the *PREDIMED* Study.

4. OBJECTIVES

4. OBJECTIVES

The main objective was to assess the associations between the key components and nutrients of the Mediterranean Diet and cardiovascular disease, cause-specific and overall mortality in Mediterranean individuals at high cardiovascular risk from the *PREDIMED* Study.

In Mediterranean individuals at high cardiovascular risk from the *PREDIMED* Study, the specific objectives were:

- To assess the associations between frequency of nut consumption, cause-specific and overall mortality in individuals from Spain, a Mediterranean country with relatively high nut intake.

- To assess the associations between the intake of total olive oil, its varieties (extra virgin olive oil and common olive oil) and the risk of cardiovascular disease, cause-specific and overall mortality.

- To assess the association between dietary magnesium intake and the risk of cardiovascular disease, cause-specific and overall mortality in Mediterranean individuals with a high average magnesium intake.

5. MATERIAL AND METHODS

5. MATERIAL AND METHODS

5.1. STUDY DESIGN

The *PREDIMED* Study is a large, multicenter, parallel-group, randomized and controlled clinical trial for the primary prevention of CVD. The main aim of the trial was to evaluate the effect of two traditional MedDiets, one supplemented with EVOO and another supplemented with nuts, compared with a low-fat control diet, on primary cardiovascular prevention (a composite of myocardial infarction, stroke and cardiovascular mortality) in a population at high cardiovascular risk. Other secondary endpoints were also evaluated in the context of the *PREDIMED* Study: death of any cause, cancer death, incidence of heart failure, diabetes mellitus, metabolic syndrome, dementia or other neurodegenerative disorders and major cancers (colorectal, breast, lung, stomach and prostate). Intermediate outcomes including changes in blood pressure, weight gain, fasting blood glucose, blood lipids and markers of inflammation were also assessed.

5.2. STUDY POPULATION

The participants were recruited between October 2003 and July 2011 in several recruiting centres from Spain (Málaga, Sevilla, Islas Baleares, Barcelona, Reus-Tarragona, Pamplona, País Vasco, Valencia, Gran Canaria). The study included men (aged 55 to 80 years) and women (aged 60 to 80 years) who were free of CVD at enrolment but at high cardiovascular risk. The inclusion criteria for the participants in the study were having either type 2 diabetes mellitus, or three or more of the following risk factors:

- Family history of premature CVD
- Overweight or obesity (BMI ≥ 25 kg/m^2)
- Current smoking (> 1 cig/day during the last month)
- Hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg 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

On the other hand, the exclusion criteria considered were:

- Documented history of previous CVD, including CHD (angina, myocardial infarction, coronary revascularization procedures or existence of abnormal Q waves in the electrocardiogram) stroke (either ischemic or hemorrhagic, including transient ischemic attacks), or clinical peripheral artery disease with symptoms of intermittent claudication
- Severe medical condition that may impair the ability of the person to participate in a nutrition intervention study (e.g. digestive disease with fat intolerance, advanced malignancy, or major neurological, psychiatric or endocrine disease)
- Any other medical condition thought to limit survival to less than 1 year
- Immunodeficiency or HIV-positive status
- Illegal drug use, chronic alcoholism or problematic use of alcohol or total daily alcohol intake >80 g/d
- Body mass index > 40 kg/m^2
- Difficulties or major inconvenience to change dietary habits
- Impossibility to follow a Mediterranean-type diet, for religious reasons or due to the presence of disorders of chewing or swallowing (e.g. difficulties to consume nuts)
- A low predicted likelihood to change dietary habits according to the Prochaska and DiClemente stages of change model
- History of food allergy with hypersensitivity to any of the components of olive oil or nuts
- Participation in any drug trial or use of any investigational drug within the last year
- Institutionalized patients for chronic care, those who lacked autonomy, were unable to walk, lacked a stable address, or were unable to attend visits in the Primary Care Health Centres every 3 months
- Illiteracy
- Patients with an acute infection or inflammation (e.g. pneumonia) were allowed to participate in the study 3 months after the resolution of their condition

The selection process started extracting names of potential participants from the clinical records. The records were reviewed by the medical doctors and the study investigators to assess the eligibility criteria of the candidates and exclude those participants with any of the exclusion criteria.

Potential participants were contacted by a telephone call or during the routine clinical visits; if they were interested in participating, a face-to face interview was scheduled. In the first interview, the characteristics and objectives of the study were explained and informed consent was obtained. The duration of the first visit was about 30 minutes and allowed to verify if the candidates met all the inclusion criteria. In this screening visit, the inclusion questionnaire (***Appendix I***) and general questionnaire (***Appendix II***) were completed by the *PREDIMED* investigators. The general questionnaire included questions on medical conditions and risk factors for the study purposes; the last electrocardiogram in the clinical history were revised or a new one was performed if the last one was not available; a 137 item food frequency questionnaire (***Appendix III***) and the Minnesota Physical Activity questionnaire (***Appendix IV***) were provided to the participants to complete at home and bring it in the next visit. Participants were also informed that they should come in fasting conditions in the next visit and written information of the study was provided. They were also informed that they might receive free olive oil or nuts for the duration of the study. After participants had signed the informed consent, both for the study participation and biochemical analysis and for the DNA recollection, they were randomized to three equally sized intervention groups. Tables of random allocation were centrally elaborated.

5.3. INTERVENTION

The participants of the *PREDIMED* Study were randomly assigned, in a 1:1:1 ratio, to one of three interventions (See **Figure 7**):

- A Mediterranean type diet supplemented with extra virgin olive oil (MedDiet + EVOO)
- A Mediterranean type diet supplemented with nuts (MedDiet + nuts)

- Advice to follow a low-fat diet (control group)

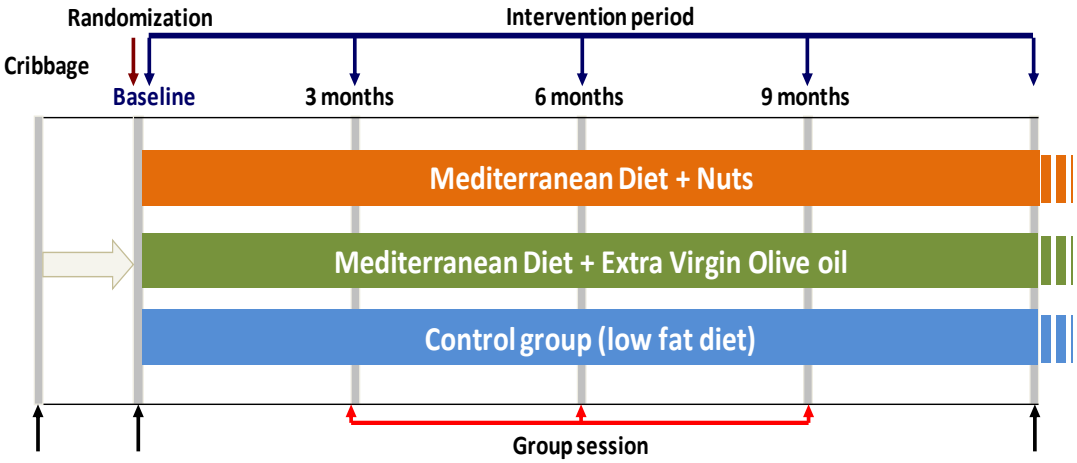


Figure 7. Design of the *PREDIMED* Study.

The *PREDIMED* dietary intervention followed a behavioural strategy focused on promoting a Mediterranean diet in the two intervention groups without specific recommendations on energy restriction or physical activity in any intervention arm. The free provision of extra virgin olive oil or nuts in the intervention groups increased the compliance and the adherence to the intervention. Participants in the control group received free small non-food gifts (kitchenware, tableware, aprons or shopping bags). Registered dieticians were responsible for the dietary intervention and they received several training sessions before and during the trial with experts in nutrition education. At baseline and at follow-up visits, dieticians gave personalized recommendations on dietary changes to the participants in order to achieve the specific aims of each intervention.

Mediterranean diet groups

The participants in the two intervention MedDiet groups, (MedDiet + EVOO) and (MedDiet + nuts) received an intensive intervention including the free provision of approximately 1 litre per week of EVOO or 30 g of mixed nuts (15 g of walnuts, 7.5 g of hazelnuts, and 7.5 g of almonds), respectively. Dieticians ran individual and group dietary-training sessions at baseline visit and quarterly thereafter encouraging the adherence to the MedDiet. Dieticians provided the following recommendations to increase the adherence to the MedDiet:

a) Use olive oil as the main fat for cooking and dressing
b) The consumption of ≥ 2 daily servings of vegetables (at least one of them as fresh vegetables)
c) ≥ 2-3 daily servings of fresh fruits (including natural juices)
d) ≥ 3 weekly servings of legumes
e) ≥ 3 weekly servings of fish or seafood (at least one serving of fatty fish)
f) ≥ 1 weekly serving of nuts or seeds
g) Select white meats (poultry without skin or rabbit) instead of red meats or processed meats (burgers, sausages)
h) Cook regularly and dress vegetables, pasta, rice and other dishes (at least twice a week) with tomato, garlic and onion adding or not other aromatic herbs (*sofrito*)

On the contrary, dieticians discourage the consumption of cream, butter, margarine, cold meat, pate, duck, carbonated and/or sugared beverages, pastries, industrial bakery products (such as cakes, donuts, or cookies), industrial desserts (puddings, custard), French fries or potato chips, and out-of-home pre-cooked cakes and sweets. Total fat intake was *ad libitum* and a high fat intake was allowed, as long as most fat is derived from fatty fish and vegetable sources, particularly olive oil or nuts. A 14-item questionnaire was used by dieticians to give personalized advice on MedDiet during the study visits. The score obtained in the questionnaire was used to assess the adherence to the MedDiet of the participants and also to focus the intervention on the points that participants were not following (**Table 3**). The maximum score was 14 points.

Table 3. Quantitative score of adherence to the Mediterranean Diet.

Foods and frequency of consumption	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 or more tablespoons
3. How many **vegetable** servings do you consume per day? *(1 serving = 200g - consider side dishes as 1/2 serving)*	2 or more *(at least 1 portion raw or as salad)*
4. How many **fruit** units (including natural fruit juices) do you consume per day?	3 or more
5. How many servings of **red meat, hamburger, or meat products** (ham, sausage, etc.) do you consume per day? *(1 serving = 100-150 g)*	Less than 1
6. How many servings of **butter, margarine, or cream** do you consume per day? *(1 serving = 12 g)*	Less than 1
7. How many **sweet/carbonated beverages** do you drink per day?	Less than 1
8. How much **wine** do you drink per week?	7 or more glasses
9. How many servings of **legumes** do you consume per week? (1 serving = 150 g)	3 or more
10. How many servings of **fish or shellfish** do you consume per week? (1 serving: 100-150 g fish, or 4-5 units or 200 g shellfish)	3 or more
11. How many times per week do you consume **commercial sweets or pastries** (not homemade), such as cakes, cookies, biscuits, or custard?	Less than 3
12. How many servings of **nuts** (including peanuts) do you consume per week? *(1 serving = 30 g)*	3 or more
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, simmered with olive oil)?	2 or more

**0 points if these criteria are not met*

Control group

The intervention in the control group was based on giving the participants dietary recommendations on a low-fat diet according to Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) [203]. The goal was to reduce the intake of all types of fat, both animal fat and vegetable fat. The dieticians encourage the consumption of low-fat dairy products, lean meats, cereals, potatoes, pasta, rice, fruits and vegetables and discourage the intake of red meat and processed meats, fatty fish, sweets and pastry, and also olive oil and nuts, for being foods which high amounts of fat. The recommendations that the dieticians gave to the control group were as follows:

a) Use the least possible amount of oil (no more than 2 table spoons per day) for cooking and dressing food

b) Remove all visible fat from meat before cooking and cool soups and broth to remove fat layer on top before heating

c) If you choose to eat red meat, eat meat cuts low in fat instead of high-fat ones such as beacon, beef and lamb

d) Do not eat butter, margarine, lard or other fat spreads

e) Do not consume fat-enriched dairy products, heavy cream, custard, ice cream and use instead low-fat dairy products

f) Cook legumes, pasta, rice and potatoes with the least amount of oil and other fats, such as fatty-meats, cold cuts, beacon, sausages, cracklings, and do not use *sofrito* (tomato sauce simmered with olive oil, garlic and herbs)

g) Eat only lean fish and avoid fatty-fish

h) Avoid eating liver, kidney and offal in general, fried foods, commercial sauces, mayonnaise and cooked foods

I) Do not eat sweets and pastry, such as cookies, cakes, pies and muffins

j) Do not eat vegetal fatty foods such as tree nuts (walnuts, hazelnuts, almonds…), peanuts, sunflower seeds, French fries and other salty snacks. The dieticians used a 9-

item quantitative score of compliance with the low-fat control diet to assess and give personalized advice in order to enhance the adherence to the intervention (**Table 4**).

Table 4. Quantitative score of compliance with the Control diet (Low-Fat).

Foods and frequency of consumption	**Criteria for 1 point***
1. How much olive oil do you consume in a given day (including oil used for frying, salads, out of house meals, etc.)?	2 or less tablespoons *(1 table spoon=10 ml)*
2. Do you remove visible fat (or the skin) of chicken, duck, pork, lamb or veal meats before cooking and the fat of soups, broths, and cooked meat dishes before consumption?	Yes
3. How many servings of fat-rich meats, hamburger, commercial ground meat, sausage, cold meat, cured ham, bacon, salami, or offal do you consume per week? meat serving: 100 g; salami or bacon: 30 g)	1 or less
4. How many servings of butter, margarine, lard, mayonnaise, milk cream, or milk-based ice cream do you consume per week? (spread fat: serving: 12 g; ice cream: 100 g)	1 or less
5. Do you exclusively consume low-fat dairy products?	Yes *(id. If no dairy consumption)*
6. How many times per week do you prepare rice, pasta, potato, or legume dishes by using "*sofrito*" sauce (based on olive oil), bacon, salami, or fatty meats such as pork or lamb ribs?	2 or less
7. How many times per week do you consume fatty fish or fish or seafood canned in oil?	1 or less
8. How many servings of commercial sweets or industrial bakery products (not homemade), such as cakes, cookies, biscuits, or custard do you consume per week? (cake serving: 80 g; 6 biscuits: 40 g)	1 or less
9. How many times per week do you consume nuts (including peanuts), potato chips, French fries, or commercial snacks?	1 or less

**0 points if these criteria are not met*

Other strategies were used by the dieticians to increase the compliance of the intervention in the three arms of the study such as specific personalized objectives taking into account the medical conditions and preferences of each participants, using the accomplishments in the previous months as support to provide further empowerment and self-reward, and try to avoid contradictory dietary advices from other health professionals external to the *PREDIMED* Study.

Group sessions

During all the follow-up, participants attended quarterly group sessions separated by intervention group (MedDiet + EVOO, MedDiet + nuts and control group) and attended by up to 20 participants per session. The sessions included:

- A briefly recall of the key points that should be followed according to 14-item questionnaire (MedDiet groups) or 9-items questionnaire (Control group)

- Informative talk and description of written material (descriptions of 4 to 5 foods typical of the dietary pattern per each study arm and year season; 1-week shopping list; weekly plan of meals and recipes)

- Clarification of any doubts regarding the instructions provided

- Free provision of extra virgin olive oil and nuts to the MedDiet intervention groups and non-food gifts to the control group

5.4. DATA COLLECTION

At baseline and yearly during the follow-up *PREDIMED* personnel completed the questionnaires and conducted anthropometric and biochemical evaluations (***Appendix II and Appendix V***).

A *general questionnaire* about CV risk factors, history of illnesses, medication use, family history of disease, smoking and alcohol use, educational achievement, occupation and socio-economic status were administered to the participants (***Appendix II***). Dieticians also completed a

validated 14-item questionnaire of adherence to the Mediterranean diet and a *quantitative score of compliance with the control (low-fat diet group)*, as detailed before. Trained dieticians completed a *137-item semi-quantitative food frequency questionnaire (FFQ)* (***Appendix III)*** in a face-to-face interview with the participant; this questionnaire has been validated before in elderly population at high cardiovascular risk from Spain[204]. Energy and nutrient intake were estimated using Spanish food composition tables[205,206]. Physical activity was assessed using the validated Spanish version of the *Minnesota Leisure-Time Physical Activity questionnaire* [207] (**Appendix IV**). A to*lerance and side effect questionnaire* was completed in the follow-up visits (***Appendix VI***).

Participants were considered to be diabetic, hypercholesterolemic or hypertensive if they had previously been diagnosed as such, and/or they were being treated with antidiabetic, cholesterol-lowering, or antihypertensive agents, respectively. Trained personnel took the anthropometric and blood pressure measurements. Weight (kg) and height (m) were measured with light clothing and no shoes with calibrated scales and a wall-mounted stadiometer, respectively, and BMI (kg/m^2) was calculated; waist circumference (cm) was measured midway between the lowest rib and the iliac crest using an anthropometric tape; blood pressure was measured using a validated oscillometer [Omron HEM705CP, Hoofddorp, Netherlands] in triplicate with a 5 minute interval between each measurement, and the mean of these values was recorded.

Blood and urine samples were collected at baseline and years 1, 3, 5 and 7 (or final visit). Tubes for EDTA plasma, citrate plasma, buffy coat and serum were collected and aliquots are kept frozen (-80ºC).

5.5. ASCERTAINMENT OF CARDIOVASCULAR DISEASE AND MORTALITY

The primary endpoint of the trial was a composite of myocardial infarction, stroke and death from cardiovascular causes. Secondary endpoints were cardiovascular, cancer and all-cause mortality, among others. Information on primary endpoint and mortality was updated once a

year by the end-point adjudication committee, whose members were blinded to treatment allocation. Different sources of information were used: 1) yearly questionnaires and examinations to all participants, 2) family physicians, 3) yearly review of medical records; and 4) linkage to the National Death Index. Medical records of deceased participants were requested, and the end-point adjudication committee adjudicated the cause of the death and confirmed cardiovascular events.

5.6. STATISTICAL ANALYSIS

The studies presented in this work are observational prospective cohort analyses in the framework of the *PREDIMED* trial. All the analyses were performed using SPSS statistical software, version 19 (SPSS Inc, Chicago, Illinois) and STATA software, version 12.0. The level of significance for all statistical tests was $P < 0.05$ for bilateral contrast.

In general for all the analyses,

1. We used standard methods (Kolmogorov-Smirnov) to test the normal distribution of the analyzed variables.
2. To compare the differences between continuous variables of the study participants we used ANOVA (parametric test) or the Pearson chi-squared (parametric test) tests for categorical variables.
3. Follow-up time was calculated as the interval between the date of cardiovascular event, death (cardiovascular, cancer or other death) or the end of follow-up (the date of the last visit or the last recorded clinical event of participants still alive) and the date of randomization.
4. We used multivariate Cox regression models to assess the risk of the main outcomes (CVD, all-cause mortality, cardiovascular mortality and cancer mortality) according to categories of the exposures. The models were adjusted for potential confounders.

5. Linear trend tests were assessed assigning the median value to each category and using it as a continuous variable in the various models.
6. Multivariable analyses with generalized estimating equation models were used to assess the associations between yearly repeated measurements of the exposure and CVD and mortality.

6. RESULTS

6. RESULTS

Three published original articles are part of the results of this thesis. All of them are observational prospective cohort studies in the context of the *PREDIMED* Study. The participants were Mediterranean individuals at high cardiovascular risk from Spain. The specific objectives and corresponding publications are summarized in **Table 5**.

Table 5. Thesis objectives and corresponding publications.

Objectives	Publications
To assess the associations between frequency of nut consumption, cause-specific and overall mortality.	Guasch-Ferré M, Bulló M, Martínez-González MA, et al. **Frequency of nut consumption and mortality risk in the PREDIMED nutrition intervention trial.** *BMC Med.* 2013 Jul 16;11:164. PMID: 23866098. **Impact factor 2013:** 7.276
To assess the associations between the intake of total olive oil, its varieties (extra virgin olive oil and common olive oil) and the risk of cardiovascular disease, cause-specific and overall mortality.	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 May 13;12(1):78. PMID: 24886626. **Impact factor 2013:** 7.276
To assess the association between dietary magnesium intake and the risk of cardiovascular disease, cause-specific and overall mortality.	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 Jan;144(1):55-60. PMID: 24259558. **Impact factor 2013:** 4.227

6.1. FREQUENCY OF NUT CONSUMPTION AND MORTALITY RISK IN THE PREDIMED NUTRITION INTERVENTION TRIAL

Guasch-Ferré M, Bulló M, Martínez-González MÁ, Ros E, Corella D, Estruch R, Fitó M, Arós F, Wärnberg J, Fiol M, Lapetra J, Vinyoles E, Lamuela-Raventós RM, Serra-Majem L, Pintó X, Ruiz-Gutiérrez V, Basora J, Salas-Salvadó J; PREDIMED study group.

BMC Med. 2013 Jul 16;11:164. PMID: 23866098; doi:10.1186/1741-7015-11-164.

Study 1 overview. Novelty and significance.

What is already known?

- Prospective studies in non-Mediterranean populations have consistently related increasing nut consumption to lower coronary heart disease mortality.
- A small protective effect on all-cause and cancer mortality has also been suggested.
- If an inverse association between nut consumption and all-cause mortality exists, the beneficial effect might be more robust in Mediterranean regions, where nut consumption per person is relatively high compared to other countries.

What this study adds?

- Our study was the first to demonstrate that consuming nuts >3 serving/week was associated with 39% (HR: 0.61, 95% CI: 0.45-0.83) reduction in the risk of total mortality after 4.8 years of follow-up, compared to non-consumers, in elderly participants at high cardiovascular risk.
- A similar protective effect against cardiovascular and cancer mortality was observed.

Summary

- This study provides further evidence of the inverse relationship between the frequency of nut consumption and the risk of mortality in a Mediterranean population at high cardiovascular risk with relatively high nut intake.

BMC Medicine

RESEARCH ARTICLE Open Access

Frequency of nut consumption and mortality risk in the PREDIMED nutrition intervention trial

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Abstract

Background: Prospective studies in non-Mediterranean populations have consistently related increasing nut consumption to lower coronary heart disease mortality. A small protective effect on all-cause and cancer mortality has also been suggested. To examine the association between frequency of nut consumption and mortality in individuals at high cardiovascular risk from Spain, a Mediterranean country with a relatively high average nut intake per person.

Methods: We evaluated 7,216 men and women aged 55 to 80 years randomized to 1 of 3 interventions (Mediterranean diets supplemented with nuts or olive oil and control diet) in the PREDIMED ('PREvención con DIeta MEDiterránea') study. Nut consumption was assessed at baseline and mortality was ascertained by medical records and linkage to the National Death Index. Multivariable-adjusted Cox regression and multivariable analyses with generalized estimating equation models were used to assess the association between yearly repeated measurements of nut consumption and mortality.

Results: During a median follow-up of 4.8 years, 323 total deaths, 81 cardiovascular deaths and 130 cancer deaths occurred. Nut consumption was associated with a significantly reduced risk of all-cause mortality (*P* for trend <0.05, all). Compared to non-consumers, subjects consuming nuts >3 servings/week (32% of the cohort) had a 39% lower mortality risk (hazard ratio (HR) 0.61; 95% CI 0.45 to 0.83). A similar protective effect against cardiovascular and cancer mortality was observed. Participants allocated to the Mediterranean diet with nuts group who consumed nuts >3 servings/week at baseline had the lowest total mortality risk (HR 0.37; 95% CI 0.22 to 0.66).

Conclusions: Increased frequency of nut consumption was associated with a significantly reduced risk of mortality in a Mediterranean population at high cardiovascular risk.
Please see related commentary: http://www.biomedcentral.com/1741-7015/11/165.

Trial registration: Clinicaltrials.gov. International Standard Randomized Controlled Trial Number (ISRCTN): 35739639. Registration date: 5 October 2005.

Keywords: Cancer, Cardiovascular, Mortality, Nuts, PREDIMED study

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Background

Nuts are an important component of the so-called Mediterranean diet (MedDiet) and a good source of unsaturated fatty acids, fiber, minerals (potassium, calcium and magnesium), vitamins (folate and tocopherols) and other bioactive compounds, such as phytosterols and polyphenols [1–4].

There is consistent evidence to suggest that the consumption of nuts has a beneficial effect on cardiovascular health, and this effect is attributable to their unique nutritional composition [5]. A pooled analysis of four large-scale observational studies showed that subjects in the highest nut consumption categories had an approximately 35% reduced risk of incident coronary heart disease (CHD) [6]. The frequency of nut consumption was also related to lower rates of sudden cardiac death in a large cohort of men [7]. Furthermore, epidemiologic studies and clinical trials have shown that frequent nut consumption is associated with a reduced load of cardiovascular disease risk factors, such as dyslipidemia, type 2 diabetes, and metabolic syndrome [4,6,8,9]. In addition, reports from the Iowa Women's Health study [10], a large Dutch cohort [11], and the US Nurses' Health Study [12], which assessed populations with relatively low overall nut intake, suggested that frequent nut consumption related inversely to total mortality, albeit the protective effect was weak, with adjusted risk reductions ranging from 5% to 15% [10–12]. If an inverse association between nut consumption and all-cause mortality exists, the beneficial effect might be more robust in Mediterranean regions, where nut consumption per person is relatively high compared to other countries [13].

The main aim of the PREDIMED study was to test the efficacy of two Mediterranean diets (one supplemented with extra-virgin olive oil and another with nuts), as compared to a control diet (advice on a low-fat diet), on primary cardiovascular prevention. In contrast, in this current manuscript our aims were only to assess the association between baseline consumption of nuts (that is, the consumption of nuts previous to starting the intervention) and total mortality (instead of cardiovascular events). We have additionally included the repeated measurements for the consumption of nuts during follow-up as another exposure, regardless of the allocated arm of the trial; this is in contrast with the original PREDIMED study, which used an intention-to-treat analysis.

We hypothesized that level of nut consumption would be strongly associated with mortality in the cohort of the PREDIMED ('PREvención con DIeta MEDiterránea') study, including older men and women at high cardiovascular risk [14]. To this end, in this cohort we longitudinally examined the association between the frequency of nut consumption at baseline and the risk of mortality at the end of follow-up.

