DIETARY FACTORS ASSOCIATED WITH METABOLIC SYNDROME AND TYPE 2 DIABETES RISK

Nerea Becerra Tomás

UNIVERSITAT ROVIRA i VIRGILI

Dietary Factors Associated With Metabolic Syndrome And Type 2 Diabetes Risk

NEREA BECERRA TOMÁS

DOCTORAL THESIS
2017

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Thesis supervised by Prof. Jordi Salas-Salvadó, and co-supervised by Dr. Nancy Babio

UNIVERSITAT ROVIRA i VIRGILI

Human Nutrition Unit
Department of Biochemistry and Biotechnology
Rovira i Virgili University
Reus, Tarragona
2017

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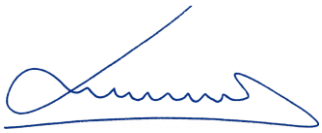
I STATE:

That the present study, entitled "DIETARY FACTORS ASSOCIATED WITH METABOLIC SYNDROME AND TYPE 2 DIABETES RISK", presented by Ms. Nerea Becerra Tomás for the award of the degree of Doctor, has been carried out under my supervision at the Department of Biochemistry and Biotechnology of this university and it is currently up for an international distinction.

Reus, 1th of September 2017

Doctoral Thesis Supervisor



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"If I have seen further, it is by standing upon the shoulders of giants"

Isaac Newton 1642-1727

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Abstract

ENGLISH

Metabolic syndrome (MetS) and type 2 diabetes (T2D) are important global health issues due to its high prevalence and its associated morbi-mortality.

Among the modifiable risk factors that have been associated with the risk of MetS and T2D, diet plays an important role in the prevention and management of both conditions. It has been shown that dietary patterns are differently associated with the risk of MetS and T2D. Whereas the Western diet seems to increase the risk of these chronic diseases, plant-based diets, such as the Mediterranean Diet (MedDiet), seem to decrease the risk. In order to better understand the different effects on disease risk among dietary patterns, it is important to analyze the role that specific food groups play on the risk of developing MetS and T2D.

This doctoral thesis has been conducted in the frame of the PREDIMED study, a parallel, multicenter, randomized clinical trial conducted in Spain between 2003 and 2011 with the aim of evaluating the effectiveness of the MedDiet in the primary prevention of cardiovascular disease.

The main aim of the present dissertation was to evaluate the association between the consumption of meat and dairy products with the risk of MetS or the incidence of some of its individual components, as well as to assess the association between legumes consumption and T2D development risk.

The results showed a non-significant inverse association between total dairy consumption and the risk of MetS incidence. The consumption of low-fat dairy products, low-fat milk and yogurt (total, low-fat and whole-fat yogurt) was associated with a lower risk of MetS and some of its individual components. Contrary, the consumption of cheese was positively associated with the MetS risk. Likewise, total meat intake was also associated with the risk of MetS and the development of some of its components. The risk differed according to the type of meat consumed. Whereas red meat and processed red meat were associated with a higher risk of MetS, poultry was associated with a lower risk. When red meat and processed red meat were replaced with eggs, fish, poultry and legumes, a decreased risk of MetS

was observed. Finally, total legume consumption, particularly lentils, was associated with a lower risk of T2D incidence. The substitution of legumes for eggs, bread, rice, and baked potato was also associated with a lower risk of T2D.

In conclusion, high consumption of low-fat dairy products and yogurt (regardless the fat content), together with the preference for poultry rather than red meat or processed red meat, and high frequency consumption of legumes, would be beneficial for the prevention of MetS and T2D among Mediterranean individuals at high CVD risk.

SPANISH

El síndrome metabólico (SM) y la diabetes tipo 2 (DT2) son un importante problema de salud mundial debido a su alta prevalencia y su morbi-mortalidad asociada.