Methods

Study population

The present study was conducted within the framework of the PREDIMED trial, the design of which has been described in detail elsewhere [14]. Briefly, the PREDIMED study is a large, multicenter, parallel-group, randomized and controlled clinical trial for the primary prevention of cardiovascular disease (CVD) (http://www.predimed.es and http://www.predimed.org). The main results of the trial on the primary endpoint have been recently published [15]. We assigned 7,447 older participants (men aged 55 to 80 years and women 60 to 80 years) to 1 of 3 interventions: a MedDiet enriched with extra-virgin olive oil (EVOO), a MedDiet supplemented with mixed nuts, or advice on a low-fat diet (control diet). Participants had no CVD at enrollment but they were at high cardiovascular risk because of the presence of type 2 diabetes or at least three of the following risk factors: current smoking, hypertension, hypercholesterolemia, low high-density lipoprotein (HDL)-cholesterol, overweight or obesity, and family history of premature CVD. Exclusion criteria were the presence of severe medical condition that may impair the ability of the person to participate in a nutrition intervention study (for example, digestive disease with fat intolerance, advanced malignancy, or major neurological, psychiatric or endocrine disease), immunodeficiency or HIV positive status, alcohol or drug abuse, body mass index (BMI) ≥40 kg/m^2, and allergy or intolerance to olive oil or nuts [16].

The primary endpoint of the main trial is a combination of several cardiovascular events (myocardial infarction, stroke or cardiovascular death). The present study was conducted as an observational cohort using baseline consumption of nuts as the exposure. The outcomes were: (1) total mortality, (2) only cardiovascular mortality, and (3) only cancer mortality. All participants provided written informed consent according to a protocol approved by the institutional review boards of the recruiting centers (Comité de Ética e Investigación Clínica (CEIC) Hospital Universitari Sant Joan de Reus, CEIC Universidad de Navarra, CEIC Hospital Clínic de Barcelona, Comité de Ética Universidad de Valencia, CEIC-Parc de Salut Mar, CEIC Hospital Universitario Araba, CEIS del distrito Sanitario Atención Primária Sevilla, IDIAP Jordi Gol, CEIC Complejo Hospitalario Materno-Insular, CEIC Facultad Medicina Universidad de Málaga, CEIC Illes Balears, and CEIC Hospital Universitari Bellvitge).

Dietary assessment

At baseline trained dietitians completed a 137-item semiquantitative food frequency questionnaire in a face-to-face interview with the participant; this questionnaire has been validated before in an older population at high cardiovascular risk from Spain [17]. Energy and nutrient

intake were estimated using Spanish food composition tables [18,19]. Information on self-reported nut intake was derived from the food frequency questionnaire. The questionnaire includes one item regarding the consumption of almonds, peanuts, hazelnuts, pistachios and pine nuts (macadamias, cashews and Brazil nuts are rarely consumed in Spain), and another question specifically inquired about the consumption of walnuts. The dietitians asked the participants if they consumed this food item never, between 1 to 3 times per month, times per week (1, 2 to 4, 5 to 6; three options) or times a day (1, 2 to 3, 4 to 6, >6; four options). For the purpose of the present study, 28 g of nuts was considered to be one serving. Peanuts, almonds, hazelnuts, walnuts, pine nuts, pistachios, Brazil nuts, macadamia and cashews were all considered nuts. In addition, dietitians administered a validated 14-item MedDiet screener designed to assess the degree of adherence to the traditional MedDiet [20]. We used the score of this brief screener to control for the overall dietary pattern, because a higher adherence to the MedDiet among frequent consumers of nuts could introduce confounding. For this purpose, the question about nut consumption was omitted from the brief screener; therefore, a 13-point score was used as a covariate (minimum 0, maximum 13).

Ascertainment of mortality

Information on mortality was updated once a year by the End-point Adjudication Committee, whose members were blinded to treatment allocation. Different sources of information were used: (1) yearly questionnaires and examinations to all participants, (2) family physicians, (3) yearly review of medical records, and (4) linkage to the National Death Index. Medical records of deceased participants were requested, and the End-point Adjudication Committee adjudicated the cause of the death.

Assessment of other covariates

At baseline, questionnaires about lifestyle variables, educational achievement, history of illnesses, and medication use were administered. Physical activity was assessed using the validated Spanish version of the Minnesota Leisure-Time Physical Activity questionnaire [21]. Participants were considered to be diabetic, hypercholesterolemic or hypertensive if they had previously been diagnosed as such, and/or they were being treated with antidiabetic, cholesterol-lowering, or antihypertensive agents, respectively. Trained personnel took the anthropometric and blood pressure measurements. Weight and height were measured with light clothing and no shoes with calibrated scales and a wall-mounted stadiometer, respectively; waist circumference was measured midway between the lowest rib and the iliac crest using an anthropometric tape; blood pressure was measured using a validated oscillometer (Omron HEM705CP; Hoofddorp, The Netherlands) in triplicate with a 5-minute interval between each measurement, and the mean of these values was recorded.

Statistical analyses

Follow-up time was calculated as the difference between the date of either death or end of follow-up (the date of the last visit or the last recorded clinical event of participants still alive) and the date of recruitment. Extremes of total energy intake (>4,000 or <800 kcal per day in men and >3,500 or <500 kcal per day in women) were excluded from the analysis [22]. Three categories of frequency of nut consumption were considered (never or almost never, 1 to 3 servings per week and >3 servings per week). We used analysis of variance (ANOVA) or the Pearson χ^2 tests to compare the quantitative or categorical baseline characteristics of the study participants, respectively, across servings of nut consumption. Results were expressed as means ± SD or percentages. Because no interaction was observed between sex and the main outcome, analyses were conducted for men and women together.

To assess the risk of total mortality by frequency of nut consumption, multivariate relative risks were computed using Cox proportional hazard models, and potential confounders were controlled for. All analyses were stratified by the recruitment center. Results are expressed as hazard ratios (HRs) with 95% confidence intervals (CIs). Given the different nutritional composition of walnuts and other nuts [1], we performed separate analyses for the frequency of total nut consumption, walnut consumption, and consumption of nuts excluding walnuts. After the unadjusted model, another model was adjusted for age (continuous), sex and intervention group. Then, a second model, was additionally adjusted for BMI (continuous), current smoking status (never, former, or current smoker), educational level (illiterate/primary education, secondary education, academic/graduate), physical activity (MET-min/day), total energy intake (kcal/day), history of diabetes (yes/no), history of hypercholesterolemia (yes/no), use of oral antidiabetic medication (yes/no), antihypertensive drugs (yes/no), and statins (yes/no). Finally, a third, fully-adjusted model, was additionally adjusted for alcohol intake (continuous, adding a quadratic term), quintiles of consumption of dietary food groups (vegetables, fruits, red meat, eggs, and fish), and adherence to the MedDiet (13-point score). The same models were used to assess the risk of cardiovascular mortality or cancer mortality, also using Cox proportional hazard models. Linear trend tests were assessed assigning the median value to each category of nut consumption and using it as a continuous variable in the various models. We evaluated the interaction between baseline nut consumption (three categories, two dummy variables) and the intervention group (three groups, two dummy variables) by introducing an interaction term with four degrees

of freedom in the model. We used Cox regression models to assess the risk of total mortality, cardiovascular mortality and cancer mortality according to the joint categories of total nut consumption and intervention group. Linear trends were also tested. We had yearly updated information on nut consumption, so to take advantage of this updated information we repeated the analysis using generalized estimating equations to assess the association between repeated measurements of nut consumption and mortality. For each 1-year period we used as exposure the average nut consumption of all repeated measurements from baseline to the beginning of that yearly period.

The level of significance for all statistical tests was P <0.05 for bilateral contrast. Analyses were performed using SPSS statistical software, version 19 (SPSS Inc, Chicago, IL, USA) and STATA software, version 12.0 (Stata Corp., College Station, TX, USA).

Table 1 Baseline characteristics of study participants by frequency of nut consumption

Variable	Baseline frequency of nut consumption			
	Never (n = 2,118)	1 to 3 servings/week (n = 2,803)	>3 servings/week (n = 2,295)	*P* value
Age, years	67 ± 6	66 ± 6	67 ± 6	<0.001
Men, % (n)	36 (773)	43 (1,219)	47 (1,079)	<0.001
BMI, kg/m^2	30.6 ± 4.0	29.9 ± 3.7	29.4 ± 3.7	<0.001
Weight, kg	77.2 ± 12.0	77.1 ± 12.1	75.8 ± 11.6	<0.001
Waist circumference, cm	101.5 ± 10.5	100.7 ± 10.4	99.2 ± 10.0	<0.001
Leisure-time energy expenditure in physical activity, MET-min/day	195 ± 220	231 ± 232	264 ± 257	<0.001
Smoking status, % (n)				<0.001
Never	64 (1,367)	61 (1,708)	59 (1,364)	
Current	14 (310)	14 (402)	12 (292)	
Former	21 (441)	24 (693)	28 (639)	
Educational level, % (n)				<0.001
Illiterate/primary education	82 (1,740)	76 (2,131)	75 (1,733)	
Secondary education	12 (266)	16 (451)	16 (379)	
Academic/graduate	5 (112)	8 (221)	8 (183)	
Diabetes, % (n)	53 (1,118)	47 (1,338)	46 (1,071)	<0.001
Hypertension, % (n)	83 (1,763)	83 (2,320)	82 (1,887)	0.670
Hypercholesterolemia, % (n)	70 (1,479)	73 (2,044)	73 (1,689)	0.012
Medication use, % (n)				
Oral antidiabetic drugs	36 (760)	31 (884)	29 (679)	<0.001
Antihypertensive drugs	75 (1,587)	72 (2,037)	71 (1,624)	0.008
Statins	48 (1,022)	48 (1,364)	47 (1,093)	0.761
Modified MedDiet score (13-point score)	8.1 ± 1.7	8.2 ± 1.8	8.6 ± 1.7	<0.001
Total energy intake, g/day	2,060 ± 529	2,222 ± 514	2,416 ± 537	<0.001
Nuts, g/day	0	4.9 ± 2.3	25.7 ± 14.4	<0.001
Alcohol, g/day	6.6 ± 13.4	8.4 ± 13.8	9.7 ± 14.7	<0.001
Vegetables, g/day	317 ± 144	329 ± 145	355 ± 149	<0.001
Fruit, g/day	344 ± 200	354 ± 195	407 ± 204	<0.001
Red meat (beef, pork, lamb), g/day	70.4 ± 44.3	79.1 ± 46.1	78.6 ± 46.2	<0.001
White meat (chicken, rabbit, turkey), g/day	44.6 ± 28.5	45.1 ± 27.1	44.5 ± 27.6	0.720
Eggs, g/day	19.4 ± 10.9	20.4 ± 11.5	20.0 ± 10.6	0.010
Fish, g/day	91.8 ± 47.8	99.9 ± 48.9	105.2 ± 53.7	<0.001
Dairy products, g/day	387 ± 224	374 ± 216	381 ± 222	0.095

Data are expressed as mean ± SD or percentage (n). *P* value for comparisons across servings of nut consumption (Pearson χ^2 test for categorical variables or one-way analysis of variance for continuous variable) as appropriate.
BMI, body mass index.

Results

After those subjects with extremes of total energy intake (n = 153) and those with incomplete dietary data (lack of food frequency questionnaire) at baseline (n = 78) had been excluded, 7,216 individuals were available for the present analysis. The mean age of the participants was 67 years and there were a total of 3,071 men and 4,145 women. Table 1 shows the baseline characteristics of study participants by frequency of total nut consumption. Subjects who ate nuts more frequently had lower BMI and waist circumference, were less likely to smoke, and were more physically active compared to those who rarely or never consumed nuts. In the upper category of nut consumption there were fewer individuals with type 2 diabetes mellitus or who used antidiabetic and antihypertensive medication. In addition, frequent nut consumption was associated with a higher intake of energy, vegetables, fruit, and fish.

Changes in total nut consumption were +15.95 ± 21.10 g/day (mean ± SD) in the MedDiet supplemented with nuts, -0.80 ± 16.31 g/day in the MedDiet supplemented with extra virgin olive oil and −3.12 ± 13.85 g/day in the control group.

During a median follow-up of 4.8 years, 323 total deaths, 81 cardiovascular deaths and 130 cancer deaths occurred. Table 2 shows HRs for total mortality by frequency of total nut consumption, walnut consumption, and consumption of other nuts. After adjustments for age, sex and intervention group (model 1), the subjects who ate nuts more frequently had a lower risk of total mortality in all the types of nuts analyzed (P for trend <0.001 for total nut and walnut consumption, and P = 0.010 for non-walnut nuts). In fully adjusted models, participants who consumed total nuts, walnuts, or non-walnut nuts >3 servings per week had significant reductions in total mortality risk of 39%, 45%, and 34%, respectively, compared to those who rarely or never consumed nuts. The relationship between nut consumption and total mortality was linear for all the models (P for trend <0.05), except for the crude model of nut consumption excluding walnuts.

Those participants who ate total nuts, walnuts or other nuts (excluding walnuts) >3 servings per week had also

Table 2 Hazard ratios of total mortality according to the frequency of nut consumption (including and not including walnuts)

Total mortality	Never	1 to 3 servings/week	>3 servings/week	*P* for trend
Frequency of total nut consumption:	n = 2,118	n = 2,803	n = 2,295	
All causes of death, % (n)	5.6 (119)	4.2 (117)	3.8 (87)	
Person-years, n	8,724	12,168	10,185	
Crude model	1 (Reference)	0.68 (0.52 to 0.88)	0.60 (0.45 to 0.79)	0.005
Multivariable model 1	1 (Reference)	0.68 (0.52 to 0.89)	0.55 (0.41 to 0.73)	0.001
Multivariable model 2	1 (Reference)	0.69 (0.53 to 0.91)	0.59 (0.43 to 0.79)	0.005
Multivariable model 3	1 (Reference)	0.71 (0.54 to 0.93)	0.61 (0.45 to 0.83)	0.012
Frequency of walnut consumption:	n = 2,916	n = 2,547	n = 1,753	
All causes of death, % (n)	5.6 (164)	3.9 (100)	3.4 (59)	
Person-years, n	12,124	11,122	7,825	
Crude model	1 (Reference)	0.64 (0.50 to 0.83)	0.54 (0.40 to 0.73)	<0.001
Multivariable model 1	1 (Reference)	0.66 (0.51 to 0.85)	0.50 (0.37 to 0.68)	<0.001
Multivariable model 2	1 (Reference)	0.65 (0.50 to 0.84)	0.53 (0.39 to 0.73)	<0.001
Multivariable model 3	1 (Reference)	0.66 (0.51 to 0.86)	0.55 (0.40 to 0.76)	<0.001
Frequency of consumption of other nuts (excluding walnuts):	n = 3,308	n = 2,643	n = 1,265	
All causes of death, % (n)	5.0 (166)	4.1 (109)	3.8 (48)	
Person-years, n	13,936	11,573	5,566	
Crude model	1 (Reference)	0.77 (0.60 to 0.98)	0.71 (0.52 to 0.98)	0.068
Multivariable model 1	1 (Reference)	0.75 (0.59 to 0.96)	0.62 (0.44 to 0.86)	0.010
Multivariable model 2	1 (Reference)	0.78 (0.61 to 1.00)	0.64 (0.45 to 0.90)	0.021
Multivariable model 3	1 (Reference)	0.80 (0.62 to 1.03)	0.66 (0.46 to 0.93)	0.031

One serving of nuts equals 28 g. Cox regression models were used to assess the risk of all-cause mortality by frequency of nut consumption. Multivariable model 1 was adjusted for age in years, sex, and intervention group. Model 2 was additionally adjusted for body mass index (BMI) in kg/m^2, smoking status (never, former, current smoker), educational level (illiterate/primary education, secondary education, academic/graduate), leisure time physical activity in MET-min/day, history of diabetes (yes/no), history of hypercholesterolemia (yes/no), use of oral antidiabetic medication (yes/no), use of antihypertensive medication (yes/no), use of statins (yes/no), and total energy intake (kcal/day). Model 3 was also adjusted for dietary variables in quintiles (vegetables, fruits, red meat, eggs, and fish), alcohol intake (continuous, adding a quadratic term) and Mediterranean diet adherence (13-point score). All models were stratified by recruitment centre. Extremes of total energy intake were excluded.

lower risk of cardiovascular mortality than those in the reference category (Table 3). The HR of cardiovascular mortality in the fully-adjusted model of total nut consumption was 0.45 (95% CI 0.25 to 0.81). Table 4 shows the HRs for cancer death by frequency of total nut consumption, walnut consumption and consumption of other nuts. Subjects in the upper category of total nut consumption had a significant 40% (95% CI −37% to −98%) reduction in cancer death, although the *P* for trend was non significant.

Figure 1 shows the multivariate adjusted HRs for total mortality by frequency of total nut consumption and intervention group. In the three arms of the trial, individuals who consumed nuts >3 servings per week tended to have a lower risk of mortality than those in the reference category. Subjects in the upper category of nut consumption at baseline allocated to the MedDiet with nuts intervention had a significant reduction in total mortality risk of 63% (95% CI −34% to −78%), while those allocated to the MedDiet with EVOO and the control diet had non-significant reductions of 34% (95% CI −64% to 10%) and 16% (95% CI −52% to 44%), respectively. The interaction between baseline total nut consumption and intervention group was significant, $P = 0.019$).

When we used generalized estimating equations to assess the association between yearly updated measurements of total nut consumption and all-cause mortality we also found a significant inverse association. The fully-adjusted relative risk (RR) was 0.68 (95% CI 0.50 to 0.93) with a significant linear trend test. When we repeated the analysis to evaluate the association between nut intake and cardiovascular mortality and cancer mortality the fully-adjusted relative risk (RR) were 0.76 (95% CI 0.42 to 1.36) and 0.63 (95% CI 0.39 to 1.03), respectively; however the linear trend tests were not significant (data not shown).

Discussion

In this longitudinal cohort study of individuals at high cardiovascular risk with relatively high nut intake living in

Table 3 Hazard ratios of cardiovascular mortality according to the frequency of nut consumption (including and not including walnuts)

Cardiovascular mortality	Never	1 to 3 servings/week	>3 servings/week	*P* for trend
Frequency of total nut consumption:	n = 2,118	n = 2,803	n = 2,295	
Cardiovascular death, % (n)	1.7 (36)	0.8 (23)	1.0 (22)	
Person-years, n	8,724	12,168	10,185	
Crude model	1 (Reference)	0.44 (0.26 to 0.74)	0.50 (0.29 to 0.85)	0.101
Multivariable model 1	1 (Reference)	0.44 (0.26 to 0.76)	0.47 (0.27 to 0.82)	0.075
Multivariable model 2	1 (Reference)	0.41 (0.24 to 0.71)	0.41 (0.23 to 0.73)	0.042
Multivariable model 3	1 (Reference)	0.42 (0.24 to 0.74)	0.45 (0.25 to 0.81)	0.091
Frequency of walnut consumption:	n = 2,916	n = 2,547	n = 1,753	
Cardiovascular death, % (n)	1.6 (46)	0.7 (18)	1.0 (17)	
Person-years, n	12,124	11,122	7,825	
Crude model	1 (Reference)	0.41 (0.24 to 0.71)	0.55 (0.31 to 0.96)	0.037
Multivariable model 1	1 (Reference)	0.42 (0.24 to 0.74)	0.54 (0.30 to 0.95)	0.040
Multivariable model 2	1 (Reference)	0.39 (0.22 to 0.69)	0.49 (0.27 to 0.88)	0.022
Multivariable model 3	1 (Reference)	0.41 (0.23 to 0.73)	0.53 (0.29 to 0.98)	0.047
Frequency of consumption of other nuts (excluding walnuts):	n = 3,308	n = 2,643	n = 1,265	
Cardiovascular death, % (n)	1.3 (43)	1.1 (28)	0.8 (10)	
Person-years, n	13,936	11,573	5,566	
Crude model	1 (Reference)	0.76 (0.47 to 1.22)	0.57 (0.28 to 1.13)	0.129
Multivariable model 1	1 (Reference)	0.73 (0.45 to 1.20)	0.48 (0.23 to 0.97)	0.056
Multivariable model 2	1 (Reference)	0.70 (0.43 to 1.15)	0.40 (0.19 to 0.83)	0.021
Multivariable model 3	1 (Reference)	0.74 (0.45 to 1.23)	0.42 (0.20 to 0.89)	0.031

One serving of nuts equals 28 g. Cox regression models were used to assess the risk of cardiovascular mortality by frequency of nut consumption. Multivariable model 1 was adjusted for age (years), sex, and intervention group. Model 2 was additionally adjusted for BMI (kg/m^2), smoking status (never, former, current smoker), educational level (illiterate/primary education, secondary education, academic/graduate), leisure time physical activity (MET-min/day), history of diabetes (yes/no), history of hypercholesterolemia (yes/no), use of oral antidiabetic medication (yes/no), use of antihypertensive medication (yes/no), use of statins (yes/no), and total energy intake (kcal/day). Model 3 was also adjusted for dietary variables in quintiles (vegetables, fruits, red meat, eggs, and fish), alcohol intake (continuous, adding a quadratic term) and Mediterranean diet adherence (13-point score). All models were stratified by recruitment centre. Extremes of total energy intake were excluded.

Table 4 Hazard ratios of cancer mortality according to the frequency of nut consumption (including and not including walnuts)

Cancer mortality	Never	1 to 3 servings/week	>3 servings/week	*P* for trend
Frequency of total nut consumption:	n = 2,118	n = 2,803	n = 2,295	
Cancer death, % (n)	2.1 (44)	1.9 (52)	1.5 (34)	
Person-years, n	8,724	12,168	10,185	
Crude model	1 (Reference)	0.82 (0.55 to 1.23)	0.64 (0.41 to 1.00)	0.070
Multivariable model 1	1 (Reference)	0.77 (0.51 to 1.16)	0.54 (0.34 to 0.86)	0.015
Multivariable model 2	1 (Reference)	0.79 (0.52 to 1.20)	0.60 (0.37 to 0.96)	0.052
Multivariable model 3	1 (Reference)	0.79 (0.52 to 1.20)	0.60 (0.37 to 0.98)	0.064
Frequency of walnut consumption:	n = 2,916	n = 2,547	n = 1,753	
Cancer death, % (n)	2.1 (62)	1.9 (48)	1.1 (20)	
Person-years, n	12,124	11,122	7,825	
Crude model	1 (Reference)	0.82 (0.56 to 1.20)	0.48 (0.29 to 0.80)	0.005
Multivariable model 1	1 (Reference)	0.76 (0.52 to 1.12)	0.41 (0.25 to 0.69)	0.001
Multivariable model 2	1 (Reference)	0.77 (0.52 to 1.14)	0.46 (0.27 to 0.77)	0.003
Multivariable model 3	1 (Reference)	0.76 (0.51 to 1.12)	0.46 (0.27 to 0.79)	0.005
Frequency of consumption of other nuts (excluding walnuts):	n = 3,308	n = 2,643	n = 1,265	
Cancer death, % (n)	2.0 (66)	1.6 (43)	1.7 (21)	
Person-years, n	13,936	11,573	5,566	
Crude model	1 (Reference)	0.77 (0.52 to 1.13)	0.79 (0.48 to 1.29)	0.439
Multivariable model 1	1 (Reference)	0.74 (0.50 to 1.10)	0.68 (0.41 to 1.14)	0.213
Multivariable model 2	1 (Reference)	0.79 (0.53 to 1.18)	0.73 (0.43 to 1.23)	0.318
Multivariable model 3	1 (Reference)	0.79 (0.53 to 1.18)	0.75 (0.44 to 1.27)	0.369

One serving of nuts equals 28 g. Cox regression models were used to assess the risk of cancer mortality by frequency of nut consumption. Multivariable model 1 was adjusted for age (years), sex, and intervention group. Model 2 was additionally adjusted for BMI (kg/m^2), smoking status (never, former, current smoker), educational level (illiterate/primary education, secondary education, academic/graduate), leisure time physical activity (MET-min/day), history of diabetes (yes/no), history of hypercholesterolemia (yes/no), use of oral antidiabetic medication (yes/no), use of antihypertensive medication (yes/no), use of statins (yes/no), and total energy intake (kcal/day). Model 3 was also adjusted for dietary variables in quintiles (vegetables, fruits, red meat, eggs, and fish), alcohol intake (continuous, adding a quadratic term) and Mediterranean diet adherence (13-point score). All models were stratified by recruitment centre. Extremes of total energy intake were excluded.

a Mediterranean country, the frequency of nut consumption was inversely related to total mortality after 4.8 years of follow-up. Compared to non-consumers, subjects who consumed >3 servings of nuts per week at baseline had a significant 39% lower risk of all-cause mortality. Relative risk reductions were similar for the upper baseline category of non-walnut nuts (34%) or walnut consumption (45%), and when we evaluated the repeated measurements of total nut consumption over time (32%).

Moreover, those in the upper category of baseline nut consumption had a 55% lower risk of cardiovascular mortality and 40% lower risk of cancer mortality compared to those who never consumed nuts. The study subjects participated in the PREDIMED study, a long-term, randomized nutrition intervention trial [14], and those consuming more nuts at baseline and allocated to intervention with a MedDiet supplemented with nuts showed a significantly reduced total mortality risk of 63%.

The inverse association between baseline nut consumption and total mortality was of borderline significance in participants in the upper category of baseline nut consumption in a MedDiet supplemented with EVOO group, while there was no significant association in those allocated to a low-fat control diet, who were advised to reduce intake of all fatty foods, including nuts. Thus, advice against eating nuts throughout the study might have counterbalanced the protective effect of a lifetime intake of these foods. We assume that the baseline assessment can be considered as a good correlate of lifetime habits in this population.

Our findings concur with those of epidemiological studies showing inverse associations between nut consumption and cardiovascular mortality. Four large prospective studies have reported consistent inverse associations between nut consumption and fatal CHD or sudden cardiac death [23–26]. In the Adventist Health Study, subjects who consumed nuts >5 times per week had a 48% reduced risk of fatal CHD [23]. The reduction in death from CHD among women who consumed nuts 2 to 4 times/week in the Iowa Women's Health Study were 57% [24]. However, a later report from the same study

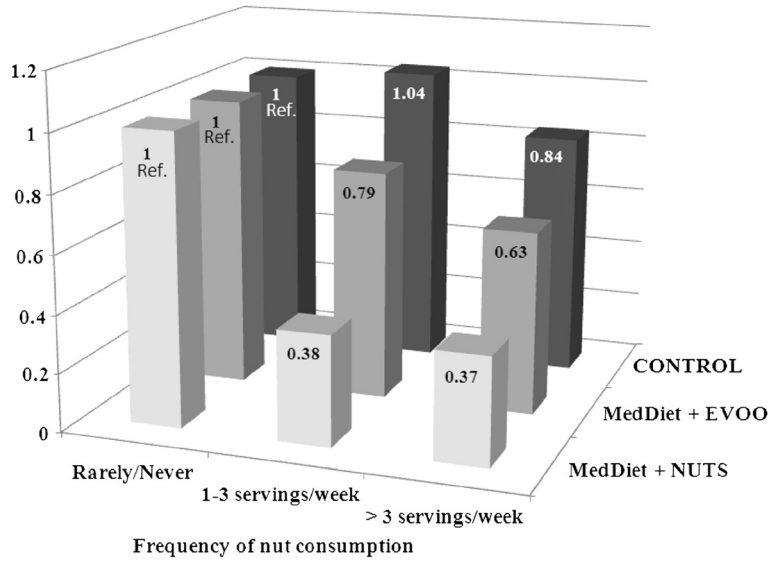

Figure 1 Adjusted hazard ratios of total mortality by frequency of nut consumption and intervention group. The Cox regression models were adjusted for age in years, sex, BMI in kg/m^2, smoking status (never, former, current smoker), educational level (illiterate/primary education, secondary education, academic/graduate), leisure time physical activity in MET-min/day, history of diabetes (yes/no), history of hypercholesterolemia (yes/no), use of oral antidiabetic medication (yes/no), use of antihypertensive medication (yes/no), use of statins (yes/no), total energy intake (kcal/d), dietary variables in quintiles (vegetables, fruits, red meat, eggs and fish), alcohol intake (continuous, adding a quadratic term), and Mediterranean diet adherence (13-point score). The model was stratified by recruitment centre. Extremes of total energy intake were excluded. Values for the two upper categories of nut consumption are 0.38 (95% CI: 0.23 to 0.63) and 0.37 (95% CI: 0.22 to 0.66) in the Mediterranean diet supplemented with nuts (MedDiet + NUTS) group; 0.79 (95% CI: 0.50 to 1.24) and 0.63 (95% CI: 0.36 to 1.1) in the Mediterranean diet supplemented with extra-virgin olive oil (MedDiet + EVOO) group; and 1.04 (95% CI: 0.64 to 1.69) and 0.84 (95% CI: 0.48 to 1.44) in the low-fat control diet group. P for the interaction between baseline nut consumption and intervention group= 0.019. P for trend: MedDiet + NUTS, p=0.01; MedDiet + EVOO, p=0.15; Control diet, p=0.42.

with longer follow-up failed to confirm that nut consumption protected from CHD death [10]. In addition, the Nurses' Health Study observed that women who consumed nuts ≥5 times/week had a 30% reduced risk of fatal CHD [25]. Finally, the Physicians' Health Study reported a 47% lower risk of sudden cardiac death and 30% lower risk of total CHD death among men who consumed nuts twice a week or more [7].

In our study, a reduced risk of cancer mortality has been observed in individuals that frequently consumed total nuts and walnuts. Few epidemiologic studies have been conducted evaluating the association between nut consumption and cancer. An ecological study showed that prostate cancer mortality was inversely associated with nuts and oilseed consumption [27]. Moreover, findings from prospective studies suggest inverse associations between nut consumption and colorectal or endometrial cancer, especially in women [28–31]. Some studies showed inverse associations of nut intake and prostate cancer [32], however the relationship between nuts and cancer incidence and mortality is insufficient and further research is needed [33]. A possible explanation that may account for the inverse relationship between walnuts and cancer mortality but not with other nuts could be that walnuts were richer in free and total polyphenols than all the other nuts [34]. As walnuts are usually consumed raw, and roasting can cause a decline in the efficacy in the antioxidant capacity, it has been shown that raw walnuts, as consumed in the PREDIMED study, had the highest antioxidant efficacy among all the nuts [34]; this could play a beneficial role in the prevention of cancer.

The present results also support those of prior observational studies suggesting that nut consumption protects against mortality. In the Iowa Women's Health Study, subjects consuming nuts ≥2 times/week had a significant 12% lower mortality risk than those who ate nuts less than once monthly after a 12-year follow-up [10]. A recent study from a large Dutch cohort followed for 10 years reported that men and women in the 75th percentile of nut intake had 8% and 5% lower risks of all-cause mortality, respectively, compared with subjects in the 25th percentile [11]. Additionally, data from the Nurses' Health Study, where participants were followed-up for nearly 18 years, showed that consuming nuts ≥2 times/week was associated with a 14% reduced risk of all-cause mortality [12]. It is noteworthy that the protection against total mortality afforded by nut consumption in our study was ≥3 orders of magnitude higher than that observed in studies of non-Mediterranean populations. A likely reason is that PREDIMED participants had a rather high self-selected nut intake before entering the study. Thus, 32% of PREDIMED participants consumed nuts >3 times/week,

compared with nearly 10% consuming nuts ≥2 times/week in both the Iowa Women's Health Study [10] and the Nurses' Health Study [12]. In the Dutch study, participants in the 75th percentile of nut consumption had rather low average daily intakes of 11.1 g for men and 6.2 g for women [11].

The healthy nutritional profile of nuts may account for the inverse association observed between nuts and mortality. Nuts are high in monounsaturated fatty acids, fiber, minerals, vitamins and many bioactive compounds; all these nutrients may partly explain the beneficial effects on health that nuts have been shown to exert [3,4]. The frequency of nut consumption has been inversely related to several chronic prevalent conditions, such as diabetes, hyperlipidemia, hypertension, obesity, metabolic syndrome, cancer, and CHD, among others [5,26]. These inverse associations can be influenced by various mechanisms: nuts improve the blood lipid profile [6] and appear to decrease insulin resistance [8], and there is also evidence suggesting that they can modulate inflammation [35], oxidative stress [36], and endothelial function [37]. As a large body of evidence supports the beneficial effects of frequent nut consumption on many health outcomes, it is plausible that nuts protect as well against all-cause mortality.

Our study has limitations. First, given its observational nature, it is not possible to firmly conclude that the inverse relationship between nut consumption and total mortality reflects cause and effect. Second, even though data were adjusted for all possible confounders, there is still the possibility of residual confounding. However, the enhanced protective effect against all-cause mortality observed in frequent nut consumers at baseline who continued eating nuts during follow-up because they were allocated to the nuts intervention arm supports a causal relationship between increasing dietary exposure to nuts and reduced mortality. However, as the study was conducted in an older Mediterranean population at high cardiovascular risk, the results cannot easily be extrapolated to the general population. Nevertheless, it is relevant to assess these associations in individuals at high cardiovascular risk because this population is the most frequently attended by primary care physicians and the segment of population that can obtain higher benefits with diet or lifestyle changes.

There are also strengths to our study, such as a large sample size, relatively long duration of follow-up, and objective and thorough ascertainment of mortality as outcome in this prospective observational assessment.

Conclusions

In summary, this study provides further evidence of the inverse relationship between the frequency of nut consumption and the risk of mortality in a Mediterranean population at high cardiovascular risk with relatively high nut intake.

Appendix: other PREDIMED Investigators

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Abbreviations

CHD: Coronary heart disease; CVD: Cardiovascular disease; EVOO: Extra-virgin olive oil; MedDiet: Mediterranean diet.

Competing interests

JS-S has received grants from Nut and Dried Fruit Foundation and is a non-paid member of the Scientific Advisory Board of the International Nut and Dried Fruit Foundation. ER has received grants from the California Walnut Commission and is a non-paid member of its Scientific Advisory Committee. No potential conflicts of interest relevant to this article were reported for any of the other authors. None of the funding sources played a role in the design, collection, analysis, or interpretation of the data or in the decision to submit the manuscript for publication.

Authors' contributions

MAM-G, DC, ER, RE, MIC, FA, JW, JL, MAM, RML-R, LS-M, XP, and JS-S designed research; MG-F, MB, MAM-G, DC, ER, RE, MIC, FA, JW, JL, MAM, RML-R, LS-M, XP, JB, JS-S conducted research; M-GF, MAM-G, and JS-S analyzed data; MG-F and JS-S wrote the paper; MAM, DC, RE, FA, JW, JL, MAM-G, LS-M, XP, and JS-S were the coordinators of subject recruitment at the outpatient clinics and MG-F and JS-S had primary responsibility for final content. All authors revised the manuscript for important intellectual content, read and approved the final manuscript.

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6.2. OLIVE OIL INTAKE AND RISK OF CARDIOVASCULAR DISEASE AND MORTALITY IN THE PREDIMED STUDY

Guasch-Ferré M, Hu FB, Martínez-González MA, Fitó M, Bulló M, Estruch R, Ros E, Corella D, Recondo J, Gómez-Gracia E, Fiol M, Lapetra J, Serra-Majem L, Muñoz MA, Pintó X, Lamuela-Raventós RM, Basora J, Buil-Cosiales P, Sorlí JV, Ruiz-Gutiérrez V, Martínez JA, Salas-Salvadó J.

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Study 2 overview. Novelty and significance.

What is already known?

- Olive oil intake is inversely associated with cardiovascular disease and all-cause mortality in healthy adults and elderly.
- Extra virgin olive oil, that contains higher amounts of polyphenols, may have cardiovascular benefits beyond lipid profile.
- Most previous studies made no distinction among the different varieties of olive oil, and the associations have not been explored in individuals at high cardiovascular risk from a Mediterranean country with higher consumption of olive oil.

What this study adds?

- For the first time a prospective study in elderly Mediterranean at high cardiovascular risk has found that total olive oil intake was associated with a 35% (HR: 0.65, 95% CI:0.47-0.89) reduction in the risk of cardiovascular disease.
- This association was even stronger for extra-virgin olive oil variety, 39% (HR: 0.61, 95% CI: 0.44-0.85) reduction in the risk of cardiovascular disease was observed in those individuals in the higher tertile of olive oil intake, compared to the reference.
- Per each 10 g/d increase in extra virgin olive oil it was associated with 10% (HR: 0.90, 95% CI: 0.85-0.95) lower risk of cardiovascular disease.

Summary

- Greater consumption of total olive oil, especially extra-virgin olive oil, was associated with reduced cardiovascular disease and mortality risk in an elderly Mediterranean population at high cardiovascular risk.

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RESEARCH ARTICLE Open Access

Olive oil intake and risk of cardiovascular disease and mortality in the PREDIMED Study

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Abstract

Background: It is unknown whether individuals at high cardiovascular risk sustain a benefit in cardiovascular disease from increased olive oil consumption. The aim was to assess the association between total olive oil intake, its varieties (extra virgin and common olive oil) and the risk of cardiovascular disease and mortality in a Mediterranean population at high cardiovascular risk.

Methods: We included 7,216 men and women at high cardiovascular risk, aged 55 to 80 years, from the PREvención con DIeta MEDiterránea (PREDIMED) study, a multicenter, randomized, controlled, clinical trial. Participants were randomized to one of three interventions: Mediterranean Diets supplemented with nuts or extra-virgin olive oil, or a control low-fat diet. The present analysis was conducted as an observational prospective cohort study. The median follow-up was 4.8 years. Cardiovascular disease (stroke, myocardial infarction and cardiovascular death) and mortality were ascertained by medical records and National Death Index. Olive oil consumption was evaluated with validated food frequency questionnaires. Multivariate Cox proportional hazards and generalized estimating equations were used to assess the association between baseline and yearly repeated measurements of olive oil intake, cardiovascular disease and mortality.

Results: During follow-up, 277 cardiovascular events and 323 deaths occurred. Participants in the highest energy-adjusted tertile of baseline total olive oil and extra-virgin olive oil consumption had 35% (HR: 0.65; 95% CI: 0.47 to 0.89) and 39% (HR: 0.61; 95% CI: 0.44 to 0.85) cardiovascular disease risk reduction, respectively, compared to the reference. Higher baseline total olive oil consumption was associated with 48% (HR: 0.52; 95% CI: 0.29 to 0.93) reduced risk of cardiovascular mortality. For each 10 g/d increase in extra-virgin olive oil consumption, cardiovascular disease and mortality risk decreased by 10% and 7%, respectively. No significant associations were found for cancer and all-cause mortality. The associations between cardiovascular events and extra virgin olive oil intake were significant in the Mediterranean diet intervention groups and not in the control group.

Conclusions: Olive oil consumption, specifically the extra-virgin variety, is associated with reduced risks of cardiovascular disease and mortality in individuals at high cardiovascular risk.

Trial registration: This study was registered at controlled-trials.com (http://www.controlled-trials.com/ISRCTN35739639). International Standard Randomized Controlled Trial Number (ISRCTN): 35739639. Registration date: 5 October 2005.

Keywords: Olive oil, Cardiovascular, Mortality, Mediterranean Diet, PREDIMED

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Background

Olive oil is a key component of the Mediterranean Diet (MedDiet), being the main source of vegetable fat, especially monounsaturated fatty acids (MUFA) [1]. Virgin olive oil, produced by mechanically pressing ripe olives, contains multiple bioactive and antioxidant components such as polyphenols, phytosterols and vitamin E [1], and has an acidity of <1.5%. Extra-virgin olive oil (EVOO) is also produced by mechanically pressing the olives but is the oil with the best quality, the most intense taste and its acidity is <1%. In contrast, common olive oil, obtained from a mixture of virgin and refined oil (usually more than 80% is refined), has fewer antioxidant and anti-inflammatory compounds. Since refined olive oil during the refining process loses phytochemicals, this oil is mixed with virgin olive oil to enhance the flavor, constituting the so-called common olive oil [2].

Evidence suggests that olive oil intake is inversely associated with cardiovascular disease (CVD) in the Spanish general population [3] and in a cohort of Italian women [4]. In the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC) study, total olive oil intake has been associated with a decreased risk of coronary heart disease, and also all-cause and cardiovascular mortality [5,6]. Similarly, a lower risk of mortality was associated with regular consumption of olive oil in an Italian population after myocardial infarction [7] and also in an elderly population [8]. A recent meta-analysis concluded that epidemiologic studies consistently found an inverse association between olive oil consumption and stroke, but there were inconsistencies between studies assessing coronary heart disease (CHD) as the end-point [9]. Of note, most of the previous studies made no distinction among the different varieties of olive oil [4,7,8]. Except for the EPIC-Spanish cohort that found a greater beneficial effect in CHD for the virgin olive oil variety than for the common variety [5] and similar effects for both varieties on all-cause mortality [6]. This distinction is important because EVOO contains much higher amounts of polyphenols than common olive oil. These polyphenols may have cardiovascular benefits beyond the lipid profile. It has also been reported that olive oil intake could be beneficial in the prevention of certain cancers, such as breast cancer [10], but the evidence is weaker.

Recently, in the context of the PREvención con DIeta MEDiterránea (PREDIMED) Study, it has been demonstrated that a MedDiet enriched with EVOO improved lipid profile, decreased blood pressure and reduced the risk of major cardiovascular events [11,12]. In this observational analysis of the PREDIMED population, we aimed to assess the associations between baseline olive oil consumption and the risk of CVD, cause-specific and overall mortality. We hypothesized that higher consumption of olive oil, especially the EVOO variety, would be associated with a decreased risk of CVD, cause-specific and overall mortality in an elderly Mediterranean population at high cardiovascular risk, independent of the allocated arm of the trial.

Methods

Study population

The present study was conducted within the framework of the PREDIMED trial, whose design has been described in detail elsewhere [13]. Briefly, the PREDIMED study is a large, multicenter, parallel-group, randomized and controlled clinical trial for the primary prevention of CVD (http://www.predimed.es). The main results of the trial on the primary endpoint have been published elsewhere [12]. We assigned 7,447 participants (men aged 55 to 80 years and women 60 to 80 years) to one of three interventions: a MedDiet supplemented with EVOO, a MedDiet supplemented with mixed nuts, or advice on a low-fat diet (control diet). Participants had no CVD at enrollment, but they were at high cardiovascular risk because of the presence of type 2 diabetes or at least three of the following risk factors: current smoking, hypertension, high low-density lipoprotein (LDL)-cholesterol, low high-density lipoprotein (HDL)-cholesterol, overweight or obesity, and family history of premature CVD. Exclusion criteria were the presence of any severe chronic illness, alcohol or drug abuse, body mass index (BMI) ≥40 kg/m^2, and allergy or intolerance to olive oil or nuts. The primary endpoint of the main trial was a composite of cardiovascular events (myocardial infarction, stroke or death from cardiovascular causes). The present analysis was conducted as an observational prospective cohort study using baseline consumption of olive oil as the exposure, and taking baseline data from the date of the first visit, before the individuals were randomized to the intervention group. The outcomes were: (a) composite of cardiovascular events, (b) cardiovascular mortality, (c) cancer mortality and (d) all-cause mortality. All participants provided written informed consent according to a protocol approved by the institutional review boards of all the recruiting centers (Comité de Ética e Investigación Clínica [CEIC] Hospital Universitari Sant Joan de Reus, CEIC Universidad de Navarra, CEIC Hospital Clínic de Barcelona, Comité de Ética Universidad de Valencia, CEIC-Parc de Salut Mar, CEIC Hospital Universitario Araba, CEIS del distrito Sanitario Atención Primaria Sevilla, IDIAP Jordi Gol, CEIC Complejo Hospitalario Materno-Insular, CEIC Facultad Medicina Universidad de Málaga, CEIC Illes Balears, CEIC Hospital Universitari Bellvitge).

Assessment of olive oil consumption and other covariates

At baseline and yearly during the follow-up, trained dieticians completed a 137-item semiquantitative food frequency questionnaire (FFQ) in a face-to-face interview

with the participants. This questionnaire has been validated before in a population at high cardiovascular risk from Spain. Reproducibility and validity of the FFQ for total olive oil consumption, estimates by the Pearson correlation coefficient (r) were 0.55 and 0.60, respectively, and the intraclass correlation coefficients for reproducibility and validity were 0.71 (P <0.001) [14]. Energy and nutrient intake were estimated using updated Spanish food composition tables [15,16]. Information on consumption of different types of olive oil intake was derived from the FFQ. The questionnaire includes three different questions regarding the consumption of olive oil: EVOO intake (produced by mechanically pressing the olives, acidity <1%), refined olive oil intake (refined olive oil, acidity <0.3%) and pomace olive oil (obtained from the residue of pressing the olives and mixed with other refined olive oils, acidity <0.3%). The dieticians asked the participants if they consumed one tablespoon of olive oil (for each of the specific varieties): never, between one to three times per month, times per week (once, two to four, five to six, three options) or times a day (once, two to three, four to six, more than six, four options). These questions were transformed to continuous variables in grams per day. The first question was used to assess EVOO intake, the sum of the second and third questions (refined olive oil and pomace olive oil) was considered common olive oil intake. The sum of all the three questions provides the total amount of olive oil consumed.

In addition, dieticians administered a validated 14-item MedDiet screener designed to assess the degree of adherence to the traditional MedDiet [17]. Two questions in the screener are related to olive oil intake (use of olive oil as the main fat for cooking (1 point if the answer is yes) and using four or more tablespoons of olive oil (1 point if the answer is yes), with 14 points the total score of the questionnaire). To control for the overall dietary pattern, we used this MedDiet screener removing the variables related to olive oil consumption; thus, a 12-point score was used as a covariate in the models.

At baseline, a questionnaire about lifestyle variables, educational achievement, history of illnesses and medication use was administered. Physical activity was assessed using the validated Spanish version of the Minnesota Leisure-Time Physical Activity questionnaire [18]. Participants were considered to be diabetic, hypercholesterolemic or hypertensive if they had previously been diagnosed as such, and/or they were being treated with antidiabetic, cholesterol-lowering or antihypertensive agents, respectively. Trained personnel took the anthropometric and blood pressure measurements. Weight and height were measured with light clothing and no shoes with calibrated scales and a wall-mounted stadiometer, respectively. Waist circumference was measured midway between the lowest rib and the iliac crest using an anthropometric tape. Blood pressure was measured using a validated oscillometer (Omron HEM705CP; Hoofddorp, The Netherlands) in triplicate with a five-minute interval between each measurement, and the mean of these values was recorded.

Ascertainment of cardiovascular disease and mortality

Information on CVD and mortality was updated once a year by the End-point Adjudication Committee, whose members were blinded to treatment allocation and to the dietary habits of participants. Different sources of information were used: (a) yearly questionnaires and examinations for all participants, (b) primary care physicians, (c) yearly review of medical records, and (d) linkage to the National Death Index. Medical records of deceased participants were requested. The End-point Adjudication Committee adjudicated the cause of the death and confirmed cardiovascular events.

Statistical analyses

Follow-up time was calculated as the interval between the date of cardiovascular events, death (cardiovascular, cancer or all-causes of death) or the end of follow-up (the date of the last visit or the last recorded clinical event of participants still alive) and the date of randomization. Extremes of reported total energy intake (>4,000 or <800 kcal per day in men and >3,500 or <500 kcal per day in women) were excluded from the present analysis [19]. Baseline characteristics of the studied population are presented according to energy-adjusted tertiles of total olive oil consumption, as means (SD) for quantitative variables and percentage (number) for categorical variables.

Multivariate Cox proportional hazard models were used to assess the associations between baseline energy-adjusted tertiles of olive oil intake and the risk of CVD, cardiovascular mortality, cancer mortality and all-cause mortality. Baseline total olive oil intake has also been analyzed as a continuous variable. All analyses were repeated using energy-adjusted tertiles of EVOO and common olive oil consumption. We also tested the associations between baseline olive fruit consumption and CVD and mortality (consumption of the whole olive fruit, not oil). All analyses were stratified by the recruitment center. Results are expressed as hazard ratios (HRs) with 95% confidence intervals (CIs). Model 1 was adjusted for age (continuous), sex and the intervention group. Model 2 was additionally adjusted for BMI (kg/m^2), smoking status (never, former, current smoker), alcohol intake (continuous, adding a quadratic term), educational level (illiterate/primary education, secondary education, academic/graduate), leisure time physical activity (Metabolic Equivalent of Task (MET)-minutes/d), prevalence of diabetes (yes/no), prevalence of hypertension (yes/no), prevalence of hypercholesterolemia (yes/no), use of antihypertensive medication (yes/no) and use of

statins (yes/no). A third model was additionally adjusted for baseline MedDiet adherence (12-point score). Additionally, myocardial infarction and stroke were used as outcomes of the analysis. Linear trend tests were assessed using the median value to each category of total olive oil, EVOO and common olive oil consumption and included as a continuous variable in the various models. We used Cox regression models to assess the association between total olive oil and CVD, cardiovascular mortality, cancer mortality and all-cause mortality separated by intervention group. Linear trends were also tested. As a secondary analysis and to take advantage of the updated information of yearly intake of total olive oil, we repeated the analysis using generalized estimating equations to assess the association between yearly repeated measurements of total olive oil consumption during follow-up and CVD and mortality. For each one-year period we used as exposure the average of total olive oil consumption of all repeated measurements from baseline to the beginning of that yearly period (between two and eight years). Sensitivity analyses were conducted including only the events observed in the two first years, between the second and fourth years, and after four years of follow-up. Also, sensitivity analyses were conducted excluding early cases (less than one year) and late cases (more than four years). The level of significance for all statistical tests was $P<0.05$ for bilateral contrast. Analyses were done using SPSS statistical software, version 19 (SPSS Inc., Chicago, IL, USA).

Results

After excluding those individuals with extremes of reported total energy intake (n = 153) and those with incomplete baseline dietary data (n = 78), a total of 7,216 participants were included in the present analysis. During a median of 4.8 y of follow-up we documented 277 incident cases of major cardiovascular events, 81 cardiovascular deaths, 130 cancer deaths and 323 all-cause deaths. The mean age of the participants was 67 y and 57.4% of them were women, respectively. The baseline characteristics of the participants according to energy-adjusted tertiles of total olive oil consumption are described in Table 1. As compared with participants in the lowest tertile of total olive oil intake, those in the highest tertile were more likely to have secondary education, lower total energy intake, lower consumption of red meat and dairy products, and also to drink less alcohol. The mean intake of total olive oil was 56.9 g/d in those participants allocated in the highest tertile compared to 21.4 g/d in those of the lowest tertile. Changes in total olive oil intake at the end of follow-up were 3.85 ± 23.02 g/d (mean ± SD) for the combined participants, 10.92 ± 22.91 g/d for those given MedDiet supplemented with EVOO, 2.36 ± 21.81 g/d for those given MedDiet supplemented with nuts and -3.03 ± 22.02 g/d in the control group. The total olive oil consumption by year during the follow-up for the total participants, and also by the intervention group is shown in Additional file 1.

After adjusting for age, sex and intervention group, there was a 34% (P for trend = 0.01) lower risk of major cardiovascular events among individuals in the highest tertile of baseline total olive oil intake as compared to those in the reference (Table 2). The inverse association remained statistically significant (P for trend = 0.01) after the adjustment for lifestyle variables and other potential confounders (36% lower risk (HR: 0.64; 95% CI: 0.46 to 0.87)) and even after further adjustment for MedDiet adherence (35% lower risk (HR: 0.65; 95% CI: 0.47 to 0.89)). For each 10 g/d (one tablespoon) higher baseline total olive oil consumption there was a 13% (HR: 0.87; 95% CI: 0.81 to 0.94) decreased risk of major cardiovascular events. A 48% reduction in the risk of cardiovascular death (HR: 0.52; 95% CI: 0.29 to 0.93) was found in the fully-adjusted model for those individuals in the highest tertile of total baseline olive oil consumption as compared to the reference. Also, for each 10 g/d (one tablespoon) higher total baseline olive oil consumption there was a 16% (HR: 0.84; 95% CI: 0.73 to 0.96) decreased risk of cardiovascular mortality. No statistically significant associations were found for cancer mortality and all-cause mortality.