Entre los factores de riesgo modificables que se han asociado con el riesgo de SM y DT2, la dieta juega un papel importante en la prevención y tratamiento de ambas condiciones. Se ha observado que los patrones dietéticos se asocian de manera distinta al riesgo de desarrollar SM y DT2. Mientras que la dieta occidentalizada parece incrementar el riesgo de estas enfermedades crónicas, dietas basadas en vegetales, como la Dieta Mediterránea, parecen disminuir el riesgo. Con el objetivo de entender mejor los distintos efectos de los patrones dietéticos sobre el riesgo de desarrollar estas enfermedades, es importante analizar el papel que juegan determinados grupos de alimentos sobre el riesgo de desarrollar el SM y la DT2.

Esta tesis doctoral se ha llevado a cabo en el contexto del estudio PREDIMED; un estudio clínico paralelo, multicéntrico, aleatorizado llevado a cabo en España entre 2003 y 2011 con el objetivo de evaluar la efectividad de la Dieta Mediterránea en la prevención primaria de la enfermedad cardiovascular.

El principal objetivo de la presente tesis ha sido evaluar la asociación entre el consumo de carne, productos lácteos y el riesgo de incidencia SM o alguno de sus componentes, así como evaluar la asociación entre el consumo de legumbres y el riesgo de desarrollar DT2.

Los resultados mostraron una asociación inversa no significativa entre el consumo total de productos lácteos y el riesgo de incidencia de SM. El consumo de productos lácteos bajos en grasa, leche desnatada y yogur (total, bajo en grasa y entero) se asoció a un menor riesgo de SM y algunos de sus componentes. Contrariamente, el consumo de queso se asoció de forma positiva al riesgo de SM. Del mismo modo, el consumo de carne total también se asoció con el riesgo de padecer SM y alguno de sus componentes. Dicho riesgo difirió en función del tipo de carne consumida. Mientras que la carne roja y la carne roja procesada se asociaron a un mayor riesgo de SM, el consumo de carne blanca se asoció a un menor riesgo. Cuando el consumo de carne roja y carne roja procesada se reemplazó por huevos, pescado, carne blanca y legumbres, se observó un menor riesgo de SM. Finalmente, el consumo de

legumbres totales, y en particular las lentejas, se asoció a un mejor riesgo de incidencia de DT2.

En conclusión, un consumo elevado de productos lácteos bajos en grasa y yogur (independientemente del contenido en grasa), conjuntamente con consumo preferente de carnes blancas en lugar de carnes rojas o carnes rojas procesadas, y una alta frecuencia de legumbres, podría ser beneficioso para la prevención del SM y la DT2 en individuos Mediterráneos con alto riesgo cardiovascular.

CATALAN

La síndrome metabòlica (SM) i la diabetis tipus 2 (DT2) són un important problema de salut mundial degut a la seva alta prevalença i la seva morbimortalitat associada. Entre els factors de risc modificables que s'han associat amb el risc de SM i DT2, la dieta juga un paper important en la prevenció i tractament d'ambdós condicions. S'ha observat que els patrons dietètics s'associen de forma diferent al risc de desenvolupar SM i DT2. Mentre que la dieta occidentalitzada sembla incrementar el risc d'aquestes malalties cròniques, les dietes basades en vegetals, com la Dieta Mediterrània, semblen disminuir el risc. Amb l'objectiu d'entendre millor els diferents efectes dels patrons dietètics sobre el risc de desenvolupar aquestes malalties, és important analitzar el paper que juguen determinats grups d'aliments sobre el risc de desenvolupar el SM i la DT2.

Aquesta tesi doctoral s'ha dut a terme en el context de l'estudi PREDIMED; un estudi clínic, paral·lel, multicèntric, aleatoritzat conduit a Espanya entre 2003 i 2011 amb l'objectiu d'avaluar l'efectivitat de la Dieta Mediterrània en la prevenció primària de la malaltia cardiovascular.

El principal objectiu de la present tesis ha estat avaluar l'associació entre el consum de carn, productes làctics i el risc d'incidència de SM o alguns dels seus components, així com avaluar l'associació entre el consum de llegums i el risc de desenvolupar DT2.

Els resultats van mostrar una associació inversa no significativa entre el consum total de productes làctics i el risc d'incidència de SM. El consum de productes làctics baixos en greix, llet descremada i iogurt (total, baix en greix i sencer) es va associar a un menor risc de SM i algun dels seus components. De la mateixa manera, el consum de carn total també es va associar amb el risc de patir SM i algun dels seus components. Aquest risc va diferir en funció del tipus de carn consumida. Mentre que la carn vermella i la carn vermella processada es van associar a un menor risc de SM, el consum de carn blanca es va associar a un menor risc. Quan el consum de carn vermella i carn vermella processada es va substituir per ous, peix, carn blanca i llegums, es va observar un menor risc de SM. Finalment, el consum de llegums totals, i en particular de llenties, es va associar a un menor risc d'incidència de DT2.

En conclusió, un consum elevat de productes làctics baixos en greix i iogurt (independentment el seu contingut en greix), conjuntament amb el consum preferent de carn blanques en lloc de carns vermelles o carns vermelles processades, i una alta freqüència de llegums podria ser beneficiós per a la prevenció del SM i la DT2 en individus Mediterranis amb alt risc cardiovascular.

Abbreviations

AHA/NHLBI; American Heart Association/National Heart, Lung, and Blood Institute

A1C; Glycosylated hemoglobin

BMI; Body mass index

CETP; Cholesteryl ester transfer protein

CRP; C-reactive protein

CVD; Cardiovascular disease

EASD; European Association for the Study of Diabetes

ESC; European Society of Cardiology

FDA; Food and Drug Administration

FFQ; Food frequency questionnaire

FPG; Fasting plasma glucose

GWAS; Genome-wide association studies

HDL; High-density lipoprotein

HSL; Hormone sensitive lipase

IDF; International Diabetes Federation

IL-6; Interleukine-6

IR; Insulin resistance

LPL; Lipoprotein lipase

MARE; Metabolic Syndrome and Arteries Research

MedDiet; Mediterranean Diet

MetS; Metabolic syndrome

MUFAs; Monounsaturated fatty acids

NCEP/ATP III; National Cholesterol Education Program-Adult Treatment Panel III

NHANES; National Health and Nutrition Examination Survey

OGTT; Oral glucose tolerance test

PREDIMED; PREvención con DIeta MEDiterránea study

PROSPER; Prospective Study of Pravastatin in the Elderly at Risk

PUFAs; Polyunsaturated fatty acids

SFAs; Saturated fatty acids

T1D; Type 1 diabetes mellitus

T2D; Type 2 diabetes mellitus

TNF-α; Tumor necrosis factor-alpha

VLDL; Very-low-density lipoprotein

WHO; World Health Organization

2-h PG; 2-h plasma glucose concentrations

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I. Introduction

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1. Metabolic syndrome

1.1. DEFINITION AND DIAGNOSIS OF METABOLIC SYNDROME

The term metabolic syndrome (MetS), traditionally also known as insulin resistance syndrome or syndrome X, is used to describe a cluster of different interrelated factors that directly increases the risk for type 2 diabetes mellitus (T2D) and cardiovascular disease (CVD).

In 1988, Gerald M. Reaven[1], after many years of investigation, hypothesized that insulin resistance (IR) was the underlying cause of a group of disorders characterized by impaired glucose tolerance, hyperinsulinaemia, increased levels of very-low-density lipoprotein (VLDL), decreased levels of high-density lipoprotein (HDL) and hypertension. However, despite the large investigations conducted in this field, in the 1990s there was no accepted international definition describing the features included in the syndrome.

The first official definition was described in 1998 by the World Health Organization (WHO)[2]. Since then, several alternative definitions have been proposed, such as that used by the National Cholesterol Education Program-Adult Treatment Panel III (NCEP/ATP III)[3], the International Diabetes Federation (IDF)[4] or the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI)[5]. However, these definitions differed in the components proposed as well as in their corresponding cut-off points used. As a consequence, in 2009 the IDF and AHA/NHLBI attempted to solve this divergence and proposed a common criteria for the diagnosis of the MetS[6].

According to both sides, abdominal obesity was not a prerequisite for its diagnosis, but it was included as 1 of the 5 criteria required. **Table 1** shows the criteria proposed for the harmonized diagnosis. The presence of at least 3 of these 5 risk factors is needed for the clinical diagnosis of MetS.