The HR and 95% CIs for the association between EVOO, CVD and also for mortality are presented in Table 3. Baseline EVOO consumption was inversely associated with major events after adjusting for potential confounders (39% lower risk (HR: 0.61; 95% CI: 0.44 to 0.85 (P for trend <0.01)). A non-significant inverse association between baseline EVOO consumption and mortality outcomes was found, specifically for overall mortality. We observed non-significant associations between the baseline intake of common olive oil and major events and mortality (Table 4).

When we screened the risk of myocardial infarction and stroke according to the different categories and varieties of olive oil consumption it was observed that the inverse associations were non-statistically significant (data not shown).

We have also analyzed the association between the consumption of olive fruit (olives) and the risk of major events. We found an association of 25% decrease in the risk of major events in the top tertile of baseline olive fruit consumption after adjusting for potential confounders (HR: 0.75; 95% CI: 0.55 to 1.01, P for trend = 0.10).

The reduction in the risk of major cardiovascular events according to tertiles of total baseline olive oil intake separated by intervention group were 57% (HR: 0.43; 95% CI: 0.25 to 0.75, P for trend <0.01) and 55% (HR: 0.45; 95% CI: 0.25 to 0.82, P for trend <0.01) in the groups of MedDiet supplemented with EVOO or nuts, respectively. In contrast, the risk in the low fat control group was increased by 9% (HR: 1.09, 95% CI: 0.63 to 1.88, P for trend = 0.24) (P-value of homogeneity test: 0.178). The association

Table 1 Baseline characteristics of study participants according to energy-adjusted tertiles of total olive oil consumption

Baseline energy-adjusted tertiles of total olive oil consumption				
Variable	**1 (n = 2,405)**	**2 (n = 2,406)**	**3 (n = 2,405)**	***P*-value**
Age (y)	67 ± 6	67 ± 6	67 ± 6	0.744
Men, % (n)	45.7 (1,099)	42.2 (1,016)	39.8 (956)	<0.001
Intervention group, % (n)				0.476
MedDiet + EVOO	33.1 (796)	34.5 (830)	35.3 (848)	
MedDiet + Nuts	32.6 (785)	32.9 (792)	32.6 (783)	
Control low fat diet	34.3 (824)	32.6 (784)	32.2 (774)	
BMI (kg/m^2)	29.9 ± 3.73	29.9 ± 3.88	30.0 ± 3.93	0.427
Weight (kg)	77.0 ± 11.7	76.6 ± 12.0	76.6 ± 12.1	0.422
Leisure-time energy expenditure in physical activity (MET minutes/d)	243.1 ± 265.2	228.6 ± 225.5	221.3 ± 223.1	0.005
Smoking status, % (n)				0.262
Never	60.3 (1,450)	60.9 (1,466)	63.3 (1,523)	
Current	14.4 (347)	14.2 (341)	13.1 (316)	
Former	25.3 (608)	24.9 (599)	23.5 (566)	
Educational level, % (n)				0.040
Illiterate/primary education	79.3 (1,907)	77.6 (1,866)	76.1 (1,831)	
Secondary education	14.1 (338)	14.7 (354)	16.8 (404)	
Academic/graduate	6.7 (160)	7.7 (186)	7.1 (170)	
Prevalence of diabetes, % (n)	49.9 (1,200)	47.9 (1,153)	48.1 (1,156)	0.312
Prevalence of hypertension, % (n)	83.1 (1,998)	82.1 (1,976)	83.0 (1,995)	0.639
Prevalence of hypercholesterolemia, % (n)	73.6 (1,769)	71.7 (1,726)	71.4 (1,716)	0.190
Family history of coronary heart disease, % (n)	22.3 (536)	22.1 (531)	22.6 (544)	0.899
Medication use, % (n)				
Oral antidiabetic drugs	33.6 (808)	31.4 (756)	31.6 (759)	0.195
Antihypertensive drugs	71.9 (1,728)	72.4 (1,743)	73.9 (1,777)	0.264
Statins	40.4 (972)	40.5 (974)	39.8 (958)	0.880
Modified MedDiet score (12-point score)	6.9 ± 1.7	7.0 ± 1.7	7.2 ± 1.7	<0.001
Total energy intake (kcal/d)	2,266 ± 479	2,242 ± 668	2,199 ± 457	<0.001
Total olive oil (g/d)	21.4 ± 8.00	38.8 ± 11.6	56.9 ± 10.8	<0.001
Extra virgin olive oil (g/d)	9.1 ± 11.2	19.5 ± 20.0	34.6 ± 27.4	<0.001
Common olive oil (g/d)	12.1 ± 11.7	18.6 ± 18.5	21.7 ± 25.9	<0.001
Alcohol (g/d)	8.51 ± 14.1	9.41 ± 15.5	7.06 ± 12.2	<0.001
Nuts (g/d)	11.3 ± 14.8	10.2 ± 14.2	8.7 ± 11.5	<0.001
Vegetables (g/d)	346 ± 156	328 ± 145	327 ± 137	<0.001
Fruit (g/d)	389 ± 219	363 ± 196	351 ± 184	<0.001
Red meat (beef, pork, lamb) (g/d)	79.6 ± 49.1	79.1 ± 46.1	70.4 ± 41.2	<0.001
White meat (chicken, rabbit, turkey) (g/d)	46.4 ± 28.2	45.9 ± 27.6	42.1 ± 27.1	<0.001
Eggs (g/d)	20.5 ± 10.2	20.1 ± 11.5	19.3 ± 11.5	<0.001
Fish (g/d)	95.7 ± 51.9	99.8 ± 52.0	102 ± 46.9	<0.001
Dairy (g/d)	407 ± 231	375 ± 220	357 ± 205	<0.001

Data are expressed as means ± SD or percentage (n). *P*-value for comparisons across tertiles of baseline energy-adjusted olive oil consumption (Pearson chi-square test for categorical variables or 1-way analysis of variance for continuous variable) as appropriate. BMI, body mass index; EVOO, extra virgin olive oil; MedDiet, Mediterranean diet; MET, Metabolic Equivalent of Task.

Table 2 Risk of cardiovascular events and mortality according to baseline total olive oil intake

	Energy-adjusted tertiles of total olive oil, g/day				
	1 (low) (n = 2,405)	**2 (n = 2,406)**	**3 (high) (n = 2,405)**	***P for trend***	**Energy-adjusted total olive oil intake (10 g/d)**
Mean total olive oil intake	**21.4 ± 8.00**	**38.8 ± 11.6**	**56.9 ± 10.8**		
Major event					
Cardiovascular event, % (n)	4.5 (108)	3.6 (86)	3.5 (83)		3.8 (277)
Multivariable model 1	1 (Ref.)	0.76 (0.57, 1.02)	0.66 (0.48, 0.90)	0.01	0.87 (0.81, 0.94)
Multivariable model 2	1 (Ref.)	0.78 (0.58, 1.04)	0.64 (0.46, 0.87)	0.01	0.87 (0.81, 0.94)
Multivariable model 3	1 (Ref.)	0.78 (0.58, 1.04)	0.65 (0.47, 0.89)	0.01	0.87 (0.81, 0.94)
Cardiovascular mortality	**1 (low) (n = 2,405)**	**2 (n = 2,406)**	**3 (high) (n = 2,405)**	***P for trend***	
Cardiovascular mortality, % (n)	1.4 (33)	1.0 (25)	1.0 (23)		1.1 (81)
Multivariable model 1	1 (Ref.)	0.68 (0.39, 1.16)	0.52 (0.29, 0.94)	0.04	0.83 (0.72, 0.96)
Multivariable model 2	1 (Ref.)	0.70 (0.41, 1.20)	0.51 (0.28, 0.92)	0.04	0.83 (0.72, 0.95)
Multivariable model 3	1 (Ref.)	0.69 (0.40, 1.18)	0.52 (0.29, 0.93)	0.04	0.84 (0.73, 0.96)
Cancer mortality	**1 (low) (n = 2,405)**	**2 (n = 2,406)**	**3 (high) (n = 2,405)**	***P for trend***	
Cancer mortality, % (n)	1.8 (44)	2.0 (49)	1.5 (37)		1.8 (130)
Multivariable model 1	1 (Ref.)	1.13 (0.74, 1.72)	0.80 (0.49, 1.30)	0.96	0.93 (0.83, 1.05)
Multivariable model 2	1 (Ref.)	1.13 (0.74, 1.72)	0.84 (0.52, 1.36)	0.95	0.95 (0.84, 1.07)
Multivariable model 3	1 (Ref.)	1.13 (0.74, 1.72)	0.84 (0.52, 1.37)	0.94	0.95 (0.85, 1.07)
All-cause mortality	**1 (low) (n = 2,405)**	**2 (n = 2,406)**	**3 (high) (n = 2,405)**	***P for trend***	
All causes of mortality, % (n)	4.8 (116)	4.4 (106)	4.2 (101)		4.5 (323)
Multivariable model 1	1 (Ref.)	0.90 (0.69, 1.19)	0.79 (0.59, 1.06)	0.21	0.93 (0.87, 1.00)
Multivariable model 2	1 (Ref.)	0.90 (0.69, 1.18)	0.77 (0.58, 1.04)	0.18	0.93 (0.87, 1.00)
Multivariable model 3	1 (Ref.)	0.90 (0.69, 1.18)	0.78 (0.58, 1.05)	0.18	0.94 (0.87, 1.00)

Cox regression models were used to assess the risk of cardiovascular events and mortality by baseline energy-adjusted tertiles of total olive oil (g/day) and as a continuous variable (10 g/d). Results were presented as Hazard Ratios (95% CI). Multivariable model 1 was adjusted for age (years), sex and theintervention group. Model 2 was also adjusted for body mass index (BMI) (kg/m^2), smoking status (never, former, current smoker), alcohol intake (continuous, adding a quadratic term), educational level (illiterate/primary education, secondary education, academic/graduate), leisure time physical activity (Metabolic Equivalent of Task (MET)-minutes/d), prevalence of diabetes (yes/no), prevalence of hypertension (yes/no), prevalence of hypercholesterolemia (yes/no), use of antihypertensive medication (yes/no) and use of statins (yes/no). Model 3 was also adjusted for Mediterranean diet adherence (Modified 12-point Mediterranean Diet score). All models were stratified by recruitment center. Extremes of total energy intake were excluded. A major event was a composite of myocardial infarction, stroke and death from cardiovascular causes.

between major events and EVOO intake showed relative risk reductions of 41% (HR: 0.59; 95% CI: 0.32 to 1.07, *P* for trend = 0.050), 63% (HR: 0.37; 95% CI: 0.20 to 0.71, *P* for trend <0.01) and 15% (HR: 0.85; 95% CI: 0.51 to 1.41, *P* for trend = 0.503) in the MedDiet supplemented with EVOO, nuts and control group, respectively (*P*-value of homogeneity test: 0.364).

The inverse association between yearly updated measurements of total olive oil consumption and CVD using generalized estimating equations were also statistically significant after adjusting for potential confounders. The fully adjusted relative risk (RR) in the highest tertile of total olive oil consumption, as compared to the reference, showed a relative risk reduction of 34% (HR: 0.66; 95% CI: 0.48 to 0.91) with a significant linear trend test (*P* for trend <0.01). When we repeated the analysis to evaluate the associations between total olive oil consumption and cardiovascular mortality and cancer mortality, the fully adjusted relative risk (RR) in the top tertile of total olive oil showed a relative risk reduction of 44% (HR: 0.56; 95% CI: 0.31 to 1.02) and 24% (HR: 0.76; 95% CI: 0.46 to 1.24), respectively. However, the linear trend tests were non-significant. Finally, the fully adjusted relative risk (RR) in the top tertile of total olive oil consumption showed a relative risk reduction of 25% (HR: 0.75; 95% CI: 0.56 to 1.00, *P* for trend <0.01) for all-cause mortality.

The results of several sensitivity analyses were consistent with the findings of the primary analysis. When only the events observed in the first two years (91 events included) were considered, the risk of a major event in the top tertile of total olive oil was: 0.87 (95% CI: 0.50 to 1.51). When only events between the second and fourth years

Table 3 Risk of cardiovascular events and mortality according to baseline extra-virgin olive oil intake

	Energy-adjusted tertiles of extra-virgin olive oil, g/day				
	1 (low) (n = 2,405)	**2 (n = 2,406)**	**3 (high) (n = 2,405)**	***P for trend***	**Energy-adjusted extra virgin olive oil intake (10 g/d)**
Mean extra-virgin olive oil intake	**9.1 ± 11.23**	**19.5 ± 20.0**	**34.6 ± 27.4**		
Major event					
Cardiovascular event, % (n)	4.6 (111)	4.2 (101)	2.7 (65)		3.8 (277)
Multivariable model 1	1 (Ref.)	1.01 (0.77, 1.33)	0.60 (0.43, 0.82)	< 0.01	0.89 (0.84, 0.95)
Multivariable model 2	1 (Ref.)	1.00 (0.76, 1.32)	0.60 (0.44, 0.84)	< 0.01	0.90 (0.85, 0.95)
Multivariable model 3	1 (Ref.)	0.99 (0.75, 1.31)	0.61 (0.44, 0.85)	< 0.01	0.90 (0.85, 0.95)
Cardiovascular mortality	**1 (low) (n = 2,405)**	**2 (n = 2,406)**	**3 (high) (n = 2,405)**	***P for trend***	
Cardiovascular mortality, % (n)	1.3 (32)	1.2 (28)	0.9 (21)		1.1 (81)
Multivariable model 1	1 (Ref.)	1.01 (0.60, 1.70)	0.64 (0.36, 1.15)	0.10	0.93 (0.84, 1.03)
Multivariable model 2	1 (Ref.)	0.99 (0.59, 1.67)	0.64 (0.36, 1.15)	0.10	0.93 (0.83, 1.03)
Multivariable model 3	1 (Ref.)	0.97 (0.58, 1.64)	0.65 (0.36, 1.17)	0.13	0.93 (0.84, 1.03)
Cancer mortality	**1 (low) (n = 2,405)**	**2 (n = 2,406)**	**3 (high) (n = 2,405)**	***P for trend***	
Cancer mortality, % (n)	2.1 (50)	1.7 (41)	1.6 (39)		1.8 (130)
Multivariable model 1	1 (Ref.)	0.90 (0.59, 1.37)	0.87 (0.56, 1.37)	0.61	0.96 (0.88, 1.04)
Multivariable model 2	1 (Ref.)	0.88 (0.58, 1.35)	0.88 (0.56, 1.39)	0.68	0.96 (0.89, 1.05)
Multivariable model 3	1 (Ref.)	0.89 (0.58, 1.35)	0.90 (0.57, 1.41)	0.73	0.97 (0.89, 1.05)
All-cause mortality	**1 (low) (n = 2,405)**	**2 (n = 2,406)**	**3 (high) (n = 2,405)**	***P for trend***	
All causes of mortality, % (n)	5.2 (125)	4.2 (100)	4.1 (98)		4.5 (323)
Multivariable model 1	1 (Ref.)	0.88 (0.67, 1.15)	0.81 (0.61, 1.07)	0.19	0.95 (0.91, 1.00)
Multivariable model 2	1 (Ref.)	0.84 (0.64, 1.10)	0.80 (0.60, 1.07)	0.20	0.95 (0.90, 1.00)
Multivariable model 3	1 (Ref.)	0.84 (0.64, 1.10)	0.82 (0.61, 1.09)	0.25	0.96 (0.91, 1.01)

Cox regression models were used to assess the risk of cardiovascular events and mortality by baseline energy-adjusted tertiles of extra virgin olive oil (g/day) and as a continuous variable (10 g/d). Results were presented as Hazard Ratios (95% CI). Multivariable model 1 was adjusted for age (years), sex and the intervention group. Model 2 was also adjusted for body mass index (BMI) (kg/m^2), smoking status (never, former, current smoker), alcohol intake (continuous, adding a quadratic term), educational level (illiterate/primary education, secondary education, academic/graduate), leisure time physical activity (Metabolic Equivalent of Task (MET)-minutes/d), prevalence of diabetes (yes/no), prevalence of hypertension (yes/no), prevalence of hypercholesterolemia (yes/no), use of antihypertensive medication (yes/no) and use of statins (yes/no). Model 3 was also adjusted for Mediterranean diet adherence (Modified 12-point Mediterranean Diet score). All models were stratified by recruitment center. Extremes of total energy intake were excluded. A major event was a composite of myocardial infarction, stroke and death from cardiovascular causes.

(99 events) were considered, the RR in the top tertile of olive oil was: 0.55 (95% CI: 0.33 to 0.93), and only including events occurred after four years the RR was: 0.56 (95% CI: 0.31 to 1.01). The RR for the top tertile of olive oil excluding early cases occurred in the first year (230 events included) was 0.60 (95% CI: 0.43 to 0.85), and excluding late cases after four years (190 events included) the RR was 0.68 (95% CI: 0.46 to 0.98).

Discussion

In this prospective study of Mediterranean individuals at high cardiovascular risk, we found that baseline total olive oil consumption, especially the extra-virgin variety, was associated with a significant reduced risk of major cardiovascular events and cardiovascular mortality in a Mediterranean population at high cardiovascular risk. Relative risk reductions in CVD and all-cause mortality were similar for the upper baseline category of total olive oil consumption when we evaluated the repeated measurements of total olive oil consumption over time. We also found a reduction in cardiovascular mortality for an increased consumption of total olive oil. Each increase of 10 g/d in EVOO intake was associated with a 10% reduction in the risk of cardiovascular events. To the contrary, consumption of common olive oil was not significantly associated with cardiovascular morbidity and mortality.

In both MedDiet groups of our study (supplemented either with EVOO or nuts), participants in the top tertile of total olive oil consumption at baseline showed a lower risk of major events compared to those in the lowest tertile, but no associations were found for those individuals

Table 4 Risk of cardiovascular events and mortality according to baseline common olive oil intake

	Energy-adjusted tertiles of common olive oil, g/day				
	1 (low) (n = 2,405)	**2 (n = 2,406)**	**3 (high) (n = 2,405)**	***P for trend***	**Energy-adjusted common olive oil intake (10 g/d)**
Mean common olive oil intake	**12.1 ± 11.7**	**18.6 ± 18.5**	**21.7 ± 25.9**		
Major event					
Cardiovascular event, % (n)	3.5 (85)	3.6 (86)	4.4 (106)		3.8 (277)
Multivariable model 1	1 (Ref.)	1.06 (0.78, 1.45)	1.20 (0.88, 1.62)	0.23	1.04 (0.99, 1.10)
Multivariable model 2	1 (Ref.)	1.01 (0.74, 1.38)	1.13 (0.83, 1.54)	0.35	1.04 (0.98, 1.10)
Multivariable model 3	1 (Ref.)	0.99 (0.73, 1.36)	1.11 (0.82, 1.51)	0.40	1.03 (0.98, 1.09)
Cardiovascular mortality	**1 (low) (n = 2,405)**	**2 (n = 2,406)**	**3 (high) (n = 2,405)**	***P for trend***	
Cardiovascular mortality, % (n)	1.3 (31)	1.0 (24)	1.1 (26)		1.1 (81)
Multivariable model 1	1 (Ref.)	0.87 (0.50, 1.51)	0.84 (0.48, 1.46)	0.60	0.98 (0.88, 1.09)
Multivariable model 2	1 (Ref.)	0.84 (0.48, 1.47)	0.84 (0.48, 1.48)	0.65	0.98 (0.88, 1.09)
Multivariable model 3	1 (Ref.)	0.83 (0.47, 1.46)	0.81 (0.46, 1.43)	0.56	0.98 (0.87, 1.09)
Cancer mortality	**1 (low) (n = 2,405)**	**2 (n = 2,406)**	**3 (high) (n = 2,405)**	***P for trend***	
Cancer mortality, % (n)	1.7 (40)	1.7 (42)	2.0 (48)		1.8 (130)
Multivariable model 1	1 (Ref.)	1.07 (0.68, 1.68)	1.16 (0.74, 1.82)	0.51	1.01 (0.92, 1.10)
Multivariable model 2	1 (Ref.)	1.04 (0.66, 1.64)	1.16 (0.74, 1.82)	0.49	1.01 (0.93, 1.10)
Multivariable model 3	1 (Ref.)	1.03 (0.65, 1.62)	1.14 (0.73, 1.79)	0.52	1.01 (0.92, 1.10)
All-cause mortality	**1 (low) (n = 2,405)**	**2 (n = 2,406)**	**3 (high) (n = 2,405)**	***P for trend***	
All causes of mortality, % (n)	4.2 (101)	4.4 (106)	4.8 (116)		4.5 (323)
Multivariable model 1	1 (Ref.)	1.14 (0.86, 1.51)	1.17 (0.88, 1.51)	0.34	1.01 (0.96, 1.07)
Multivariable model 2	1 (Ref.)	1.10 (0.83, 1.47)	1.16 (0.87, 1.54)	0.37	1.01 (0.96, 1.07)
Multivariable model 3	1 (Ref.)	1.09 (0.82, 1.45)	1.14 (0.85, 1.51)	0.44	1.01 (0.96, 1.07)

Cox regression models were used to assess the risk of cardiovascular events and mortality by baseline energy-adjusted tertiles of common olive oil (g/day) and as a continuous variable (10 g/d). Results were presented as Hazard Ratios (95% CI). Multivariable model 1 was adjusted for age (years), sex and the intervention group. Model 2 was also adjusted for body mass index (BMI) (kg/m^2), smoking status (never, former, current smoker), alcohol intake (continuous, adding a quadratic term), educational level (illiterate/primary education, secondary education, academic/graduate), leisure time physical activity (Metabolic Equivalent of Task (MET)-minutes/d), prevalence of diabetes (yes/no), prevalence of hypertension (yes/no), prevalence of hypercholesterolemia (yes/no), use of antihypertensive medication (yes/no) and use of statins (yes/no). Model 3 was also adjusted for Mediterranean diet adherence (Modified 12-point Mediterranean Diet score). All models were stratified by recruitment center. Extremes of total energy intake were excluded. A major event was a composite of myocardial infarction, stroke and death from cardiovascular causes.

allocated to the control group. One explanation could be that the advice against eating fat food such as olive oil in the low fat control group throughout the study might have counterbalanced the protective effect of a lifetime intake of olive oil. However, we found inverse associations between olive oil consumption and CVD not only in the group supplemented with EVOO but also in the nuts group. Although it is difficult to isolate the effect of a single food because a range of foods are consumed in the whole diet, our study was able to distinguish the effects attributed to olive oil, a food that it is clearly a key component of the MedDiet. The positive association found in the MedDiet groups seems to confirm these effects.

Recent findings of the PREDIMED Study have demonstrated that adherence to the Mediterranean dietary pattern, as a whole and enriched with EVOO or nuts, reduce the incidence of major cardiovascular events by 30% within the context of primary prevention [12]. Our results further confirm the important role that olive oil consumption may play, even though other key components of the MedDiet, such as nuts, vegetables, fruits, legumes, fish and wine could also contribute to the observed beneficial effects. In agreement with our results, previous observational studies found an inverse association between olive oil consumption and CVD. Thus, in the EPICOR study conducted in 30,000 Italian women followed for 7.8 years, a 44% reduction in the risk of CHD was observed in those women in the top quartile of total olive oil consumption compared to those in the lowest quartile [4]. Similarly, the hazard ratio of CHD in

participants in the top quartile of olive oil consumption in the Spanish EPIC cohort was 0.78 (95% CI: 0.59 to 1.03) compared to the reference, after a 10.4-year follow-up. In this study, the reduction of CHD risk was greater for virgin olive oil than for the common variety [5].

We found a strong relationship between total olive oil consumption and the composite of cardiovascular major events, but when we analyzed separately myocardial infarction and stroke, the associations were non-significant. This could be explained by the lack of statistical power, but, according to our results, two previous case-control studies conducted in an Italian population also reported no associations between olive oil intake and myocardial infarction [20,21].

Our data suggest that total olive oil intake was inversely associated with cardiovascular mortality but not with cancer mortality: each 10 g/d increase in total olive oil consumption is associated with a 16% reduction in cardiovascular mortality. These results are supported by the findings of EPIC-Spain, where a 44% reduction in CVD mortality was found in participants at the top quartile of total olive oil consumption compared to those in the bottom quartile [6] and they can contribute to solving the inconsistencies reported by a recent meta-analysis [9]. In the same study of EPIC-Spain, total olive oil intake was not associated with cancer mortality [6]. However, like our study, this study did not examine specific types of cancer. A recent review of epidemiological studies reported some evidence suggesting that olive oil can decrease the risk of upper digestive and respiratory tract neoplasms, breast cancer and probably cancer in other sites [10]. Therefore, future larger studies or meta-analyses may need to focus on incidence and mortality of specific cancer sites.

The outcomes regarding olive oil consumption and all-cause mortality have been inconsistent, as reported in a recent meta-analysis including a large number of participants [9]. A previous study conducted in participants from Italy, who have suffered a myocardial infarction, showed that there was a 24% (HR: 0.76; 95% CI: 0.64 to 0.91) reduction in the risk of overall mortality for those consuming olive oil regularly compared to those who never consumed olive oil [7]. The results from the EPIC-cohort indicated a 26% (HR: 0.74; 95% CI: 0.64 to 0.87) reduction in the risk of overall mortality for those in the highest quartile of total olive oil intake compared to the lowest quartile [6]. On the contrary, no associations were found between total olive oil intake and all-cause mortality in a free-living Greek population [22]. Our findings suggest a non-significant possible inverse relation between each 10 g/d (one tablespoon of oil) increase in total olive oil and EVOO consumption and all-cause mortality.

The inverse associations between olive oil consumption and CVD could be explained by several mechanisms. The beneficial effects of olive oil are mainly attributed to its high content on MUFAs (that are less susceptible to oxidation than other type of fatty acids) but also to other minor components with important biological properties, such as phenolic compounds, vitamin E and other lipid-derivate molecules (squalene, tocopherols, triterpenic alcohols and so on), especially occurring in EVOO [2,3]. Evidence suggests that olive oil has anti-inflammatory and anti-atherogenic effects and it may also play a beneficial role in reducing oxidative stress and improving endothelial function [3,23,24]. Moreover, it has been observed that EVOO, particularly in a context of a MedDiet, improved lipid profile, insulin sensitivity, glycemic control, decreased blood pressure [11,24-27] and also was inversely associated with new-onset diabetes [28], all of them considered strong risk factors for CVD.