Table 1. Clinical Diagnosis of the Metabolic Syndrome[6]	
Measurement	**Categorical cut-off**
Abdominal adiposity*	Population and country specific definitions
Elevated triglycerides or drug treatment†	≥150mg/dL (1.7 mmol/L)
Reduced HDL-c or drug treatment†	<40mg/dL (1.0 mmol/L) in man <50mg/dL (1.3 mmol/L) in woman
Elevated blood pressure or drug treatment‡	Systolic ≥130 and/or diastolic ≥85 mmHg
Elevated fasting glucose or drug treatment	≥100mg/dL

*It is recommended that the IDF cut points be used for non-Europeans and either the IDF of AHA/NHLBI cut points used for people of European origin until more data are available.

†The most commonly used drugs for elevated triglycerides and reduced HDL-c are fibrates and nicotinic acid. Patients taking 1 of these drugs can be presumed to have high triglycerides and low HDL-c. High-dose ω-3 fatty acid presumes high triglycerides.

‡Most patients with type 2 diabetes mellitus will have the metabolic syndrome by the proposed criteria.

1.2. EPIDEMIOLOGY OF METABOLIC SYNDROME

Elucidating the prevalence trends of the MetS has become a big challenge due to the multiple definitions existing over time, as commented above, as well as the fact that data is limited to certain populations. Nonetheless, different attempts to describe the epidemiological features of this condition in different regions of the world have been made in the last decade.

In the United States, data coming from a cross-sectional sample of the National Health and Nutrition Examination Survey (NHANES)[7] showed an overall prevalence of MetS of 33% among adults aged ≥20 years from 2003 to 2012. Significant differences were observed between women (35.6%) and men (30.3%). When trends from 2003-2004 to 2011-2012 were evaluated, an increased prevalence from 32.9% to 34.7% was found. However, it seemed that MetS prevalence from 2007-2008 to

2011-2012 remained stable. It is important to point out that more than 50% of adults aged 60 years or older had MetS[7].

In the Asia-Pacific region, a recent publication showed a prevalence of MetS ranging from 11.9% to 37.1%. In the majority of the countries, 1 or more of each 5 adults suffered from MetS[8].

In Europe, similar trends have been observed. In the Metabolic Syndrome and Arteries Research (MARE) consortium cohort, which included information of 34,821 subjects from 12 different cohorts representing 10 countries, a 24.3% general prevalence of MetS was observed, increasing the percentage in an age-dependent fashion (more than 30% in subjects aged ≥ 70 years). Lithuania, Belgium, Spain, UK and Sardinia (Italy) had the highest prevalence, whereas Greece had the lowest[9]. Another study conducted in the Mediterranean population of Catalonia, confirmed this alarming data showing an increased prevalence of MetS from 18.4% in 1992-1993 to 24.8% in 2002-2003[10].

Therefore, independently of the definition used for its diagnostic, approximately 25% of adult population suffers from MetS, which is in accordance to the IDF figure[11], and its prevalence increases with age. To date, all studies agree that MetS is a condition with a clearly increasing prevalence, which makes it a critical public health problem worldwide.

1.3. PHYSIOPATHOLOGY OF METABOLIC SYNDROME

The physiopathology process underlying the MetS is an important ongoing debate. Although certainly all the components of the metabolic syndrome appear together at a higher frequency than expected, the underlying pathophysiology of the syndrome it is not yet clear. For many years it has been thought that MetS is caused by a single basic defect: the IR[12,13]. Nowadays, this hypothesis is questioned and it has been proposed that features of the MetS occur as a consequence of the abdominal adiposity[14]. However, it is impossible to discern which one of these two factors plays a predominant role in the pathogenesis and progression of the MetS due to their high interrelation.

Insulin resistance

Insulin hormone is well known to be a major regulator of carbohydrates, proteins and lipids metabolism by activating their synthesis and storage, as well as by inhibiting their hydrolysis and their subsequent release to the circulation[15]. Therefore, alterations in insulin action may have important metabolic consequences. The concept IR is used to describe the inability of normal insulin concentrations to produce an insulin response in peripheral tissues[16].

The pathophysiological implications of IR in the carbohydrates metabolism will be discussed extensively in the diabetes section of the present doctoral thesis. In brief, this defect in insulin action supposes the inability of the hormone to mediate the glucose uptake in different tissues, as well as to diminish the liver glucose production, which eventually affects blood glucose levels. The association between IR, impaired fasting glucose and impaired glucose tolerance has been well documented[17].

Apart from controlling glucose metabolism, insulin is also involved in lipid metabolism. The lipoprotein lipase (LPL) activity in adipose tissue is predominantly regulated by insulin[18,19]. This enzyme acts on the triglycerides content from chylomicrons and VLDL, releasing free fatty acids that are subsequently uptaken by adipocytes[20] for future storage. In normal conditions, insulin stimulates LPL[21] while inhibiting lipolysis through the repression of the hormone sensitive lipase (HSL)- an enzyme that hydrolyzes triglycerides into free fatty acids and glycerol in the adipose tissue[22]. Under IR condition, an impaired lipid metabolism has been observed, mainly due to a reduced LPL activity, as well as a reduced LPL mRNA levels in adipose tissue[23,24], hence affecting the clearance of triglycerides-rich lipoproteins in the circulation. Moreover, in this setting of IR, insulin cannot properly inhibit lipolysis, leading to a higher free fatty acids secretion into the plasma[25]

Abdominal obesity

The inclusion of waist circumference instead of body mass index (BMI) as 1 of the 5 criteria required for the diagnosis of the MetS, illustrates the importance of abdominal adiposity in the pathogenesis of the syndrome. However, it is important

to highlight that the clinical definition of the MetS does not distinguish between subcutaneous and visceral adipose tissue, which may have large different metabolic affections.

Traditionally, adipose tissue has been considered as a simple passive energy storage organ. Nowadays, this conception is obsolete and it is well known that it is a dynamic organ expressing and secreting bioactive components called adipokines. These proteins not only can act at paracrine level but also can be secreted into the circulation, hence acting at systemic level in the brain, liver or skeletal muscle[26]. Therefore, adipose tissue communicates with other organs and is involved in different processes including immune and reproductive function, energy metabolism, appetite and inflammatory signaling[27]. Considering the foregoing, an excess of adipose tissue, especially at visceral level, can lead to adverse metabolic consequences.

In individuals with excess visceral adiposity, reduced circulating levels of adiponectin have been observed[28–30]. This protein is synthesized predominantly in adipose tissue and it seems to play an important role in the metabolism of carbohydrates and lipids. Moreover, purportedly it also has anti-atherogenic and anti-inflammatory properties[31]. Adiponectin has been inversely associated with some CVD risk factors such as LDL cholesterol, triglycerides and blood pressure[32,33]. As a consequence of all this, it is not surprising that adiponectin has been associated with the risk of CVD events[34,35] and T2D[36].

Whereas levels of adiponectin are reduced in individuals with excess of visceral adipose tissue, other pro-inflammatory adipokines such as C-reactive protein (CRP), interleukine-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are increased[30,37,38]. CRP is considered an inflammatory marker that could be predictive of CVD events[39] and T2D incidence[40]. Moreover, it has been associated with impaired endothelial vasoreactivity[41]. In regard to IL-6, it has been shown that in adipose tissue reduces the activity of LPL, as well as increases lipolysis[42,43] and it is also a predictor of both T2D[44] and myocardial infarction[45]. Finally, TNF-α exerts different actions in adipose

tissue among which stands out the alteration of lipid storage and oxidative capacity through the inhibition of lipogenesis and the activation of lipolysis[46].

Other proteins with different inflammatory, endothelial and thrombotic properties are also produced by adipocytes or the central adipose tissue, which contains infiltrated macrophages in the obesity states (**Figure 1**).

Visceral adipose tissue also contributes to increase free fatty acids levels into the systemic circulation. In women with upper body obesity, but not with lower body obesity, a higher free fatty acids release from adipose tissue has been observed compare to non-obese postmenopausal women[47]. This could be explained through the higher lipolysis activity in visceral adipocytes compare to subcutaneous adipocytes, as observed in several *in vivo* and *in vitro* studies[48]. Elevated concentrations of free fatty acids can directly affect liver functions increasing hepatic glucose production and resulting in fasting hyperglycemia[49]. In fact, higher concentrations of free fatty acids have been associated with an increased risk of T2D in a large case-cohort study[50].