The strengths of the present study are its prospective design and a relatively long duration of follow-up. In addition, previous studies made no distinctions among different varieties of olive oil, but we analyzed the associations for EVOO and common olive oil separately. The present study was conducted in a population where the intake of total olive oil was relatively high, allowing a better assessment of the association between olive oil consumption and CVD or mortality.

Some limitations of our study deserve attention. First, the generalizability of our results may be limited, as the studied population was composed of Mediterranean elderly individuals at high cardiovascular risk who increased their intake of olive oil due to the intervention. However, the findings from our study are broadly consistent with those from other populations. Although the individuals changed their oil consumption during the study and this could have affected the observed beneficial effects of olive oil, it should be noted that the baseline intake of olive oil was high and the baseline assessment can be considered as a good correlate of lifetime habits in this population. Second, because of the observational nature of the study, residual confounding remains a possibility even though our analyses were extensively adjusted for a wide range of cardiovascular risk factors. Nonetheless, our observational findings are consistent with the intervention effects observed in the olive oil enriched arm in the PREDIMED trial. Finally, although the FFQ used was validated, measurement errors are inevitable, especially regarding self-reported different varieties of olive oil.

Conclusions

In summary, we found that greater consumption of total olive oil, especially EVOO, was associated with reduced cardiovascular disease and mortality risk in an elderly Mediterranean population at high cardiovascular risk. Our findings underscore olive oil consumption as one of the key components of the MedDiet for cardiovascular disease prevention.

Additional file

Additional file 1: Total olive oil intake during follow-up. Changes in total olive oil consumption by year during the follow-up for the total participants, and also by intervention group.

Abbreviations

BMI: Body mass index; CHD: Coronary heart disease; CVD: Cardiovascular disease; EPIC: European Prospective Investigation into Cancer and Nutrition; EVOO: Extra virgin olive oil; FFQ: Food frequency questionnaires; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; MedDiet: Mediterranean diet; MUFA: Monounsaturated fatty acids.

Competing interests

No potential conflicts of interest relevant to this article were reported by any of the authors.

Authors' contributions

MAM-G, RE, ER, DC, EG-G, MF, JL, LS-M, MAM, XP, RML-R, VR-G and JS-S designed the research. MG-F, FBH, MAM-G, MF, MB, RE, ER, DC, JR, EG-G, MF, JL, LS-M, MAM, XP, RML-R, JB, PB-C, JVS, VR-G, JAM and JS-S conducted the research. M-GF, FBH and JS-S analyzed the data. MG-F and JS-S wrote the paper. MAM-G, RE, ER, DC, MF, JL, LS-M, MAM, XP and JS-S were the coordinators of subject recruitment at the outpatient clinics. MG-F and JS-S had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors revised the manuscript for important intellectual content, and read and approved the final manuscript.

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6.3. DIETARY MAGNESIUM INTAKE IS INVERSELY ASSOCIATED WITH MORTALITY IN ADULTS AT HIGH CARDIOVASCULAR DISEASE RISK

Guasch-Ferré M, Bulló M, Estruch R, Corella D, Martínez-González MA, Ros E, Covas M, Arós F, Gómez-Gracia E, Fiol M, Lapetra J, Muñoz MÁ, Serra-Majem L, Babio N, Pintó X, Lamuela-Raventós RM, Ruiz-Gutiérrez V, Salas-Salvadó J; PREDIMED Study Group.

J Nutr. 2014 Jan;144(1):55-60. PMID: 24259558; doi: 10.3945/jn.113.183012.

Study 3 overview. Novelty and significance.

What is already known?

- The relation between dietary magnesium intake and cardiovascular disease and mortality was evaluated in several prospective studies, but few of them have assessed the risk of all-cause mortality.
- This has never been evaluated in Mediterranean individuals at high cardiovascular risk.

What this study adds?

- In our prospective study of Mediterranean individuals at high risk followed for a median of 4.8 years, we found that dietary magnesium intake was associated with cardiovascular, cancer and all-cause mortality.
- Compared with lower consumers, individuals in the highest tertile of magnesium intake had 37% (HR: 0.63, 95% CI: 0.46-0.86) reduction in all-cause mortality risk.

Summary

- Our findings suggest an inverse association between dietary magnesium intake and cardiovascular, cancer, and all-cause mortality in participants at high cardiovascular risk.

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Nutrition and Disease

Dietary Magnesium Intake Is Inversely Associated with Mortality in Adults at High Cardiovascular Risk[1–3]

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Abstract

The relation between dietary magnesium intake and cardiovascular disease (CVD) or mortality was evaluated in several prospective studies, but few of them have assessed the risk of all-cause mortality, which has never been evaluated in Mediterranean adults at high cardiovascular risk. The aim of this study was to assess the association between magnesium intake and CVD and mortality risk in a Mediterranean population at high cardiovascular risk with high average magnesium intake. The present study included 7216 men and women aged 55–80 y from the PREDIMED (Prevención con Dieta Mediterránea) study, a randomized clinical trial. Participants were assigned to one of two Mediterranean diets (supplemented with nuts or olive oil) or advice on a low-fat control diet. Mortality was ascertained by linkage to the National Death Index and medical records. We fitted multivariable-adjusted Cox regressions to assess associations between baseline energy-adjusted tertiles of magnesium intake and relative risk of CVD and mortality. Multivariable analyses with generalized estimating equation models were used to assess the associations between yearly repeated measurements of magnesium intake and mortality. After a median follow-up of 4.8 y, 323 total deaths, 81 cardiovascular deaths, 130 cancer deaths, and 277 cardiovascular events occurred. Energy-adjusted baseline magnesium intake was inversely associated with cardiovascular, cancer, and all-cause mortality. Compared with lower consumers, individuals in the highest tertile of magnesium intake had a 34% reduction in mortality risk (HR: 0.66; 95% CI: 0.45, 0.95; $P < 0.01$). Dietary magnesium intake was inversely associated with mortality risk in Mediterranean individuals at high risk of CVD. This trial was registered at controlled-trials.com as ISRCTN35739639. J. Nutr. doi: 10.3945/jn.113.183012.

Introduction

Magnesium is an essential mineral for the human body, acting as a coenzyme in different ATP-dependent reactions and in the production and transport of energy and proteins (1). The major food sources of magnesium are vegetables, fruits, legumes, nuts, soy products, and whole grains. Some evidence suggests that high dietary magnesium intake plays a protective role not only in cardiovascular risk factors, such as diabetes mellitus (2),

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[3] Supplemental Appendix is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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hypertension (3), and metabolic syndrome (4), but also in cardiovascular disease (CVD)[20] (5).

A meta-analysis evaluating the association between magnesium and the risk of cardiovascular events demonstrated that both dietary and serum magnesium were inversely related with the risk of total CVD events (6). Similarly, inverse associations between dietary magnesium intake and risk of stroke or ischemic heart disease were also demonstrated in two other recent meta-analyses (7,8).

Prospective studies have also been made into the relation between magnesium and cardiovascular and cancer mortality. Some suggest inverse associations (9,10) between magnesium intake and cardiovascular mortality, but others have found no significant associations (4,11,12). Two prospective studies have evaluated the association between dietary magnesium intake and cancer death (11,12). Finally, an inverse association between dietary or plasma magnesium concentrations and sudden cardiac death was reported recently (13), and serum magnesium has also been inversely associated with cardiovascular, cancer, and all-cause mortality in middle-aged men (14).

Although some studies have shown that dietary magnesium intake is inversely related to CVDs and mortality, very few have evaluated the risk of all-cause mortality (11), and these associations have not been evaluated previously in Mediterranean individuals.

We hypothesized that dietary magnesium intake is inversely associated with CVDs and mortality. Therefore, the main aim of the present study was to assess the association between dietary magnesium intake and the risk of CVD (a composite including stroke, myocardial infarction, and cardiovascular death) and cardiovascular, cancer, and all-cause mortality in Mediterranean individuals at high risk of CVD and with a high average magnesium intake.

Materials and Methods

Study population. The PREDIMED (Prevención con Dieta Mediterránea) study is a multicenter, randomized, parallel-group clinical trial conducted in Spain. Details of the cohort, design of the study, and methods were described previously (15–18). The study was registered at controlled-trials.com as ISRCTN35739639 (19). A complete list of the PREDIMED investigators is listed in the **Supplemental Appendix**. The study included men (aged 55–80 y) and women (aged 60–80 y) who were free of CVD at enrollment but at high CVD risk. The inclusion criteria for the participants in the trial were having type 2 diabetes mellitus or three or more of the following cardiovascular risk factors: *1)* family history of premature CVD; *2)* overweight or obesity; *3)* current smoking; *4)* hypertension; *5)* hypercholesterolemia; and *6)* low HDL-cholesterol. The exclusion criteria included the following: *1)* presence of BMI $\geq$ 40 kg/m^2; *2)* alcohol or drug abuse; *3)* any severe chronic illness; and *4)* allergy or intolerance to olive oil or nuts. The study included 7447 participants who were randomized to one of two Mediterranean diets (MedDiets) [supplemented with either extra-virgin olive oil (EVOO) or mixed nuts] or to a control diet (advice on a low-fat diet). All participants included in the study provided written informed consent according to a protocol approved by the institutional review boards of the recruiting centers. In the present study, we analyzed data as in an observational prospective cohort.

Assessment of magnesium intake and other covariates. Dietary magnesium intake was assessed by a validated baseline 137-item FFQ completed by trained dieticians (20). We used Spanish food composition tables to estimate energy and nutrient intake (21,22). Reproducibility and validity of the FFQ for dietary magnesium intake estimated by the Pearson's correlation coefficient (r) was 0.71, and the intraclass correlation coefficients for reproducibility and validity were 0.83 and 0.67, respectively ($P < 0.001$). At baseline, we administered several questionnaires about history of illnesses, medication use, lifestyle variables, and educational achievement. The validated Spanish version of the Minnesota Leisure-Time Physical Activity questionnaire was used to assess physical activity (23). If participants were being treated with antidiabetic, cholesterol-lowering, or antihypertensive agents, or if they had been diagnosed previously, they were considered to be diabetic, hypercholesterolemic, or hypertensive, respectively. Anthropometric and blood pressure measurements were taken by trained personnel. We used calibrated scales and a wall-mounted stadiometer to measure weight and height, respectively, with participants in light clothing and no shoes. Waist circumference was measured midway between the lowest rib and the iliac using an anthropometric tape. We used a validated oscillometer (HEM705CP; Omron) to measure blood pressure in triplicate with a 5-min interval between each measurement, and we recorded the mean of these three values.

Ascertainment of CVD and mortality. For the present analysis, we used the following endpoints: *1)* a composite of cardiovascular events (myocardial infarction, stroke, and death from cardiovascular causes); and *2)* cardiovascular, cancer, and all-cause mortality. The endpoint adjudication committee, whose members were unaware of treatment allocation, updated information on these endpoints once a year. The committee used different sources of information: *1)* yearly questionnaires and examinations for all participants; *2)* family physicians; *3)* yearly review of medical records; and *4)* linkage to the National Death Index. Medical records of deceased participants were requested, and the endpoint adjudication committee determined the cause of death.

Statistical analyses. ANOVA or the Pearson's χ^2 tests were used to compare the quantitative or categorical baseline characteristics of the study participants, respectively, across baseline energy-adjusted tertiles of magnesium intake. The results were expressed as means $\pm$ SEs or percentages.

Follow-up time was calculated as the difference between the date of the cardiovascular event, death, or end of follow-up (the date of the last visit or the last recorded clinical event of participants who were still alive) and the date of recruitment.

Multivariate Cox regression models were fitted to estimate the hazard ratios (HRs) and 95% CIs of the cardiovascular events and cardiovascular, cancer, and all-cause mortality. We stratified all analyses by the recruitment center. The first multivariate model was adjusted for sex, age (years), and intervention group. The second model was also adjusted for the following covariates: *1)* BMI; *2)* smoking status (never, former, current smoker); *3)* leisure time physical activity [metabolic equivalent task (MET)-min/d, MET, 1 MET-min is approximately equivalent to 1 kcal, METs-min/d); *4)* educational level (illiterate/primary education, secondary education, academic/graduate); *5)* prevalence of hypertension (yes/no); *6)* prevalence of diabetes (yes/no); *7)* prevalence of hypercholesterolemia (yes/no); *8)* family history of coronary heart disease (CHD) (no, yes before 55 y, yes after 55 y); *9)* use of aspirin (yes/no); *10)* use of oral antidiabetic medication (yes/no); *11)* use of antihypertensive medication (yes/no); *12)* use of hypocholesterolemic medication (yes/no); and *13)* alcohol intake (continuous, adding a quadratic term). A third model was adjusted for the total of dietary fiber and calcium intake. When myocardial infarction and stroke were used as the outcomes of the analysis, the models were adjusted for the same potential confounders used in model 2. We also separated the analysis by intervention group. We assigned the median value to each tertile of magnesium intake and used it as a continuous variable to assess linear trend tests in various models. During the follow-up, we obtained information on magnesium intake yearly. We repeated the analysis using generalized estimating equations to evaluate the association between repeated measurements of energy-adjusted magnesium intake and mortality. For each 1-y period, the exposure indicator we used was the average magnesium intake of all repeated measurements from baseline to the beginning of that yearly period.

The level of significance for all statistical tests was $P < 0.05$ for bilateral contrast. Analyses were performed using SPSS statistical software (version 19; SPSS).

[20] Abbreviations used: CVD, cardiovascular disease; EVOO, extra-virgin olive oil; MedDiet, Mediterranean Diet; MET, metabolic equivalent task.

Results

For the present analysis, we excluded those participants with high or low energy intake (<500 or >3500 kcal/d for women; >800 or >4000 kcal/d for men) and those with incomplete dietary data at baseline (n = 78). As a result, a total of 7216 participants were included. There were no significant interactions between dietary magnesium intake and sex, alcohol intake, smoking status, or the use of medication.

The baseline characteristics of study participants according to baseline energy-adjusted tertiles of dietary magnesium are shown in **Table 1**. The participants in the highest tertile of dietary magnesium intake were mainly women, had lower body weight, were more physically active, and were less likely to smoke or drink alcohol. These participants also had intakes of dietary fiber and calcium. The mean intake of magnesium in the lowest and the highest energy-adjusted tertile, respectively, was 318 and 454 mg/d.

The median follow-up time of the study was 4.8 y; after this period, 323 total deaths, 81 cardiovascular deaths, 130 cancer deaths, and 277 cardiovascular events occurred (**Table 2**). Of the total deaths, 145 were in the lower energy-adjusted tertile of magnesium intake and 78 in the upper tertile.

Table 2 shows the HRs and 95% CIs for CVD and mortality according to baseline energy-adjusted tertiles of magnesium intake. After adjusting for potential confounders (model 2), those participants with the highest magnesium intake had 37% less risk of all-cause mortality (HR: 0.63; 95% CI: 0.46, 0.86; P-trend < 0.01) than those participants in the lower tertile. Magnesium intake was also inversely associated with cardiovascular and cancer mortality. The respective multivariable HRs in model 2 for the highest energy-adjusted tertile of magnesium intake were 0.53 (95% CI: 0.28, 0.99), P-trend = 0.06 and 0.55 (95% CI: 0.33, 0.91), P-trend = 0.04. Additional adjustment for total intake of dietary fiber and calcium intake (model 3) did not appreciably alter these results [HR for all-cause mortality for the highest compared with the lowest tertile: 0.66 (95% CI: 0.45, 0.95); HR for cardiovascular mortality: 0.41 (95% CI: 0.19, 0.88); HR for cancer mortality: 0.63 (95% CI: 0.35, 1.15)]. To exclude the potential bias effect of those individuals who took

TABLE 1 Baseline characteristics of study participants according to energy-adjusted tertiles of dietary magnesium intake[1]

	Baseline energy-adjusted tertiles of magnesium intake			
Variable	1 (n = 2405)	2 (n = 2406)	3 (n = 2405)	P values[2]
Median magnesium intake, *mg/d*	312	341	442	
Age, *y*	67 ± 6	67 ± 6	67 ± 6	0.05
Men, *% (n)*	54.2 (1303)	38.7 (932)	34.8 (836)	<0.01
BMI, *kg/m²*	30.0 ± 3.70	29.9 ± 3.86	29.9 ± 4.00	0.54
Weight, *kg*	78.2 ± 11.9	76.2 ± 12.1	75.8 ± 11.7	<0.01
Leisure-time energy expenditure in physical activity, *MET-min/d*	225 ± 227	224 ± 226	244 ± 262	<0.01
Smoking status, *% (n)*				<0.01
Never	53.6 (1290)	63.6 (1530)	67.3 (1619)	
Current	18.9 (454)	12.4 (299)	10.4 (251)	
Former	27.5 (661)	24.0 (577)	22.2 (535)	
Educational level, *% (n)*				0.15
Illiterate/primary education	76.8 (1847)	78.3 (1885)	77.8 (1872)	
Secondary education	16.5 (396)	14.8 (355)	14.3 (345)	
Academic/graduate	6.7 (162)	6.9 (166)	7.8 (188)	
Prevalence of diabetes, *% (n)*	46.2 (1111)	49.5 (1191)	50.9 (1225)	<0.01
Prevalence of hypertension, *% (n)*	83.1 (1998)	83.6 (2011)	81.5 (1961)	0.14
Prevalence of hypercholesterolemia, *% (n)*	68.8 (1654)	72.5 (1745)	75.4 (1813)	<0.01
Family history of myocardial infarction, *% (n)*	19.5 (468)	23.4 (563)	24.1 (580)	<0.01
Medication use, *% (n)*				
Aspirin	21.3 (512)	21.9 (527)	23.9 (574)	0.08
Oral antidiabetic drugs	29.6 (712)	33.7 (812)	33.2 (799)	<0.01
Antihypertensive drugs	72.9 (1753)	74.1 (1784)	71.1 (1711)	0.06
Statins	37.2 (894)	40.4 (972)	43.2 (1038)	<0.01
Total energy intake, *kcal/d*	2340 ± 565	2110 ± 496	2260 ± 542	<0.01
Magnesium intake, *mg/d*	318 ± 73.0	347 ± 67.8	454 ± 102	<0.01
Alcohol intake, *g/d*	12.1 ± 17.9	6.97 ± 11.7	5.94 ± 10.6	<0.01
Total protein, *g/d*	86.9 ± 20.9	88.1 ± 19.4	98.9 ± 21.6	<0.01
Total carbohydrates, *g/d*	240 ± 78.9	218 ± 66.4	244 ± 71.4	<0.01
Total fat, *g/d*	105 ± 28.0	92.8 ± 26.0	93.9 ± 29.0	<0.01
Saturated fat, *g/d*	26.9 ± 8.84	23.8 ± 7.67	23.7 ± 8.45	<0.01
Monounsaturated fat, *g/d*	53.3 ± 14.9	46.2 ± 14.2	44.9 ± 15.2	<0.01
Polyunsaturated fat, *g/d*	15.8 ± 6.4	14.6 ± 5.90	16.2 ± 7.26	<0.01
Total fiber, *g/d*	20.4 ± 5.55	23.0 ± 5.57	32.3 ± 9.19	<0.01
Calcium intake, *mg/d*	890 ± 306	1010 ± 321	1190 ± 375	<0.01

[1] Values are mean ± SE or percentage (n). MET, metabolic equivalent task.
[2] P values for comparisons across baseline energy-adjusted magnesium intake (Pearson χ^2 test for categorical variables or one-factor analysis of variance for continuous variable) as appropriate.

TABLE 2 Hazard ratios and 95% CIs for cardiovascular event and mortality according to tertiles of magnesium intake[1]

	Baseline energy-adjusted tertiles of dietary magnesium intake, HR (95% CI)			
	1 (low) (n = 2405)	2 (n = 2406)	3 (high) (n = 2405)	P-trend
Major event[2]				
Median magnesium intake, *mg/d*	312	341	442	
Cardiovascular event, *% (n)*	4.6 (111)	3.8 (91)	3.1 (75)	
Crude model	1 (Reference)	0.86 (0.65, 1.14)	0.73 (0.54, 0.98)	0.04
Multivariable model 1[3]	1 (Reference)	0.96 (0.72, 1.28)	0.80 (0.59, 1.09)	0.15
Multivariable model 2[4]	1 (Reference)	0.92 (0.68, 1.23)	0.83 (0.60, 1.14)	0.27
Cardiovascular mortality				
Cardiovascular death, *% (n)*	1.7 (40)	1.0 (23)	0.7 (18)	
Crude model	1 (Reference)	0.60 (0.36, 1.01)	0.49 (0.28, 0.85)	0.02
Multivariable model 1	1 (Reference)	0.70 (0.41, 1.18)	0.51 (0.28, 0.95)	0.04
Multivariable model 2	1 (Reference)	0.67 (0.39, 1.16)	0.53 (0.28, 0.99)	0.06
Cancer death				
Cancer death, *% (n)*	2.6 (63)	1.5 (37)	1.2 (30)	
Crude model	1 (Reference)	0.62 (0.42, 0.94)	0.52 (0.34, 0.81)	0.01
Multivariable model 1	1 (Reference)	0.67 (0.44, 1.02)	0.57 (0.35, 0.91)	0.04
Multivariable model 2	1 (Reference)	0.65 (0.42, 1.01)	0.55 (0.33, 0.91)	0.04
All-cause mortality				
All causes of death, *% (n)*	6.0 (145)	4.2 (100)	3.2 (78)	
Crude model	1 (Reference)	0.73 (0.57, 0.94)	0.59 (0.45, 0.78)	<0.01
Multivariable model 1	1 (Reference)	0.79 (0.61, 1.03)	0.64 (0.48, 0.86)	<0.01
Multivariable model 2	1 (Reference)	0.77 (0.59, 1.01)	0.63 (0.46, 0.86)	<0.01

[1] Cox regression models were used to assess the risk of mortality by tertiles of dietary magnesium intake (mg/d).
[2] Major event was a composite of myocardial infarction, stroke, and death from cardiovascular causes.
[3] Multivariable model 1 was adjusted for age in years, sex, and intervention group.
[4] Model 2 was also adjusted for body mass index (kg/m^2), smoking status (never, former, current smoker), educational level (illiterate/primary education, secondary education, academic/graduate), leisure time physical activity (metabolic equivalent task-min/d), prevalence of diabetes (yes/no), prevalence of hypertension (yes/no), prevalence of hypercholesterolemia (yes/no), family history of coronary heart disease (no, yes before 55 y, yes after 55 y), use of aspirin (yes/no), use of antihypertensive medication (yes/no), use of oral antidiabetic medication (yes/no), use of hypocholesterolemic medication (yes/no), alcohol intake (continuous, adding a quadratic term). All models were stratified by recruitment center. Extremes of total energy intake were excluded.

magnesium supplements and multivitamins, we conducted a sensitivity analysis from which they were excluded (seven individuals from the total population, 0.09%), and the results did not change.

After adjusting for all potential confounders included in the previous model 2, the HRs for baseline dietary magnesium intake and major cardiovascular event were 0.83 (95% CI: 0.6, 1.14; P-trend = 0.27) and 0.62 (95% CI: 0.36, 1.06; P-trend = 0.08) when we analyzed only the risk of myocardial infarction. Neither was any association found when we analyzed the risk of stroke (HR: 1.10; 95% CI: 0.70, 1.74; P-trend = 0.64).

When we separated the analysis by intervention group, we observed a significant reduction in the risk of all-cause mortality in those individuals in the highest tertile of magnesium consumption and assigned to the control low-fat diet (HR: 0.42; 95% CI: 0.22, 0.78; P-trend < 0.01). There were no associations for those who were assigned to the MedDiet with nuts and EVOO. Compared with the individuals in the lowest tertile of magnesium intake, we observed a significant reduction in the risk of cardiovascular mortality in the individuals in the highest tertile of magnesium intake and assigned to the MedDiet supplemented with nuts (HR: 0.27; 95% CI: 0.08, 0.87; P-trend = 0.04). No associations were observed for the other intervention groups and other outcomes (major event and cancer death).

When we used generalized estimating equations to assess the association between yearly updated measurements of energy-adjusted magnesium and all-cause mortality, we observed a fully-adjusted RR of 0.70 (95% CI: 0.52, 0.95), but the linear trend test was nonsignificant (P-trend = 0.32). The analysis to assess the relation between magnesium intake and cardiovascular mortality and cancer mortality showed a fully-adjusted RR of 0.77 (95% CI: 0.40, 1.48) and 0.50 (95% CI: 0.30, 0.83), respectively. The linear trend tests were also nonsignificant (P-trend = 0.91 and 0.12, respectively).

Discussion

In this prospective study of Mediterranean individuals at high risk of CVD, an inverse association was found between dietary magnesium intake and risk of mortality. No significant associations were observed between magnesium intake and major cardiovascular events. When we evaluated the repeated measurements of magnesium intake over time, we found a reduction in the RR of all-cause and cancer mortality in those individuals in the higher tertile of magnesium intake compared with those in the lower tertile.

There were no associations between baseline magnesium intake and mortality in participants in the highest tertile of magnesium intake in either of the MedDiet groups (supplemented with EVOO or nuts). However, a significant association was found in those randomized to a low-fat control diet; they were advised to reduce the intake of all sources of fat. The lower magnesium intake in this group, then, probably resulted in a stronger inverse association between magnesium intake and mortality. In fact, previous evidence has suggested an inverse relation between marginal-to-moderate dietary magnesium deficiency and several chronic diseases (24).

A meta-analysis of 532,979 participants from 19 studies has shown that the pooled RR of total CVD events was 15% and 33% lower in the highest category of dietary magnesium and

serum magnesium, respectively, than in the lowest (6). Results were similar for ischemic stroke in another meta-analysis of seven prospective studies with 241,378 participants (7). Significant associations between circulating magnesium and CVD events were shown by the most recent meta-analysis, which also associated dietary magnesium with 22% lower risk of ischemic heart disease and showed an inverse significant association between dietary magnesium and fatal ischemic heart disease up to a threshold of ~250 mg/d compared with higher intakes (8). The findings of these meta-analyses consistently showed inverse associations between magnesium intake or status and cardiovascular events. However, in the present study of the PREDIMED cohort, we have not demonstrated a significant decreased risk in the composite of cardiovascular events.