Therefore, an excess of adipose tissue, mainly in the visceral area, contributes to the physiopathology associated to MetS, through an alteration of free fatty acids metabolism and its endocrine function leading not only to an impaired glucose and lipid metabolism but also to a state of low-grade inflammation.

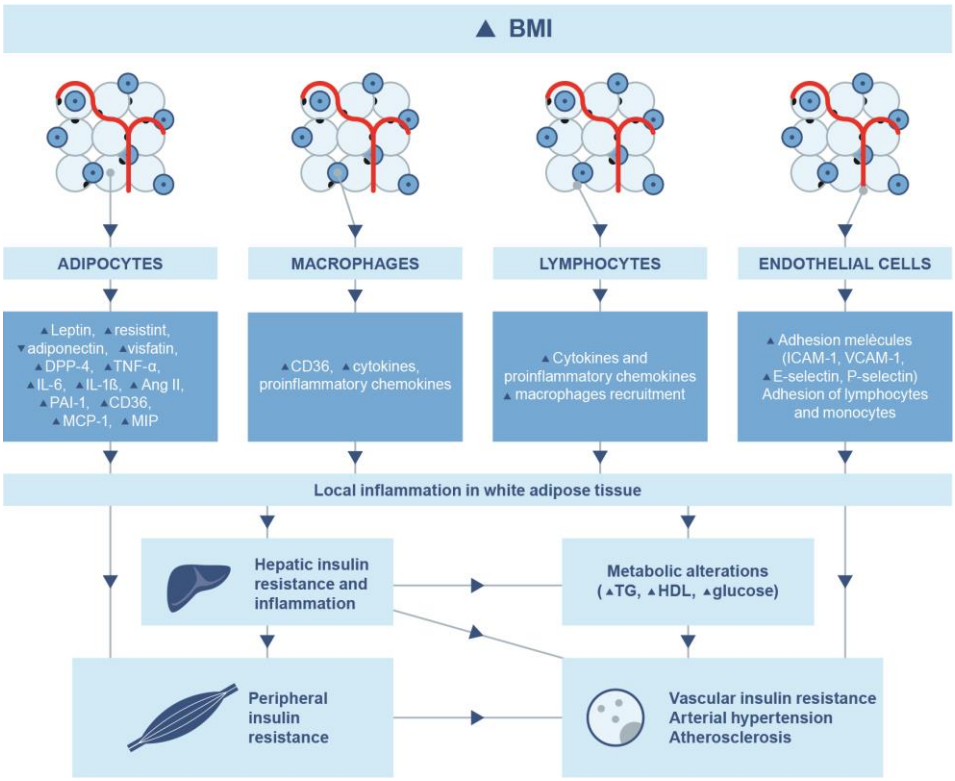

Figure 1. Contribution of white adipose tissue to metabolic and vascular complications. Adapted from: Gómez-Hernández et al**,** Int J Endocrinol. 2016; 2016: 1216783

Dyslipidemia

Individuals with MetS present a lipid profile characterized by a combination of low levels of HDL-c and high concentrations of triglycerides. As stated before, IR and abdominal adiposity can lead to an impaired lipid metabolism through different pathways. Under both conditions, lipolysis in adipocytes is enhanced and a higher release of free fatty acids into the liver is produced. This increased flux of free fatty acids promotes hepatic production of triglycerides and secretion of VLDL[51]. Under normal conditions, triglycerides from VLDL particles are hydrolyzed to be storage in adipose tissue. However, in individuals with MetS, an impaired activity of LPL is presented favoring the accumulation of lipoproteins rich in triglycerides in the circulation.

Low levels of HDL-c are highly correlated with hypertriglyceridemia and could be as a result of both increased production and reduced catabolism of VLDL observed in MetS individuals. In normal conditions, LPL hydrolyzes triglycerides from VLDL and chylomicrons. As pointed previously, LPL activity is reduced in individuals with MetS producing a reduction in the clearance of VLDL from the circulation. The large concentrations of these triglyceride-rich lipoproteins during a prolonged time produce an exchange, mediated by cholesteryl ester transfer protein (CETP), of cholesteryl ester and triglycerides between HDL-c and VLDL. As a results, enriched triglycerides HDL-c particles are generated, which are faster catabolized than normal HDL particles[22,52]. Besides, the activity of lipase hormone, responsible for the catalysis of HDL-c, is increased[53]. Taking together all the aforementioned aspects, irredeemably levels of circulating HDL-c are reduced.

Hypertension

High blood pressure is considered another feature of the MetS although its relation with the syndrome is complex. Both IR and abdominal adiposity have been associated with hypertension[54–56]. Although different possible mechanisms have been proposed, the association between these conditions is not well understood.

The main hypothesis linking the three conditions seems to be the contribution of visceral adiposity and IR to the endothelial dysfunction by lowering nitric oxide bioavailability. In normal conditions, insulin produces vasorelaxation and increases capillarity in the skeletal muscle through the stimulation of endothelial nitric oxide production[57]. In the setting of IR condition, the production of nitric oxide is impaired and the resulting compensatory hyperinsulinemia may enhance vasoconstriction[57], hereby contributing to the hypertension in MetS. Different studies supported this theory. The activity of the nitric oxide synthase, stimulated by insulin in muscle, is impaired in individuals with T2D[58]. Moreover, lower levels of adiponectin, as has been observed in individuals with abdominal obesity, also can contribute to the reduced bioavailability of nitric oxide. It seems that this adipokine directly stimulates its production in endothelial cells[59,60]. In this line, reduced levels of adiponectin have

been associated with hypertension[61,62], suggesting a possible role of this protein in the pathophysiology of hypertension in the MetS.

Insulin also plays a role stimulating renal sodium reabsorption[63,64]. In fact, in IR conditions, the stimulated activity seems to be maintained or even enhanced. This is supported by a study showing an increased sodium retention in diabetics participants with severe IR, compared to those participants with less severe impaired insulin sensitivity and controls[65]. In consonance, individuals with MetS have an increased rate of proximal fractional sodium reabsorption[66,67], which may be relevant in the pathophysiology of hypertension.

The increased circulating levels free fatty acids observed in individuals with MetS also could contribute to the raise of blood pressure through the endothelial dysfunction, the stimulation of vascular cell growth and the increase of oxidant stress[68].

Finally, an increased sympathetic neuron system activity has been also observed in individuals with MetS[69], although the mechanisms leading to an hyperactivity of sympathetic neuron system in the setting of MetS are not well established, it seems that may contribute to the initiation and progression of hypertension[70].

1.4. METABOLIC SYNDROME AS A RISK FACTOR FOR CARDIOVASCULAR DISEASE AND TYPE 2 DIABETES

Each component of the MetS has been associated independently with the risk of T2D and CVD development. Some evidence suggests that, regardless the definition used, MetS is more than the sum of its individual components and it also represents an additional risk of T2D and CVD incidence. However, some authors suggested that although the MetS can predict the development of T2D or CVD, its ability is less effective than the Framingham risk score or the Diabetes Predicting Model, which are models designed specifically for this purpose[71]. In any case, MetS can be considered as a useful and simple phenotypic clinical tool for the identification of individuals predisposed to T2D and CVD.