To the best of our knowledge, five previous prospective studies have directly assessed the associations between magnesium intake and the risk of cardiovascular death with diverging results (4,9–11,13). In agreement with our results, a decreased risk in mortality by CVD and sudden cardiac death was found in the women of the Japan Collaborative Cohort and the Nurses' Health Study, respectively (10,13). Conversely, a nonsignificant inverse association was found for fatal CHD in the men of the Health Professional Follow-Up Study cohort (9), and a nonsignificant effect was also found between dietary magnesium and cardiovascular death in the Women's Health Study and the Cohort of Swedish Men (4,11). The mean intake of magnesium in the lowest categories in these studies was apparently adequate for healthy individuals but probably not for individuals at high cardiovascular risk, such as those in the PREDIMED study (24).

Only two prospective studies—the Cohort of Swedish Men and the EPIC-Heidelberg Study—have assessed the association between dietary magnesium intake and cancer mortality. Neither of them showed any associations (11,12), so our study is the first to find a significant inverse association between dietary magnesium intake and cancer mortality in individuals at high cardiovascular risk. However, middle-aged men with higher serum magnesium concentrations had a 50% lower risk of cancer death than those in the lower quartile of serum magnesium intake (14). Magnesium is involved in several biochemical reactions modulating cell proliferation, differentiation, and apoptosis (25). It was also shown to play a key role in genetic stability and DNA synthesis (26), and supplemental magnesium was shown to reduce the incidence of cancer, possibly by means of inhibition of *c-myc* oncogene expression in cancer cells (27,28). Intake of magnesium was also reported to reduce insulin resistance and the risk of type 2 diabetes, which is a potential risk factor for cancer (29).

Finally, only one prospective study has related dietary magnesium intake and all-cause mortality. The results of this study concluded that there was no significant association between magnesium intake and all-cause mortality in the men of the Cohort of Swedish Men (11). However, in agreement with our results, there was a 60% reduction in all-cause mortality in individuals with a high concentration of serum magnesium compared with those in the reference category (14). In our study, we also found a significant reduction in the risk of all-cause mortality. It should be mentioned that the enrollment conditions of the present study—i.e., individuals at high cardiovascular risk, most of whom are overweight or obese and who apparently have increased requirements of dietary magnesium—have contributed to the positive associations observed between magnesium intake and disease or mortality. Moreover, magnesium deficiency, through exacerbating chronic inflammatory stress, may play a role in the onset of such chronic diseases as atherosclerosis, hypertension, osteoporosis, diabetes mellitus, and cancer (24).

There may be several explanations for these associations. In particular, hypertension is a strong risk factor for CVD, and it is known that magnesium can lower blood pressure (30). Also, magnesium intake may inhibit platelet aggregation, modulate inflammation, and improve endothelial function. All of these mechanisms can have a beneficial effect on lowering the risk of CVD and death (5,31).

We acknowledge that magnesium is an isolated nutrient, and it is important to investigate the associations between the whole diet and health. However, to establish appropriate dietary patterns, it is essential to know which nutrients and food play an important role in the target pathologies, in this case CVD and mortality.

Several potential limitations of our study deserve comment. First, the present results may not be extrapolatable to the general population because the study was conducted in participants with a high risk of CVD. Second, there is the possibility of residual confounding, especially of dietary fiber or other nutrients. To prevent this from happening, we adjusted the analysis for possible confounders. The FFQ may also lead to the misclassification of magnesium intake despite having been validated and providing a reasonable reflection of dietary intake (20). Finally, given the observational nature of the study, it is not possible to firmly establish a cause-and-effect relation of the variables studied. Conversely, the strengths of our study are that the sample studied is large, the follow-up relatively long, and the ascertainment of mortality accurate.

In conclusion, the findings from this prospective study suggest an inverse association between dietary magnesium intake and cardiovascular, cancer, and all-cause mortality. Additional studies in different populations should confirm these results.

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7. DISCUSSION

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The aim of the present study was to assess the effect of key components of the MedDiet on the risk of cardiovascular disease and mortality in a Mediterranean population at high cardiovascular risk. The focus is on three MedDiet components: total nuts, olive oil and its varieties, and dietary magnesium intake. Nuts and olive oil make MedDiet unique and different from other dietary patterns. These have been recognised, among others, as the key components that might explain the cardio-protective effect of MedDiet. On the other hand, magnesium is a nutrient which is found in high amounts in the MedDiet because it is present in most of the characteristic components of the MedDiet such as nuts, vegetables, fruits, legumes and whole grains.

7.1. GENERAL DISCUSSION

The research findings obtained in this project make an important contribution to the existing scientific evidence in the field of MedDiet and cardiovascular health. In particular, we have analysed, for the first time, the effect of MedDiet components on CVD and mortality in the context of the primary cardiovascular prevention. This is crucial because it could avoid further complications associated with CVD, reduce the financial and human burden and importantly decrease the risk of death from CVD.

The *PREDIMED* Study has demonstrated that a MedDiet supplemented with extra-virgin olive oil or nuts reduced the incidence of major cardiovascular events[68]. The present work was carried out as a post-hoc analysis of the *PREDIMED* data treating the individuals as an observational cohort. The current prospective study was conducted on Mediterranean individuals at high cardiovascular risk from Spain, where the intake of nuts, olive oil and dietary magnesium is relatively high compared to other regions. We have observed that after a median of 4.8 years of follow-up, the frequency of nut consumption was inversely associated with cause-specific and all-cause mortality; the baseline total olive oil consumption, specifically the extra-virgin variety,

was inversely associated with cardiovascular events and cardiovascular mortality; and dietary magnesium was inversely associated with cause-specific and all-cause mortality.

Our results expand the existing literature on the importance of consuming olive oil and nuts as part of a healthy dietary pattern. First of all, it is important to mention that there is still a popular belief that nuts and olive oil may increase body weight because they are an energy-dense and high-fat food. However, the scientific evidence suggests that frequent nut consumption and olive oil intake does not lead to any appreciable weight gain[208,209]. Probably this could be explained by the fact that olive oil and nuts may increase satiety, diet-induced thermogenesis, daily energy expenditure and post-prandial fat oxidation that decrease energy intake from other sources[208,210,211]. Moreover, it has been shown that the quality of fat is more important rather than the total fat of the diet[140]. In general, nuts and olive oil are vegetal fat rich in MUFA and some nuts (e.g. walnuts) in PUFA, which have been associated with several health benefits[87,212]. Besides not being associated with weight gain, nuts and olive oil have been consistently related to be beneficial in the prevention of cardiovascular and chronic diseases[76,213].

Even though there is an emerging body of evidence demonstrating the inverse associations between nuts and olive oil, CVD and mortality[93,168,169]; to date, none of the previous studies have analysed these associations in a Mediterranean population at high cardiovascular risk. In addition, most of the studies have made no distinction between different types of nuts (walnuts and non-walnuts) or different varieties of olive oil (extra-virgin olive oil and common olive oil). In contrast, our studies have differentiated between these varieties in the analyses.

In agreement with our results showing an inverse association between nut consumption and all-cause mortality, a recent meta-analysis including 11 prospective cohort studies has concluded that increasing intake of 1-serving of nuts was associated with 17% reduced risk of all-cause mortality (pooled RR was 0.83, and 95% CI: 0.76-0.91)[94]. Our research findings have also demonstrated an inverse relationship between the frequency of nut consumption and cardiovascular and cancer death. Large prospective studies have reported consistent inverse associations between nut consumption and fatal CHD or sudden cardiac death[214–216].

Furthermore, a recent pooled analysis, using the cohorts of NHS and HPFS including 76,464 women and 42,498 men, has observed that consuming nuts two or more times per week versus never consuming nuts was associated with 24% lower risk of dying from heart disease (pooled RR 0.76, 95% CI 0.67-0.85) and 17% lower risk of dying from cancer (pooled RR 0.83, 95% CI 0.76-0.90)[93].

The findings of this thesis demonstrate an inverse association between olive oil intake and the risk of major cardiovascular diseases. These results are consistent with those from prior studies[78,79]. A 44% reduction in the risk of CHD was observed in Italian women in the top quartile of olive oil consumption[78] and 22% reduction in the risk of CHD was also found in the Spanish *EPIC* cohort participants in the top quartile of olive oil intake[79]. A recent meta-analysis including studies which have investigated the association between olive oil consumption and CHD has found a non-significant reduction in the risk of CHD for 25 g increase in olive oil intake[168]. The main results of the *PREDIMED* trial have demonstrated that a MedDiet supplemented with EVOO reduced the risk of composite cardiovascular events by 30% (HR: 0.70, 95% CI 0.54-0.92) as compared to the control group[68]. Our data suggested an inverse association between olive oil intake and all-cause mortality but the results were not significant.

The results on this outcome are not well established; although some studies have found a reduction in the risk of all-cause mortality[61,67] others have found no significant associations[43]. In line with a previous report of the Spanish *EPIC*-Study,[61] we found an inverse relation between olive oil intake and cardiovascular mortality but not with cancer mortality. EVOO has been associated with even more reduced risk of CVD in our analyses, probably because it contains higher amounts of bioactive and antioxidant components than other varieties and it is considered as the best quality olive oil.

Finally, we observed inverse associations between dietary magnesium intake and cause-specific and overall mortality. Few previous studies have assessed the association between dietary magnesium and all-cause mortality. No significant association was found in a cohort of Swedish men,[190] but a 60% reduction was observed in individuals with a high concentration of serum magnesium intake[217]. Despite we found a 47% reduction in the risk of cardiovascular mortality

for the individuals in the higher tertile of magnesium intake, though the data on cardiovascular mortality seem to be inconsistent. Our results are in line with two prior prospective studies,[189,218] but there are three others that found non-significant associations[190,219,220]. Since two previous studies failed to demonstrate inverse associations between cancer mortality and magnesium intake,[190,191] our study is the first one that shows significant data on the inverse association between cancer death and magnesium intake. Even though meta-analyses of prospective cohorts found inverse associations between dietary and circulating magnesium intake,[187,221,222] the results for the composite of cardiovascular events and dietary magnesium intake in the *PREDIMED* cohort were not significant.

The research findings of the present project further confirmed the important role that key components of MedDiet—nuts and olive oil, and also dietary magnesium—may play in the prevention of CVD and mortality. However, other components including vegetables, fruits, legumes, fish and wine could also contribute to the observed benefits suggesting that the adherence to a traditional MedDiet that includes all these foods might be the best option for the primary prevention of CVD.

Several explanations can account for the observed inverse associations between nuts, olive oil, magnesium and CVD and mortality. The beneficial effects of nuts and olive oil are mainly attributed to its high contain of MUFA (in nuts, also PUFA), and minor components that have shown to have biological properties such as magnesium (in nuts), phytosterols, vitamin E, phenolic compounds and lipid molecules[86,211,212]. Evidence suggests that olive oil and nuts intake could improve blood lipid profile[88,223] and insulin sensitivity, and modulate inflammation, oxidative stress and endothelial function[70,74,89,224]. Consequently, they reduce the risk of diabetes, hypertension, obesity, metabolic syndrome and CHD, among others[75,76,87,216,225].

Magnesium, which is found in high amounts in nuts, may exert similar effects on health. It is thought that it could lower blood pressure that reduces the risk of hypertension, which is a strong risk factor for CHD[226]. Magnesium also plays a role in improving inflammation status and endothelial function, thus decreasing the risk of CVD and death[227,228].

7.2. STRENGTHS AND LIMITATIONS

Several strengths and potential limitations of this study need to be addressed. First, one of the main limitations is the studied population. The population consisted of elderly individuals at high cardiovascular risk; because of this, it was probably easier to detect changes in metabolic parameters as compared to a healthier population. The extrapolation of our results to others may be limited. However, the results are broadly consistent with those from other studies. In addition, our participants were from a Mediterranean country, where the intake of the analysed food was relatively high. Further studies are needed to evaluate, if lower intakes of nuts, olive oil and magnesium are inversely associated with cardiovascular risk factors, cardiovascular disease and mortality.

Second, given to the observational nature of our studies and the potential residual confounding, it is not possible to firmly conclude that the associations reflect cause and effect. Nevertheless, our observational findings are consistent with the intervention effects found in the *PREDIMED* trial.

Third, although the FFQ was validated, its use to assess the intake of food could lead to a misclassification of the food and nutrient consumption and measurement errors, particularly, to the varieties of olive oil and magnesium intake.

On the other hand, our study also has a number of strengths. The prospective design and the relatively long follow-up (a median of about 5 years) could be highlighted. Our sample is relatively large and individuals are well characterised, since they are frequently attended by primary care physicians for being at high cardiovascular risk; therefore, they can get higher benefits of diet and lifestyle changes. Finally, the ascertainment of cardiovascular disease and mortality was objective and accurate. An End-point Adjudication Committee, whose members were blinded to the treatment allocation, updated the information once a year.

7.3. GLOBAL PERSPECTIVE

As a global perspective, CVD and death due to non-communicable diseases are among the important public health concerns worldwide. The results of this research are relevant because they emphasise the importance of nutrition for the primary prevention of CVD and mortality. Of note, the intakes of some key components of MedDiet seem to be beneficial for CVD prevention. These findings support and expand the existing scientific literature on the associations between MedDiet—nuts, olive oil and magnesium—and the risk of CVD and mortality, especially confirming these data in individuals at high cardiovascular risk. There is a need of preserving and promoting traditional MedDiet as a cultural heritage and a healthy dietary pattern.

In the last years, globalisation, industrialisation, a rapid increase in fast-food restaurants and advances in food industry have transformed the MedDiet of most Mediterranean regions into a more global and "Western" dietary pattern[229]. The intake of fresh fruit and vegetables, nuts, legumes, whole-grain and non-processed meat and fish have been replaced by processed energy-dense food, rich in refined carbohydrates, sugar and fat[230]. The diet changes combined with a more sedentary lifestyle, both at work and in leisure time activities, are a possible explanation of the increase in obesity and CVD epidemic of the last decades. Therefore, scientific studies demonstrating and supporting the benefits of MedDiet and its components are essential to make the different populations aware about it. We have contributed to investigate these associations in a Mediterranean population at high cardiovascular risk. Public health policies and appropriate nutritional recommendations to promote MedDiet and a healthy lifestyle at different levels (schools, universities, primary care centres, hospitals, etc.) should be a cornerstone for prevention of chronic disease at a national and international level.

Another point to address is the possibility that non-Mediterranean regions could adopt a Mediterranean dietary pattern, which would imply similar health benefits for others. Indeed, some of the studies suggesting inverse associations between MedDiet and also nuts with CVD and mortality have been conducted in non-Mediterranean populations[47,53,55]. In contrast, studies on olive oil have been conducted mainly in Mediterranean regions, so its extrapolation to other regions in the world remains to be elucidated. The food globalisation, the availability of most

foods out of season and in different regions from where they are produced, as well as an adaptation of the MedDiet to the habits and culture of the different populations could make it feasible to transfer the MedDiet to other regions[231]. Actually, some countries and regions, such as Australia, Chile or California, because of the similar weather and agricultural conditions of the Mediterranean regions, already consume and produce some of the characteristic components of the MedDiet, particularly wine, olive oil and nuts.

7.4. FUTURE PERSPECTIVES

From the findings of the present work, we could consider several future hypotheses that will help complete and understand the current knowledge on olive oil, nuts, magnesium and Mediterranean diet on the risk of CVD, its related risk factors and mortality. In addition, a better understanding of the mechanisms that could explain the associations reported in the current thesis will be an important point to investigate.

Further epidemiological studies to extrapolate our results to other populations (healthy adults or individuals with overweight or obesity) will be of great interest to corroborate the associations in people without cardiovascular risk factors to evaluate if it is effective in conveying a message to these populations. Analyses of the transferability of the Mediterranean diet and its components, olive oil and nuts in non-Mediterranean individuals, where the consumption of these foods are lower, will be also an interesting line of research to explore. Regarding magnesium intake, it would be interesting to analyse the associations in healthy and non-Mediterranean populations with different levels of magnesium intake. Besides the magnesium intake, it would also be important to analyse the effect that serum magnesium has on CVD and death to avoid the possible interactions of magnesium with other components of the diet.

Further clinical trials similar to the *PREDIMED* Study but with longer follow-up, larger number of participants and in different populations will be very helpful to expand knowledge in the area of Mediterranean diet and cardiovascular health.

Moreover, the results of this work suggest inverse associations between olive oil, nuts and magnesium and CVD and mortality, but the mechanisms involved in these associations have not been clearly demonstrated. An innovative and appropriate approach to understand the possible mechanisms could be conducting of metabolomics analyses in the context of the *PREDIMED* Study. Using metabolomics we can identify biomarkers, in an earlier stage, that are associated with an increased risk of suffering from diabetes, cardiovascular disease and other chronic disease. It would be interesting to evaluate if the dietary intervention conducted in the *PREDIMED* modifies the association between baseline metabolite profiles and diabetes and cardiovascular risk, and if an improvement in the metabolite profile resulting from the dietary intervention mediates the benefits of the Mediterranean diet on cardiovascular risk. Also, to identify the roles that olive oil and nuts may play in these associations. Investigating the metabolites that could influence in these associations through metabolomic techniques will allow us to provide effective public health messages for the primary prevention and management of cardiovascular disease.

Furthermore, and because of the importance that genetics and the interaction between genetics and dietary habits have gained in the recent years, it would be compelling to apply other techniques such as nutrigenomics. This will provide a new insight to assess if the main components of the MedDiet, like olive oil or nuts, play a role in modulating genetic expression and consequently, if this association affects health and cardiovascular risk factors. To date, some genetic variants and single nucleotide polymorphisms have been related to cardiovascular and mortality. Therefore, it is essential to identify if some of these genetic loci are also associated with cardiovascular disease and mortality in a population at high cardiovascular risk; and, to evaluate the interactions between MedDiet and its components and these genetic variants. The techniques, such as lipidomics, proteomics, metabolomics, nutrigenomics, etc., will provide new tools that can lead to a more personalised nutrition.

Finally, the findings of the present thesis combined with the near-future research on this topic will help provide strong scientific evidence to develop and promote public health actions based on diet, lifestyle and behavioural changes.

8. CONCLUSIONS

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From the results of this prospective cohort study of Mediterranean elderly individuals at high cardiovascular risk, following conclusions can be made:

1. EFFECTS OF NUT INTAKE ON MORTALITY

The frequency of nut consumption was inversely related to cardiovascular, cancer and total mortality after 4.8 years of follow-up.

2. EFFECTS OF OLIVE OIL INTAKE ON CARDIOVASCULAR DISEASE MORTALITY

Olive oil consumption, specifically the extra-virgin variety, was associated with reduced risk of cardiovascular disease and cardiovascular mortality after 4.8 years of follow-up.

3. EFFECTS OF MAGNESIUM INTAKE ON CARDIOVASCULAR DISEASE AND MORTALITY

Dietary magnesium intake was inversely associated with cardiovascular, cancer and total mortality risk after 4.8 years of follow-up.

Final comments

The results of this work support the healthy benefits of the components of MedDiet on the primary prevention of cardiovascular disease and mortality. The key components of MedDiet play important roles in these associations. The observed benefits of MedDiet are probably explained by the sum of all the effects of its individual components. This work has contributed to add knowledge on the effect of specific foods of MedDiet —nuts, olive oil, magnesium and other nutrients—on cardiovascular disease and mortality. It has public health relevance due to the benefits that could be gained, both for financial and human burden, if more attention is paid on the primary prevention of cardiovascular disease.

9. REFERENCES

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10. APPENDICES

10.1. PREDIMED QUESTIONNAIRES

APPENDIX I.

Inclusion questionnaire. PREDIMED Study.

ESTUDIO PREDIMED

Inclusión / exclusión

Identificador del participante: Nodo | C.Salud | Médico | Paciente | Visita

Nodo: anotar el número de nodo correspondiente.
01. Andalucía - Málaga / 02. Andalucía - Sevilla - S.Pablo / 03. Andalucía - Sevilla - V.Rocío / 04. Baleares / 05. Cataluña - Barcelona norte / 06. Cataluña - Barcelona Sur / 07. Cataluña - Reus - Tarragona / 08. Madrid Norte / 09. Madrid Sur / 10. Navarra / 11. País Vasco / 12. Valencia

C.Salud: anotar el número del centro de salud correspondiente.

Médico: anotar el número del médico correspondiente.

Paciente: anotar el número del paciente correspondiente.

Visita: anotar el número de visita correspondiente.
00. Inclusión - exclusión / 01. Visita Inicial / 02. Visita 3 meses / 03. Visita 1 año / 04. Visita 2 años / 05. Visita 3años

Fecha del examen Día / Mes / Año

Primer apellido | Segundo apellido | Nombre

Dirección
Calle, Plaza, Paseo, Avenida | Número | Piso | Puerta

Población | Código postal

Teléfono | Teléfono

Fecha de nacimiento Día / Mes / Año

Sexo: ◯ Hombre ◯ Mujer

¿Evita usted habitualmente comer con mucha grasa de origen animal (mantequilla, manteca, bolleria industrial...)? En caso de no ser así, ¿estaría usted dispuesto a intentarlo?

- ◯ Sí, lo hago desde hace MÁS de 6 meses
- ◯ Sí, lo hago desde hace MENOS de 6 meses
- ◯ No lo hago, pero lo intentaré en los próximos 30 días
- ◯ No lo hago, pero lo intentaré en los próximos 6 meses
- ◯ No lo hago, y no lo intentaré en los próximos 6 meses
- ◯ datos insuficientes

¿Sigue usted una alimentación rica en fibra, es decir con abundante fruta, verdura y legumbres? En caso de no ser así, ¿estaría usted dispuesto a intentarlo?

- ◯ Sí, lo hago desde hace MÁS de 6 meses
- ◯ Sí, lo hago desde hace MENOS de 6 meses
- ◯ No lo hago, pero lo intentaré en los próximos 30 días
- ◯ No lo hago, pero lo intentaré en los próximos 6 meses
- ◯ No lo hago, y no lo intentaré en los próximos 6 meses
- ◯ datos insuficientes

Procedencia: ◯ Europea ◯ Latinoamericana ◯ Norteafricana ◯ Subsahariana ◯ Asiática ◯ Otras

¿Piensa mudarse a otro municipio en los próximos años o tiene alguna limitación que le impida o dificulte poder acudir a los controles y reuniones programados?

◯ sí ◯ no ◯ datos insuficientes

¿Ha sido usted informado por personal sanitario, que padezca una enfermedad que le impida seguir alguna dieta determinada que incluya aceite de oliva y/o frutos secos ?

○ sí ○ no ○ datos insuficientes

¿Ha sido usted informado por personal sanitario, que haya tenido alguna vez un infarto de miocardio?

○ sí ○ no ○ datos insuficientes

¿Ha sido usted informado por personal sanitario, que haya tenido alguna vez una angina de pecho?

○ sí ○ no ○ datos insuficientes

¿Ha sido usted informado por personal sanitario, que haya tenido alguna vez una embolia o un accidente vascular cerebral?

○ sí ○ no ○ datos insuficientes

¿Ha sido usted informado por personal sanitario, que haya tenido alguna vez una claudicación intermitente?

○ sí ○ no ○ datos insuficientes

¿Ha sido usted informado por personal sanitario, que haya tenido una diabetes?

○ sí ○ no ○ datos insuficientes

¿Ha sido usted informado por personal sanitario, que tenga el colesterol alto?

○ sí ○ no ○ datos insuficientes

¿Sigue usted algún tratamiento hipolipemiante?

○ sí ○ no

En caso afirmativo, anotar:

Col. total ☐☐☐ **Col. HDL** ☐☐ **Col. LDL** ☐☐☐ **Triglicéridos** ☐☐☐

¿Ha sido usted informado por personal sanitario, que tenga la presión alta?

○ sí ○ no ○ datos insuficientes

¿Sigue usted algún tratamiento antihipertensivo?

○ sí ○ no

En caso afirmativo, anotar:

Presión arterial sistólica: ☐☐☐ **Presión arterial diastólica:** ☐☐☐

¿Algun familiar directo (padres, hermanos, hijos, tíos) ha sufrido o fallecido un infarto de miocardio o angina a una edad inferior a 55 años (varones)/65 años (mujeres) ?

○ sí ○ no ○ datos insuficientes

¿Fuma usted cigarrillos actualmente?

○ sí, regularmente ○ ex-fumador de 0 a 1 año ○ ex-fumador de 1 a 5 años ○ ex-fumador > de 5 años
○ nunca fumador ○ datos insuficientes

En caso afirmativo, ¿cuantos años hace que fuma? ☐☐ 88 = no procede 99 = datos insuficientes

¿Aproximadamente, ¿cuántos cigarrillos, puros o pipas fuma al día?

☐☐ cigarrillos/día ☐☐ puros/día ☐☐ pipas/día 88 = no procede 99 = datos insuficientes

¿Es usted capaz de cambiar/seguir la dieta que le aconsejen los médicos del estudio?

○ sí ○ no ○ datos insuficientes

INCLUSIÓN ○ sí ○ no

MOTIVO de exclusión:

○ No cumplir criterios de inclusión
○ Dificultad de seguimiento del estudio o cambio de hábitos alimenticios
○ Falta de interés de participación en el estudio
○ Datos insuficientes
○ Otros .....
○ Enfermedad Cardiovascular previa
○ Enfermedad médica grave
○ Imposibilidad para cambiar de hábitos

APPENDIX II.

General questionnaire. PREDIMED Study.

ESTUDIO PREDIMED

Cuestionario general

Identificador del participante: Nodo | C.Salud | Médico | Paciente | Visita

Nodo: anotar el número de nodo correspondiente.
01. Andalucía - Málaga / 02. Andalucía - Sevilla - S.Pablo / 03. Andalucía - Sevilla - V.Rocío / 04. Baleares / 05. Cataluña - Barcelona norte / 06. Cataluña - Barcelona Sur / 07. Cataluña - Reus - Tarragona / 08. Madrid Norte / 09. Madrid Sur / 10. Navarra / 11. País Vasco / 12. Valencia

C.Salud: anotar el número del centro de salud correspondiente.

Médico: anotar el número del médico correspondiente.

Paciente: anotar el número del paciente correspondiente.

Visita: anotar el número de visita correspondiente.
00. Inclusión - exclusión / 01. Visita Inicial / 02. Visita 3 meses / 03. Visita 1 año / 04. Visita 2 años / 05. Visita 3años

Información de contacto (Pariente o amigo):

Primer apellido | Segundo apellido | Nombre

Teléfono | Teléfono

GRUPO asignado: ◯ Aceite de oliva virgen ◯ Frutos secos ◯ Control

VARIABLES SOCIO DEMOGRÁFICAS

Lugar de nacimiento:

◯ Galicia ◯ La Rioja ◯ Murcia ◯ Castilla la Mancha
◯ Asturias ◯ Aragón ◯ Madrid ◯ Andalucía
◯ Cantabria ◯ Cataluña ◯ Castilla-León ◯ Canarias
◯ País Vasco ◯ Comunidad Valenciana ◯ Extremadura ◯ Baleares
◯ Navarra

País (solo rellenar en caso de extranjeros):

Estado Civil: ◯ Soltero/a ◯ Casado/a ◯ Viudo/a ◯ Divorciado/a ◯ Separado/a ◯ Religioso

¿Cúal es el nivel más alto de escolarización que ha completado?

◯ Titulado Superior o similares ◯ Técnico Escuela Universitaria ◯ Escuela secundaria o Bachiller ◯ Escuela primaria
◯ No sabe leer ni escribir ◯ Datos insuficientes

Número de personas con las que comparte el hogar:

¿Cúal es su situación laboral actual?

◯ Está trabajando ◯ Incapacidad permanente ◯ Ama de casa ◯ Estudiante ◯ Jubilado
◯ Trabaja pero tiene una baja laboral de más de tres meses ◯ Paro con subsidio ◯ Paro sin subsidio ◯ Datos insuficientes

¿Se considera una persona tensa y/o agresiva? Puntuase de 0 (más relajado) a 10 (más competitivo)

Qué trabajo concreto hace o hacía

Qué trabajo concreto hace o hacía el/la cabeza de familia

Durante el último mes, ¿Ha tomado algún medicamento de los siguientes?

Aspirina, Adiro o similar	○ sí	○ no	○ no sabe / no contesta
Otras medicinas para aliviar el dolor o la fiebre	○ sí	○ no	○ no sabe / no contesta
Tranquilizantes, sedantes, pastillas para la ansiedad, pastillas para dormir.	○ sí	○ no	○ no sabe / no contesta
Vitaminas o minerales	○ sí	○ no	○ no sabe / no contesta
Medicamentos para el corazón	○ sí	○ no	○ no sabe / no contesta
Medicamentos para la presión arterial	○ sí	○ no	○ no sabe / no contesta
Medicamentos para el colesterol	○ sí	○ no	○ no sabe / no contesta
Insulina	○ sí	○ no	○ no sabe / no contesta
Medicamentos parala diabetes (diferentes de la insulina)	○ sí	○ no	○ no sabe / no contesta
Solo mujeres: Tratamiento hormonal	○ sí	○ no	○ no sabe / no contesta
Otros	○ sí	○ no	○ no sabe / no contesta

En caso afirmativo, nombre del medicamento/s

indicar el nombre del fármaco, la dosis y el tiempo del tratamiento en años

LOS TRATAMIENTOS ANOTADOS POR EL PACIENTE DEBEN SER CONFIRMADOS POR LA ENFERMERA A PARTIR DE LA HISTORIA CLÍNICA DEL CENTRO DE SALUD

¿Algún familiar directo (padres, hermanos, hijos, etc...) ha fallecido por causas cardíacas, o ha tenido algún problema cardíaco?

◯ sí, antes de los 55 años (varones) / 65 años (mujeres) ◯ sí, después de los 55 años (varones) / 65 años (mujeres)
◯ no ◯ Datos insuficientes

¿Ha sido usted informado por personal sanitario, que haya tenido alguna vez arritmias o alguna enfermedad cardíaca?

◯ sí ◯ no ◯ datos insuficientes

Diagnóstico

¿Algún familiar directo (padres, hermanos, hijos...) ha tenido algún accidente vascular cerebral?

◯ sí, antes de los 55 años ◯ no ◯ sí, después de los 55 años ◯ datos insuficientes

¿Algún familiar directo (padres, hermanos, hijos...) tiene el colesterol elevado?

◯ sí, antes de los 55 años ◯ sí, después de los 55 años ◯ no ◯ datos insuficientes

¿Algún familiar directo (padres, hermanos, hijos...) tiene la tensión arterial alta?

◯ sí, antes de los 55 años ◯ sí, después de los 55 años ◯ no ◯ datos insuficientes

¿Algún familiar directo (padres, hermanos, hijos...) tiene o ha tenido cáncer?

◯ sí, antes de los 55 años ◯ sí, después de los 55 años ◯ no ◯ datos insuficientes

¿Se cansa excesivamente o le falta el aire al realizar alaún eiercicio (subir escaleras, caminar, etc.)?

◯ No disnea
◯ Disnea a grandes esfuerzos (bailar, caminar durante media hora, trabajos de jardinería, etc.)
◯ Disnea a moderados esfuerzos (ducharse, vestirse,etc.)
◯ Disnea a mínimos esfuerzos (cualquier actividad, levantarse de la cama)
◯ Disnea sin especificar grado
◯ Datos insuficientes

¿Algún médico le ha diagnosticado de alguna de estas enfermedades? Puede haber más de una respuesta.

◯ Embolia pulmonar ◯ Trombosis venosa profunda ◯ Cataratas
◯ Aneurisma de aorta ◯ Bronquitis crónica - Enfisema ◯ Apneas del sueño
◯ Insuficiencia cardíaca izquierda ◯ Depresión ◯ Cáncer o Tumores

Edad del diagnóstico: ☐☐ años

Solo mujeres: **¿Que edad tenía cuando inició la menopausia?** ☐☐ años

¿Le ha molestado a ud. alguna vez la gente criticándole su forma de beber?

◯ sí ◯ no ◯ datos insuficientes

¿Ha tenido ud. la impresión de que debería beber menos?

◯ sí ◯ no ◯ datos insuficientes

¿Se ha sentido alguna vez mal o culpable por su costumbre de beber?

◯ sí ◯ no ◯ datos insuficientes

¿Alguna vez lo primero que ha hecho por la mañana ha sido beber para calmar los nervios o para librarse de una resaca?

◯ sí ◯ no ◯ datos insuficientes

EXPLORACIÓN FÍSICA

Altura ☐☐☐ cm **Cintura** ☐☐☐ cm **Cadera** ☐☐☐ cm

Peso ☐☐☐ kg **Índice tobillo-brazo** ☐☐☐ cm

		PAS	PAD	FC
Brazo no dominante	**1**	☐☐☐	☐☐☐	☐☐☐
(paciente sentado)	**2**	☐☐☐	☐☐☐	☐☐☐
Brazo izquierdo	**1**	☐☐☐	☐☐☐	☐☐☐
(paciente decubito supino	**2**	☐☐☐	☐☐☐	☐☐☐
Brazo derecho	**1**	☐☐☐	☐☐☐	☐☐☐
(paciente decubito supino)	**2**	☐☐☐	☐☐☐	☐☐☐
Tobillo izquierdo	**1**	☐☐☐	☐☐☐	☐☐☐
(paciente decubito supino)	**2**	☐☐☐	☐☐☐	☐☐☐
Tobillo derecho	**1**	☐☐☐	☐☐☐	☐☐☐
(paciente decubito supino)	**2**	☐☐☐	☐☐☐	☐☐☐

ITB Izquierdo (PAS mayor del tobillo izquierdo / PAS mayor de los brazos) ☐,☐☐

ITB Derecho (PAS mayor del tobillo derecho / PAS mayor de los brazos) ☐,☐☐

APPENDIX III.

Food frequency questionnaire. PREDIMED Study.

IDENTIFICACIÓN DEL PARTICIPANTE

NODO
01. Andalucía-Málaga
02. Andalucía-Sevilla-San Pablo
03. Andalucía-Sevilla-V. Rocío
04. Baleares
05. Catalunya-Barna Norte
06. Catalunya-Barna Sur
07. Catalunya-Reus-Tarragona
08. Madrid Norte
09. Madrid Sur
10. Navarra
11. País Vasco
12. Valencia

marque así

así no marque

NODO	CENTRO	MÉDICO	PACIENTE	VISITA

PÁGINA 1

Por favor, marque una única opción para cada alimento.

Para cada alimento, marque el recuadro que indica la frecuencia de consumo **por término medio** durante el **año pasado**. Se trata de tener en cuenta también la variación verano/invierno. Por ejemplo, si toma helados 4 veces/semana sólo durante los 3 meses de verano, el uso promedio al año es 1/semana

CONSUMO MEDIO DURANTE EL AÑO PASADO

	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6+
I. LÁCTEOS									
1. Leche entera (1 taza, 200 cc)									
2. Leche semidesnatada (1 taza, 200 cc)									
3. Leche descremada (1 taza, 200 cc)									
4. Leche condensada (1 cucharada)									
5. Nata o crema de leche (1/2 taza)									
6. Batidos de leche (1 vaso, 200 cc)									
7. Yogurt entero (1, 125 gr.)									
8. Yogurt descremado (1, 125 gr.)									
9. Petit suisse (1, 55 gr.)									
10. Requesón o cuajada (1/2 taza)									
11. Queso en porciones o cremoso (1, porción 25 gr.)									
12. Otros quesos: curados, semicurados (Manchego, Bola, Emmental...) (50 gr.)									
13. Queso blanco o fresco (Burgos, cabra...) (50 gr.)									
14. Natillas, flan, puding (1, 130 cc)									
15. Helados (1 cucurucho)									

Un plato o ración de 100-150 gr, excepto cuando se indique otra cosa	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6+
II. HUEVOS, CARNES, PESCADOS									
16. Huevos de gallina (uno)									
17. Pollo o pavo CON piel (1 ración o pieza)									
18. Pollo o pavo SIN piel (1 ración o pieza)									
19. Carne de ternera o vaca (1 ración)									
20. Carne de cerdo (1 ración)									
21. Carne de cordero (1 ración)									
22. Conejo o liebre (1 ración)									
23. Hígado (ternera, cerdo, pollo) (1 ración)									
24. Otras vísceras (sesos, corazón, mollejas) (1 ración)									
25. Jamón serrano o paletilla (1 loncha, 30 gr.)									
26. Jamón York, jamón cocido (1 loncha, 30 gr.)									
27. Carnes procesadas (salchichón, chorizo, morcilla, mortadela, salchichas, butifarra, sobrasada, 50 gr.)									
28. Patés, foie-gras (25 gr.)									
29. Hamburguesa (una, 50 gr.), albóndigas (3 unidades)									
30. Tocino, bacon, panceta (50 gr.)									
31. Pescado blanco: mero, lenguado, besugo, merluza, pescadilla,... (1 plato, pieza o ración)									
32. Pescado azul: sardinas, atún, bonito, caballa, salmón (1 plato, pieza o ración 130 gr.)									
33. Pescados salados: bacalao, salazones (1 ración, 60 gr. en seco)									
34. Ostras, almejas, mejillones y similares (6 unidades)									
35. Calamares, pulpo, chipirones, jibia (sepia) (1 ración, 200 gr.)									
36. Crustáceos: gambas, langostinos, cigalas, etc. (4-5 piezas, 200 gr.)									
37. Pescados y mariscos enlatados al natural (sardinas, anchoas, bonito, atún) (1 lata pequeña o media lata normal, 50 gr.)									
38. Pescados y mariscos en aceite (sardinas, anchoas, bonito, atún) (1 lata pequeña o media lata normal, 50 gr.)									

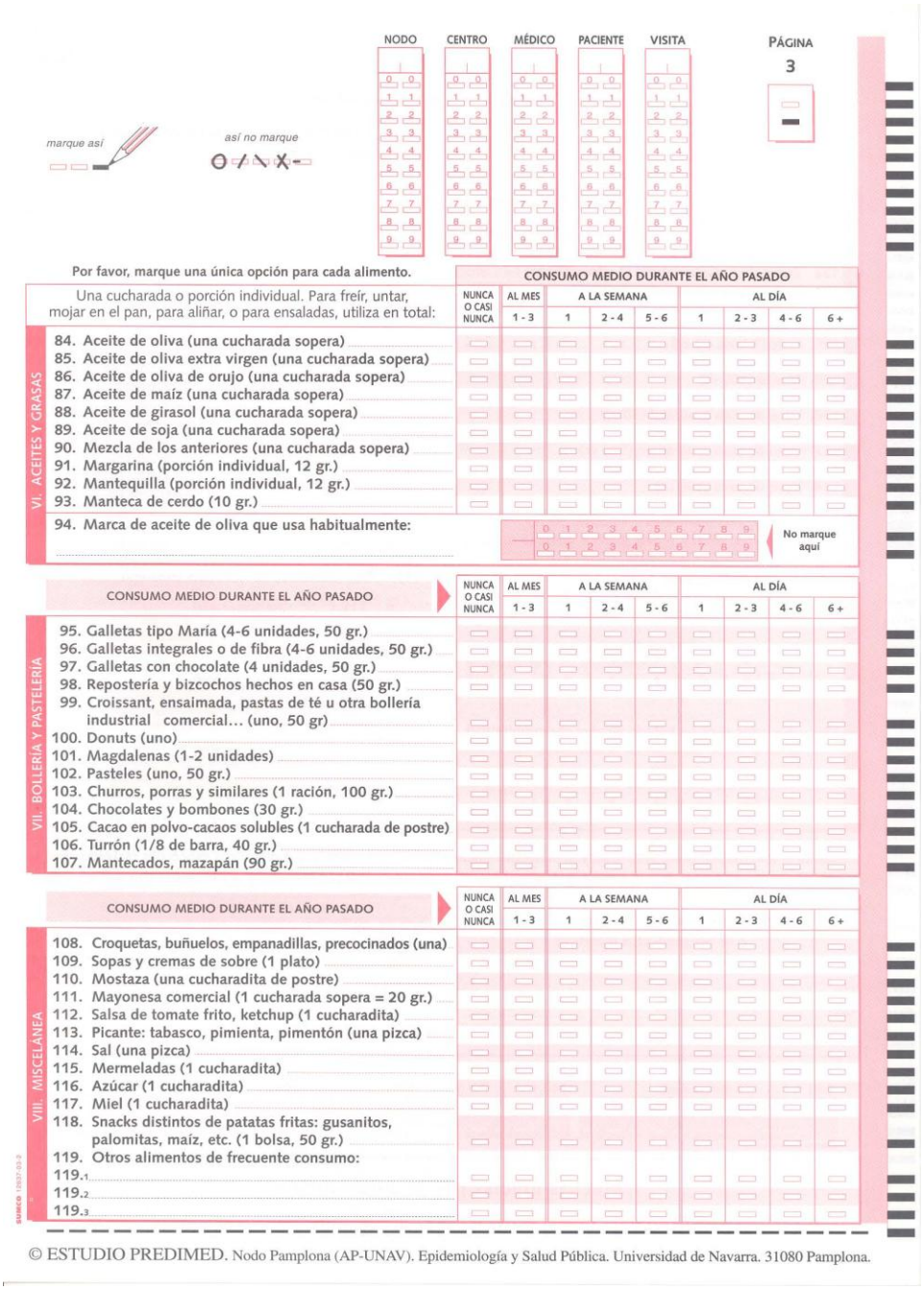
Por favor, marque una única opción para cada alimento.

	Un plato o ración de 200 grs, excepto cuando se indique	CONSUMO MEDIO DURANTE EL AÑO PASADO								
		NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
			1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
III. VERDURAS Y HORTALIZAS	39. Acelgas, espinacas									
	40. Col, coliflor, brócoles									
	41. Lechuga, endivias, escarola (100 gr.)									
	42. Tomate crudo (1, 150 gr)									
	43. Zanahoria, calabaza (100 gr.)									
	44. Judías verdes									
	45. Berenjenas, calabacines, pepinos									
	46. Pimientos (150 gr.)									
	47. Espárragos									
	48. Gazpacho andaluz (1 vaso, 200 gr.)									
	49. Otras verduras (alcachofa, puerro, cardo, apio)									
	50. Cebolla (media unidad, 50 gr.)									
	51. Ajo (1 diente)									
	52. Perejil, tomillo, laurel, orégano, etc. (una pizca)									
	53. Patatas fritas comerciales (1 bolsa, 50 gr.)									
	54. Patatas fritas caseras (1 ración, 150 gr.)									
	55. Patatas asadas o cocidas									
	56. Setas, níscalos, champiñones									

	Una pieza o ración	CONSUMO MEDIO DURANTE EL AÑO PASADO								
		NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
			1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
IV. FRUTAS	57. Naranja (una), pomelo (una), o mandarinas (dos)									
	58. Plátano (uno)									
	59. Manzana o pera (una)									
	60. Fresas/fresones (6 unidades, 1 plato postre)									
	61. Cerezas, picotas, ciruelas (1 plato de postre)									
	62. Melocotón, albaricoque, nectarina (una)									
	63. Sandía (1 tajada, 200-250 gr.)									
	64. Melón (1 tajada, 200-250 gr.)									
	65. Kiwi (1 unidad, 100 gr.)									
	66. Uvas (un racimo, 1 plato postre)									
	67. Aceitunas (10 unidades)									
	68. Frutas en almíbar o en su jugo (2 unidades)									
	69. Dátiles, higos secos, uvas-pasas, ciruelas-pasas (150 gr.)									
	70. Almendras, cacahuetes, avellanas, pistachos, piñones (30 gr.)									
	71. Nueces (30 gr.)									

	72. ¿Cuántos días a la semana toma fruta como postre?		0	1	2	3	4	5	6	7

	Un plato o ración (150 gr.)	CONSUMO MEDIO DURANTE EL AÑO PASADO								
		NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
			1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
V. LEGUMBRES Y CEREALES	73. Lentejas (1 plato, 150 gr. cocidas)									
	74. Alubias (pintas, blancas o negras) (1 plato, 150 gr. cocidas)									
	75. Garbanzos (1 plato, 150 gr. cocidos)									
	76. Guisantes, habas (1 plato, 150 gr. cocidas)									
	77. Pan blanco, pan de molde (3 rodajas, 75 gr.)									
	78. Pan negro o integral (3 rodajas, 75 gr.)									
	79. Cereales desayuno (30 gr.)									
	80. Cereales integrales: muesli, copos avena, all-bran (30 gr.)									
	81. Arroz blanco (60 gr. en crudo)									
	82. Pasta: fideos, macarrones, espaguetis, otras (60 gr. en crudo)									
	83. Pizza (1 ración, 200 gr.)									



NODO | CENTRO | MÉDICO | PACIENTE | VISITA

PÁGINA 3

marque así

así no marque

Por favor, marque una única opción para cada alimento.

CONSUMO MEDIO DURANTE EL AÑO PASADO

Una cucharada o porción individual. Para freír, untar, mojar en el pan, para aliñar, o para ensaladas, utiliza en total:	NUNCA O CASI NUNCA	AL MES 1 - 3	A LA SEMANA 1	A LA SEMANA 2 - 4	A LA SEMANA 5 - 6	AL DÍA 1	AL DÍA 2 - 3	AL DÍA 4 - 6	AL DÍA 6+
VI. ACEITES Y GRASAS									
84. Aceite de oliva (una cucharada sopera)									
85. Aceite de oliva extra virgen (una cucharada sopera)									
86. Aceite de oliva de orujo (una cucharada sopera)									
87. Aceite de maíz (una cucharada sopera)									
88. Aceite de girasol (una cucharada sopera)									
89. Aceite de soja (una cucharada sopera)									
90. Mezcla de los anteriores (una cucharada sopera)									
91. Margarina (porción individual, 12 gr.)									
92. Mantequilla (porción individual, 12 gr.)									
93. Manteca de cerdo (10 gr.)									
94. Marca de aceite de oliva que usa habitualmente:									

No marque aquí

CONSUMO MEDIO DURANTE EL AÑO PASADO

	NUNCA O CASI NUNCA	AL MES 1 - 3	A LA SEMANA 1	A LA SEMANA 2 - 4	A LA SEMANA 5 - 6	AL DÍA 1	AL DÍA 2 - 3	AL DÍA 4 - 6	AL DÍA 6+
VII. BOLLERÍA Y PASTELERÍA									
95. Galletas tipo María (4-6 unidades, 50 gr.)									
96. Galletas integrales o de fibra (4-6 unidades, 50 gr.)									
97. Galletas con chocolate (4 unidades, 50 gr.)									
98. Repostería y bizcochos hechos en casa (50 gr.)									
99. Croissant, ensaimada, pastas de té u otra bollería industrial comercial... (uno, 50 gr)									
100. Donuts (uno)									
101. Magdalenas (1-2 unidades)									
102. Pasteles (uno, 50 gr.)									
103. Churros, porras y similares (1 ración, 100 gr.)									
104. Chocolates y bombones (30 gr.)									
105. Cacao en polvo-cacaos solubles (1 cucharada de postre)									
106. Turrón (1/8 de barra, 40 gr.)									
107. Mantecados, mazapán (90 gr.)									

CONSUMO MEDIO DURANTE EL AÑO PASADO

	NUNCA O CASI NUNCA	AL MES 1 - 3	A LA SEMANA 1	A LA SEMANA 2 - 4	A LA SEMANA 5 - 6	AL DÍA 1	AL DÍA 2 - 3	AL DÍA 4 - 6	AL DÍA 6+
VIII. MISCELÁNEA									
108. Croquetas, buñuelos, empanadillas, precocinados (una)									
109. Sopas y cremas de sobre (1 plato)									
110. Mostaza (una cucharadita de postre)									
111. Mayonesa comercial (1 cucharada sopera = 20 gr.)									
112. Salsa de tomate frito, ketchup (1 cucharadita)									
113. Picante: tabasco, pimienta, pimentón (una pizca)									
114. Sal (una pizca)									
115. Mermeladas (1 cucharadita)									
116. Azúcar (1 cucharadita)									
117. Miel (1 cucharadita)									
118. Snacks distintos de patatas fritas: gusanitos, palomitas, maíz, etc. (1 bolsa, 50 gr.)									
119. Otros alimentos de frecuente consumo:									
119.1									
119.2									
119.3									

Por favor, marque una única opción para cada alimento.

IX. BEBIDAS

	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6+
120. Bebidas carbonatadas con azúcar: bebidas con cola, limonadas, tónicas, etc. (1 botellín, 200 cc)									
121. Bebidas carbonatadas bajas en calorías, bebidas light (1 botellín, 200 cc)									
122. Zumo de naranja natural (1 vaso, 200 cc)									
123. Zumos naturales de otras frutas (1 vaso, 200 cc)									
124. Zumos de frutas en botella o enlatados (200 cc)									
125. Café descafeinado (1 taza, 50 cc)									
126. Café (1 taza, 50 cc)									
127. Té (1 taza, 50 cc)									
128. Mosto (100 cc)									
129. Vaso de vino rosado (100 cc)									
130. Vaso de vino moscatel (50 cc)									
131. Vaso de vino tinto joven, del año (100 cc)									
132. Vaso de vino tinto añejo (100 cc)									
133. Vaso de vino blanco (100 cc)									
134. Vaso de cava (100 cc)									
135. Cerveza (1 jarra, 330 cc)									
136. Licores, anís o anisetes... (1 copa, 50 cc)									
137. Destilados: whisky, vodka, ginebra, coñac (1 copa, 50 cc)									

138. ¿A que edad empezó a beber alcohol (vino, cerveza o licores), incluyendo el que toma con las comidas con regularidad (más de siete "bebidas" a la semana)?

Edad (años) — Decena / Unidad

139. ¿Cuántos años ha bebido alcohol con regularidad (más de siete "bebidas" a la semana)?

Años — Decena / Unidad

119. Otros alimentos de frecuente consumo

119.1 (No marque aquí)

119.2 (No marque aquí)

119.3 (No marque aquí)

Si durante el año pasado tomó vitaminas y/o minerales (incluyendo calcio) o productos dietéticos especiales (salvado, aceite de onagra, leche con ácidos grasos omega-3, flavonoides, etc.), por favor indique la marca y la frecuencia con que los tomó:

Marcas de los suplementos de vitaminas o minerales o de los productos dietéticos	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6+
140.									
140.1									
140.2									

140 (No marque aquí)

140.1 (No marque aquí)

140.2 (No marque aquí)

sumco 12637-03-(Ref. 2)

Muchas gracias por su colaboración

APPENDIX IV.

Physical activity questionnaire.

PREDIMED Study.

ESTUDIO PREDIMED — **Cuestionario de actividad física**

Identificador del participante: Nodo | C.Salud | Médico | Paciente | Visita

Fecha del examen Dia / Mes / Año

DNI

CIP

CUESTIONARIO DE ACTIVIDAD FÍSICA EN EL TIEMPO LIBRE DE MINNESOTA

A continuación encontrara un cuadro con un listado de actividades fisicas y unas columnas con periodos de tiempo de realización de las mismas(semana,mes,trimestre y año). Cada columna esta dividida en dias y minutos.
La forma de rellenar el cuestionario es la siguiente:
1. Se lee atentamente cada actividad una a una y cuando se encuentre una que se haya realizado durante la última semana,con números claros y sin salirse del recuadro se rellenan las casillas correspondientes a los dias y minutos.
2. Seguidamente se repite la misma acción para el último mes,el último trimestre y el último año.
Ha de tener en cuenta que si ha realizado algúna actividad la última semana supone tambíen que la ha realizado el último mes,trimestre y año.
Para asegurar la uniformidad de la información recogida consideramos que:
- cada piso de escaleras = 1/2 min.
- una vuelta en esqui acuático = 5 mn.
- un set de tenis indivdual = 20 min.
- un set de tenis dobles = 15 min.
- golf 9 hoyos = 90 min.

Ejemplo:

Una persona que:

- durante la última semana ha ido a caminar media hora cada dia menos el fin de semana, ha de anotar un 5 en la columna de dias de práctica a la semana y 30 en minutos/dia de practica. Si durante el último año también ha ido a caminar pero durante 2 meses en el verano no ha hecho esta actividad , tendra que anotar 200 en la columna de dias de practica al año y 30 en minutos / dia de practica .
- durante la última semana ha subido 2 veces al dia 2 pisos por la escalera a de anotar un 7 en la columna de dias de práctica a la semana y 2 a minutos/ dia de práctica. Si esta actividad la repite todo el año,tendra que anotar 365 en la columna dias de práctica al año y 2 en minutos / dia de práctica.

ACTIVIDADES FÍSICAS	SEMANA		AÑO	
	DIAS DE PRACTICA	MINUTOS/DIA DE PRACTICA	DIAS DE PRACTICA	MINUTOS/DIA DE PRACTICA
ANDAR/BAILAR/SUBIR ESCALERAS				
1.Pasear	5	30	200	30
5.Subir escaleras	7	2	365	2

ACTIVIDADES FÍSICAS	SEMANA		AÑO	
	DIAS DE PRACTICA	MINUTOS/DIA DE PRACTICA	DIAS DE PRACTICA	MINUTOS/DIA DE PRACTICA
ANDAR/BAILAR/SUBIR ESCALERAS				
1.Pasear				
2.Andar de casa al trabajo y del trabajo a casa o en periodos de descanso del mismo				
3.Andar (llevando carrito de la compra)				
4.Andar (llevando bolsas de la compra)				
5.Subir escaleras				
6.Andar campo a través				
7.Excursiones con mochila				
8.Escalar montañas				
9.Ir en bicicleta al trabajo				
10.Bailar				
11.Aeróbic o ballet				
12.Jugar con los niños (corriendo, saltando,..)				
EJERCICIOS DE MANTENIMIENTO GENERAL				
13.Hacer ejercicio en casa				
14.Hacer ejercicio en un gimnasio				
15.Caminar deprisa				
16.Trotar ("Jogging")				
17.Correr 8-11 km/h				
18.Correr 12-16 km/h				
19.Levantar pesas				
ACTIVIDADES ACUÁTICAS				
20.Esquí acuático				
21.Surf				
22.Navegar a vela				
23.Ir en canoa o remar (por distracción)				
24.Ir en canoa o remar (en competición)				
25.Hacer un viaje en canoa				

ACTIVIDADES FÍSICAS	SEMANA		AÑO	
	DIAS DE PRACTICA	MINUTOS/DIA DE PRACTICA	DIAS DE PRACTICA	MINUTOS/DIA DE PRACTICA
26.Nadar (más de 150 metros en piscina)				
27.Nadar en el mar				
28.Bucear				
DEPORTES DE INVIERNO				
29.Esquiar				
30.Esquí de fondo				
31.Patinar (ruedas o hielo)				
OTRAS ACTIVIDADES				
32.Montar a caballo				
33.Jugar a los bolos				
34.Balonvolea				
35.Tenis de mesa				
36.Tenis individual				
37.Tenis dobles				
38.Badminton				
39.Baloncesto (sin jugar partido)				
40.Baloncesto (jugando un partido)				
41.Baloncesto (actuando de árbitro)				
42.Squash				
43.Fútbol				
44.Golf (llevando el carrito)				
45.Golf (andando y llevando los palos)				
46.Balonmano				
47.Petanca				
48.Artes marciales				
49.Motociclismo				
50.Ciclismo de carretera o montaña				

ACTIVIDADES FÍSICAS	SEMANA		AÑO	
	DIAS DE PRACTICA	MINUTOS/DIA DE PRACTICA	DIAS DE PRACTICA	MINUTOS/DIA DE PRACTICA
ACTIVIDADES EN EL JARDÍN				
51.Cortar el césped con máquina				
52.Cortar el césped manualmente				
53.Limpiar y arreglar el jardín				
54.Cavar el huerto				
55.Quitar nieve con pala				
TRABAJOS Y ACTIVIDADES CASERAS				
56.Trabajos de carpintería dentro de casa				
57.Trabajos de carpinteria (exterior)				
58.Pintar dentro de casa				
59.Pintar fuera de casa				
60.Limpiar la casa				
61.Mover muebles				
CAZA Y PESCA				
62.Tiro con pistola				
63.Tiro con arco				
64.Pescar en la orilla del mar				
65.Pescar con botas altas dentro del río				
66.Caza menor				
67.Caza mayor (ciervos, osos...)				
OTROS (ESPECIFICAR)				

APPENDIX V.

Follow-up general questionnaire.

PREDIMED Study.

ESTUDIO PREDIMED

Cuestionario de seguimiento

Identificador del participante:

Nodo | C.Salud | Médico | Paciente | Visita

Nodo: anotar el número de nodo correspondiente.
01. Andalucía - Málaga / 02. Andalucía - Sevilla - S.Pablo / 03. Andalucía - Sevilla - V.Rocío / 04. Baleares / 05. Cataluña - Barcelona norte / 06. Cataluña - Barcelona Sur / 07. Cataluña - Reus - Tarragona / 08. Madrid Norte / 09. Madrid Sur / 10. Navarra / 11. País Vasco / 12. Valencia
C.Salud: anotar el número del centro de salud correspondiente.
Médico: anotar el número del médico correspondiente.
Paciente: anotar el número del paciente correspondiente.
Visita: anotar el número de visita correspondiente.
00. Inclusión - exclusión / 01. Visita Inicial / 02. Visita 3 meses / 03. Visita 1 año / 04. Visita 2 años / 05. Visita 3años

Fecha del examen

____ / ____ /20____
Día | Mes | Año

NIF

CIP

Información de contacto (Pariente o amigo):

Primer apellido | Segundo apellido | Nombre

Teléfono | Teléfono

Ha cambiado su estado civil desde la última visita: ◯ sí ◯ no

Estado Civil: ◯ Soltero/a ◯ Casado/a ◯ Viudo/a ◯ Divorciado/a ◯ Separado/a ◯ Religioso

Ha cambiado el númeo de personas con las que comparte el hogar desde la última visita: ◯ sí ◯ no

Número de personas con las que comparte el hogar: ____

Ha cambiado su situación laboral desde la última visita: ◯ sí ◯ no

¿Cúal es su situación laboral actual?

◯ Está trabajando ◯ Incapacidad permanente ◯ Ama de casa ◯ Estudiante ◯ Jubilado
◯ Trabaja pero tiene una baja laboral de más de tres meses ◯ Paro con subsidio ◯ Paro sin subsidio ◯ Datos insuficientes

Qué trabajo concreto hace o hacía

Qué trabajo concreto hace o hacía el/la cabeza de familia

EXPLORACIÓN FÍSICA

Altura __ , _____ m **Peso** _____ , __ kg **IMC** _____ , __ kg/m² **Cintura** _____ , ___ cm

		Presión Arterial Sistólica	Presión Arterial Diastólica	Frecuéncia Cardíaca
Extremidad superior izquierda	**1**			
(paciente sentado)	**2**			
Extremidad superior derecha	**1**			
(paciente sentado)	**2**			
DOPPLER (paciente decubito supino)				
Extremidad superior derecha	**1**			
Extremidad inferior izquierda	**1**			
Extremidad inferior derecha	**1**			

Anotación de incidéncias

_____

Tipo de recogida de la información:

última fecha en história clínica

◯ Presencial ◯ Teléfono ◯ História Clínica ____ / ____ /20____ (Dia / Mes / Año) ◯ Otros

Adhesión a la intervención:

◯ Buena ◯ Regular ◯ Mala

Muestra biológica en este seguimiento:

◯ Si ◯ No

En pacientes diabéticos, ¿le han diagnosticado en el último año algunas de las siguientes complicaciones?

Afectación renal ◯ sí ◯ no ◯ datos insuficientes

En caso de afectación renal, el empeoramiento de la función renal ha motivado que entrara en un programa de diálisis

◯ sí ◯ no ◯ datos insuficientes

Afectación de la retina por la diabetes (Retinopatía diabética) que haya motivado un tratamiento con laser

◯ sí ◯ no ◯ datos insuficientes

¿Le han diagnosticado en el último año de cataratas?

◯ sí ◯ no ◯ datos insuficientes

¿Ha sido sometido a algún tipo de intervención quirúrgica este último año? *(En caso afirmativo indique cual)*

◯ sí ◯ no ◯ datos insuficientes ______________________

¿Ha desarrollado en este último año algún tipo de enfermedad que no se le hubiera diagnosticado previamente? *(En caso afirmativo indique cual)*

◯ sí ◯ no ◯ datos insuficientes ______________________

Durante el último mes, ¿Ha tomado algún medicamento de los siguientes?

Aspirina, Adiro o similar	◯ sí	◯ no	◯ no sabe / no contesta
Otras medicinas para aliviar el dolor o la fiebre	◯ sí	◯ no	◯ no sabe / no contesta
Tranquilizantes, sedantes, pastillas para la ansiedad, pastillas para dormir.	◯ sí	◯ no	◯ no sabe / no contesta
Vitaminas o minerales	◯ sí	◯ no	◯ no sabe / no contesta
Medicamentos para el corazón	◯ sí	◯ no	◯ no sabe / no contesta
Medicamentos para la presión arterial	◯ sí	◯ no	◯ no sabe / no contesta
Medicamentos para el colesterol	◯ sí	◯ no	◯ no sabe / no contesta
Insulina	◯ sí	◯ no	◯ no sabe / no contesta
Medicamentos parala diabetes (diferentes de la insulina)	◯ sí	◯ no	◯ no sabe / no contesta
Solo mujeres: Tratamiento hormonal	◯ sí	◯ no	◯ no sabe / no contesta
Otros	◯ sí	◯ no	◯ no sabe / no contesta

En caso afirmativo, nombre del medicamento/s y dosis		mañana	mediodia	noche
______________	____,__ mg	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1
______________	____,__ mg	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1
______________	____,__ mg	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1
______________	____,__ mg	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1
______________	____,__ mg	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1
______________	____,__ mg	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1
______________	____,__ mg	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1
______________	____,__ mg	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1
______________	____,__ mg	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1
______________	____,__ mg	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1
______________	____,__ mg	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1	◯ 0 ◯ 1/2 ◯ 1

¿Ha cambiado este tipo de medicación en el último año? ◯ sí ◯ no ◯ datos insuficientes

LOS TRATAMIENTOS ANOTADOS POR EL PACIENTE DEBEN SER CONFIRMADOS POR LA ENFERMERA A PARTIR DE LA HISTORIA CLÍNICA DEL CENTRO DE SALUD

ENVIAR

¿Fuma usted cigarrillos actualmente?

◯ sí ◯ ex-fumador de 0 a 1 año ◯ ex-fumador de 1 a 5 años ◯ ex-fumador > de 5 años ◯ nunca fumador ◯ datos insuficientes

¿Aproximadamente, ¿cuántos cigarrillos, puros o pipas fuma al día?

_____ cigarrillos/día _____ puros/día _____ pipas/día 88 = no procede 99 = datos insuficientes

Ha cambiado su hábito tabáquico en los últimos 6 meses: ◯ sí ◯ no

EN LAS SIGUIENTES PREGUNTAS, en caso afirmativo solicitar informe médico y rellenar hoja de eventos.

¿Se le ha diagnosticado en el último año de diabetes?

◯ sí ◯ no ◯ datos insuficientes

¿Ha sido usted informado por personal sanitario, que se le hayan hallado cifras de colesterol alto?

◯ sí ◯ no ◯ datos insuficientes

¿Ha sido usted informado por personal sanitario, que tenga la tensión alta?

◯ sí ◯ no ◯ datos insuficientes

¿Ha sido usted informado por personal sanitario, que haya tenido alguna vez un infarto de miocardio en el último año? ◯ sí ◯ no ◯ datos insuficientes

¿Ha sido usted informado por personal sanitario, que haya tenido alguna vez una angina de pecho en el último año? ◯ sí ◯ no ◯ datos insuficientes

¿Ha sido usted informado por personal sanitario, que le hayan practicado una angioplastia coronaria con o sin implantación de stent o realizado una intervención de bypass coronario quirúrgico?

◯ sí ◯ no ◯ datos insuficientes

¿Ha sido usted informado por personal sanitario, que haya sufrido un paro cardíaco del que haya recuperdo en el último año?

◯ sí ◯ no ◯ datos insuficientes

¿Ha sido usted informado por personal sanitario, que haya sufrido alguna arrítmia en el último año?

◯ sí ◯ no ◯ datos insuficientes

¿Ha sido usted informado por personal sanitario, que haya sufrido un accidente vascular cerebral en el último año? ◯ sí ◯ no ◯ datos insuficientes

¿Le han diagnosticado en el último año de aneurisma de aorta?

◯ sí ◯ no ◯ datos insuficientes

¿Ha notado en el último año, que se cansa excesivamente o le falta el aire al realizar algún ejercicio (subir escaleras, caminar, etc.)?

◯ No disnea
◯ Disnea a grandes esfuerzos (bailar, caminar durante media hora, trabajos de jardinería, etc.)
◯ Disnea a moderados esfuerzos (ducharse, vestirse,etc.)
◯ Disnea a mínimos esfuerzos (cualquier actividad, levantarse de la cama)
◯ Disnea sin especificar grado
◯ Datos insuficientes

¿En el último año, le han diagnosticado de un trastorno circulatorio en las piernas?

◯ sí ◯ no ◯ datos insuficientes

¿Ha motivado este trastorno circulatorio que le intervinieran quirúrgicamente o que le amputaran parte de una extremidad? ◯ sí ◯ no ◯ datos insuficientes

Última ingesta de aceite de oliva virgen ___ (Día) / ___ (Mes) / 20___ (Año) ◯ desayuno ◯ comida ◯ cena ◯ 8

Última ingesta de bebida alcohólica o vino ___ (Día) / ___ (Mes) / 20___ (Año) ◯ desayuno ◯ comida ◯ cena ◯ 8

APPENDIX VI.

Tolerance questionnaire. PREDIMED Study.

ESTUDIO PREDIMED

Tolerancia

Identificador del participante: _____ _____ _____ _____ _____
Nodo C.Salud Médico Paciente Visita

Fecha del examen: ____ / ____ /20____
Día Mes Año

GRUPO asignado:
- ◯ Aceite de oliva virgen
- ◯ Frutos secos
- ◯ Dieta baja en grasa

Con respecto a su situación antes de empezar el estudio:

1. Estado general de salud - Se encuentra:	◯ Peor	◯ Igual	◯ Mejor	◯ No sabe
2. Las comidas le sientan:	◯ Peor	◯ Igual	◯ Mejor	◯ No sabe
3. Hace la digestión:	◯ Peor	◯ Igual	◯ Mejor	◯ No sabe
4. Tiene el ritmo intestinal (deposiciones):	◯ Peor	◯ Igual	◯ Mejor	◯ No sabe

4.1 (Sólo si ha cambiado) - Ahora defeca:

- más blando:	◯ Sí	◯ No
- más duro:	◯ Sí	◯ No
- más volumen:	◯ Sí	◯ No
- más veces al día:	◯ Sí	◯ No

Los participantes de la Dieta Baja en Grasa (DBG) sólo deberan responder hasta la pregunta 4.1 inclusive.

5. ¿Ha tenido alguna dificultad para ingerir los alimentos que le hemos dado?

5.1 Aceite virgen de oliva: ◯ Sí ◯ No

Si la respuesta es afirmativa: (marcar la opción que corresponda):

- ◯ Demasiada cantidad
- ◯ No lo tolera
- ◯ No le gusta
- ◯ Otros motivos. Indique cuáles: _____

Otras observaciones (ej., calidad del aceite) _____

5.2 Frutos secos: ◯ Sí ◯ No

Si la respuesta es afirmativa: (marcar la opción que corresponda):

- ◯ Demasiada cantidad
- ◯ Dificultad para masticarlos... ¿Cómo se ha solucionado? _____
- ◯ No los tolera
- ◯ Otros motivos. Indique cuáles: _____
- ◯ No le gustan

Otras observaciones (ej., calidad de los frutos secos)

_____

6. ¿Cómo ha tomado los frutos secos?

Combinados con alimentos	◯ Sí	◯ No	**En una sola toma**	◯ Sí	◯ No
Los ha tomado solos	◯ Sí	◯ No	**Varias tomas**	◯ Sí	◯ No

10.2. SCIENTIFIC CONTRIBUTIONS

Scientific contributions

PUBLICATIONS DERIVED FROM THE PRESENT WORK

Original, Peer Reviewed Articles

Guasch-Ferré M, Bulló M, Martínez-González MÁ, Ros E, Corella D, Estruch R, Fitó M, Arós F, Wärnberg J, Fiol M, Lapetra J, Vinyoles E, Lamuela-Raventós RM, Serra-Majem L, Pintó X, Ruiz-Gutiérrez V, Basora J, Salas-Salvadó J; PREDIMED study group**. Frequency of nut consumption and mortality risk in the PREDIMED nutrition intervention trial.** BMC Med. 2013; **11:**164. PMID: 23866098.

Guasch-Ferré M, Bulló M, Estruch R, Corella D, Martínez-González MA, Ros E, Covas M, Arós F, Gómez-Gracia E, Fiol M, Lapetra J, Muñoz MÁ, Serra-Majem L, Babio N, Pintó X, Lamuela Raventós RM, Ruiz-Gutiérrez V, Salas-Salvadó J; PREDIMED Study Group. **Dietary magnesium intake is inversely associated with mortality in adults at high cardiovascular disease risk.** J Nutr 2014; **144(1):**55-60. PMID: 24259558.

Guasch-Ferré M, Hu FB, Martínez-González MA, Fitó M, Bulló M, Estruch R, Ros E, Corella D, Recondo J, Gómez-Gracia E, Fiol M, Lapetra J, Serra-Majem L, Muñoz MA, Pintó X, Lamuela Raventós RM, Basora J, Buil-Cosiales P, Sorlí JV, Ruiz-Gutiérrez V, Martínez JA, Salas-Salvadó J. **Olive oil intake and risk of cardiovascular disease and mortality in the PREDIMED Study.** *BMC Med* 2014; **12:**78. PMID: 24886626.

OTHER PUBLICATIONS

Original, Peer Reviewed Articles

Guasch-Ferré M, Bulló M, Costa B, Martínez-Gonzalez MÁ, Ibarrola-Jurado N, Estruch R, Barrio F, Salas-Salvadó J; PREDI-PLAN Investigators. **A risk score to predict type 2 diabetes mellitus in an elderly Spanish Mediterranean population at high cardiovascular risk**. *PLoS One* 2012;**7(3):**e33437. PMID: 22442692.

Díaz-López A, Bulló M, Martínez-González MÁ, **Guasch-Ferré M**, Ros E, Basora J, Covas MI, Del Carmen López-Sabater M, Salas-Salvadó J; PREDIMED (Prevención con Dieta Mediterránea) Reus Study Investigators. **Effects of Mediterranean diets on kidney function: a report from the PREDIMED trial.** *Am J Kidney Dis* 2012; **60(3)**:380-9. PMID: 22541738.

Guasch-Ferré M, Bulló M, Martínez-González MÁ, Corella D, Estruch R, Covas MI, Arós F, Wärnberg J, Fiol M, Lapetra J, Muñoz MÁ, Serra-Majem L, Pintó X, Babio N, Díaz-López A, Salas-Salvadó J. **Waist-to-height ratio and cardiovascular risk factors in elderly individuals at high cardiovascular risk.** *PLoS One* 2012; **7(8):**e43275. PMID: 22905246.

Díaz-López A, Bulló M, Basora J, Martínez-González MA, **Guasch-Ferré M**, Estruch R, Wärnberg J, Serra-Majem L, Arós F, Lapetra J, Ros E, Pintó X, Covas MI, Salas-Salvadó J. **Cross-sectional associations between nutrient intake and chronic kidney disease in a population at high cardiovascular risk.** *Clin Nutr* 2013; **32(4):**606-12. PMID: 23141101.

Guasch-Ferré M, Bulló M, Babio N, Martínez-González MA, Estruch R, Covas MI, Wärnberg J, Arós F, Lapetra J, Serra-Majem L, Basora J, Salas-Salvadó J. **Mediterranean diet and risk of hyperuricemia in subjects at high cardiovascular risk.** *J Gerontol A Biol Sci Med Sci* 2013; **68(10):**1263-70. PMID: 23599357.

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Babio N, Ibarrola-Jurado N, Bulló M, Martínez-González MA, Wärnberg J, Salaverría I, Ortega-Calvo M, Estruch R, Serra-Majem L, Covas MI, Salas-Salvadó J, **for the PREDIMED Study Investigators. White blood cell counts as risk markers of developing metabolic syndrome and its components in the PREDIMED study.** *PLoS One* 2013; **8(3):**e58354. PMID: 23526980.

Juanola-Falgarona M, Cándido-Fernández J, Salas-Salvadó J, Martínez-González MA, Estruch R, Fiol M, Arija-Val V; Mònica Bulló; **PREDIMED Study Investigators**. **Association between serum ferritin and osteocalcin as a potential mechanism explaining the iron-induced insulin resistance.** *PLoS One* 2013; **8(10):**e76433. PMID: 24167545.

Mayneris-Perxachs J, Sala-Vila A, Chisaguano M, Castellote AI, Estruch R, Covas MI, Fitó M, Salas-Salvadó J, Martínez-González MA, Lamuela-Raventós R, Ros E, López-Sabater MC; **PREDIMED Study Investigators. Effects of 1-year intervention with a mediterranean diet on plasma fatty acid composition and metabolic syndrome in a population at high cardiovascular risk.** *PLoS One* 2014; **9(3):**e85202. PMID: 24651160.

Juanola-Falgarona M, Salas-Salvadó J, Ibarrola-Jurado N, Rabassa-Soler A, Díaz-López A, **Guasch-Ferré M,** Hernández-Alonso P, Balanza R, Bulló M. **Effect of the glycemic index of the diet on weight loss, modulation of satiety, inflammation, and other metabolic risk factors: a randomized controlled trial**. *Am J Clin Nutr* 2014; **100(1):**27-35. PMID: 24787494.

Salas-Salvadó J, **Guasch-Ferré** M, Bulló M, Sabaté J. **Nuts in the prevention and treatment of metabolic syndrome.** *Am J Clin Nutr* 2014; **100(Supplement 1):**399S-407S. PMID: 24898227.

Martínez-González MÁ, Toledo E, Arós F, Fiol M, Corella D, Salas-Salvadó J, Ros E, Covas MI, Fernández-Crehuet J, Lapetra J, Muñoz MA, Fitó M, Serra-Majem L, Pintó X, Lamuela-Raventós RM, Sorlí JV, Babio N, Buil-Cosiales P, Ruiz-Gutierrez V, Estruch R, Alonso A; **PREDIMED Investigators**. **Extravirgin olive oil consumption reduces risk of atrial fibrillation: the PREDIMED (Prevención con Dieta Mediterránea) trial.** *Circulation* 2014; **130(1)**:18-26. PMID: 24787471.

Mobility

Length: 6 months (August 2013 – February 2014).

Institution: Harvard School of Public Health (Boston, MA, USA).

Supervisor: Frank B. Hu.

Objective: Participate in the confection of scientific papers in the context of the PREDIMED Study and also in the context of the Harvard Cohorts.

Participation in national and international conferences

Conference: 11th European Nutrition Conference (FENS). Madrid, 26-29 October 2011.
Authors: ***Guasch-Ferré M***, Bulló M, Salas-Salvadó J, Basora J, Covas MI, Garcia-Aloy M.
Title: *Bone quantitative ultrasound measurements in relation to the metabolic syndrome and type-2 diabetes mellitus.*
Format: Poster.
Publication: *Ann Nutr Metab* 2011; **58(suppl 3):**279.

Conference: IV Symposium Ciber Fisiopatología de la Obesidad y Nutrición (CIBERobn): Nuevos avances en Obesidad y Nutrición. Málaga, 10-11 of November 2011.
Authors: Díaz-López A, Bulló M, Martínez-González MA, ***Guasch-Ferré M***, Ros E, Basora J, Covas MI, Salas-Salvadó J, for the PREDIMED study investigators.
Title: *Mediterranean diet and renal function: Cross-sectional evaluation and results of one-year intervention in the Reus cohort of the PREDIMED Study.*
Format: Poster.
Publication: Abstract Book; p.35. M-43-380-2011.

Conference: 19th European Congress on Obesity (ECO2012). Lyon, France, 9-12 of May 2012.
Authors: ***Guasch-Ferré M***, Bulló M, Costa B, Martínez-Gonzalez MÁ, Ibarrola-Jurado N, Estruch R, Barrio F, Salas-Salvadó J.
Title: *A risk score to predict type 2 diabetes mellitus in an elderly Spanish Mediterranean population at high cardiovascular risk.*
Format: Poster.
Publication: *Obes Facts* 2012; **5(suppl 1)**: 216.

Conference: 1st World Forum for Nutrition Research Conference. Reus, Spain, 20-21 of May 2013.
Authors: ***Guasch-Ferré M***, Bulló M, Martínez-González, Corella D, Ros E, Estruch R, Wärnberg J, Serra-Majem L, Basora J, Salas-Salvadó J.
Title: *Frequency of Nut Consumption and Risk of Total Mortality in the PREDIMED Study.*
Format: Poster.
Publication: *Annals of Nutrition and Metabolism*. **62(suppl 2):**1–90

Conference: 1st World Forum for Nutrition Research Conference. Reus, Spain, 20-21 of May 2013.
Authors: ***Guasch-Ferré M***, Bulló M, Babio N, Martínez-González MA, Estruch R, Covas MI, Wärnberg J, Serra-Majem L, Basora J, Salas-Salvadó J.
Title: *Mediterranean Diet and Risk of Hyperuricemia in Elderly Subjects at High Cardiovascular Risk.*
Format: Poster.
Publication: Annals of Nutrition and Metabolism. **62(suppl 2)**:1–90.

Conference: 2nd World Forum for Nutrition Research Conference. Brisbane, Australia, 14-18 of May 2014.
Authors: ***Guasch-Ferré M.***
Title: *Olive oil and cardiovascular disease and mortality in the PREDIMED Study.*
Format: Oral communication.