Metabolic syndrome as a predictor of cardiovascular disease

The main objective to create a definition for the MetS was to have a clinical tool to identify individuals at high risk of CVD. However, whether MetS is actually associated or not with the risk of developing CVD has been a subject of discussion in the last decade. In 2005 the American Diabetes Association jointly with the European Association for the Study of Diabetes stated a big concern regarding the MetS given that the CVD risk associated with it appeared to be not greater than the sum of its parts[72]. Since then, several different studies in large populations have been conducted in order to increase the knowledge in this area, despite the fact that differences in the Mets diagnosis represented a big limitation. In 2010, Mottillo et al. published a systematic review and meta-analysis evaluating the association between MetS, CVD risk and all-cause mortality. The 87 prospective studies included were published between 2002 and 2009 and used NCEP/ATPIII definition for the diagnosis of the MetS. The results revealed a 2-fold increased risk of total CVD outcomes, CVD mortality and stroke associated with the MetS[73]. These findings are in line with the IDF, which affirms that individuals with MetS are twice as likely to die from and three times as likely to have heart attack or stroke than those individuals without the syndrome[11]. However, although few, there are some exceptions to these findings. The Casale Monferrato Study[74], the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER)[75] and the Strong Heart Study[76] did not find an association between the MetS and CVD risk. It seems that the ability to predict CVD vary by sex and the numbers of components that are present. For instance, a study showed a higher relative risk (RR) of coronary heart diseases in women than in men even in the presence of the same number of MetS components[77]. In the same line, another study conducted in Korean participants aged 20-78 years observed a higher odd ratios for CVD in women (4.04; 95%CI 1.78-9.14) than men (1.98; 95%CI 1.30-3.03)[78]. Regarding the number of components of the MetS, according to the Beaver Dam Study, those individuals with three risk factors had an odd ratio for CVD of 1.95 (95%CI 0.91-4.16) whereas in those individuals with four risk factors it was 5.86 (95%CI 2.51-13.66)[79]. Similar results were observed in the

Framingham Heart Study, where the relative risk for CVD increased exponentially, regardless gender, with the number of presented risk factors[80].

The predictability of CVD incidence may differ according to the definition used[81], however, evidence so far suggests that individuals suffering from MetS are at a higher risk of CVD development. Nonetheless, it is important to point out that to date no study has evaluated the association between the MetS, diagnosed with the harmonized criteria, and the risk of CVD development. Therefore, although criteria included in the definition did not vary a lot from the previous definitions, further studies are needed to confirm the ability of the new clinical tool to identify individuals at high CVD risk and to elucidate the mechanisms by which MetS may increase the risk.

Metabolic syndrome as a predictor of type 2 diabetes

One of the gold standard methods in research to identify individuals at increased risk of T2D is the oral glucose tolerance test (OGTT). However, because of its cost, it is not widely used in clinical practice. For this reason, is important to identify new tools to predict the risk of T2D. In this field, the MetS has been proposed as a useful and good predictor instrument of T2D.

The evidence supporting the ability of the MetS to predict T2D is well recognized. According to the IDF individuals with MetS have a 5-fold increased risk of T2D development[11]. Different studies conducted in large populations support this statement. In 2008 a meta-analysis of 16 prospective studies of several different populations showed a strong positive association between MetS, independently of the definition used, and the risk of T2D incidence[82]. Nonetheless, because impaired fasting glucose levels is one component of the MetS, is not surprising to observe a higher risk of T2D associated to this condition. It is noteworthy that in the Kuopio Ischemic Heart Disease Risk Factor Study, the sensitivity for the prediction of T2D incidence decreased after excluding impaired fasting glycaemia from the definitions of the MetS, especially for the NCEP definition[83]. In the same line, in another longitudinal study conducted in Korean population, the ability of MetS to predict T2D decreased dramatically after controlling the analysis for fasting plasma glucose[78].

Different studies have also analyzed the association between the number of MetS components and the risk of T2D. In the comparisons, the risk associated rose in proportion with the number of MetS features present. In the British Regional Heart Study[84] the risk of T2D increases from 4.56 (95%CI 2-48-8,78), in those individuals with 3 or 4 criteria, to 10.88 (95%CI 5.77-20.50) in those participants with 5. Results were in the same line in the West of Scotland Coronary Prevention study[85] and the Beaver Dam study[79].

As with CVD, to date no previous studies have analyzed the association between the MetS defined by the harmonized criteria and the risk of T2D. Therefore, although strong evidence has shown an increased risk, studies using the new clinical criteria are warranted to corroborate these findings.

2. Diabetes

2.1. DEFINITION, CLASSIFICATION AND DIAGNOSTIC OF DIABETES

Diabetes is considered, rather than an isolated pathological entity, a group of metabolic diseases whose main common characteristic is a hyperglycemic status resulting from defects in insulin action, secretion, or a combination of both. High levels of sustained plasma glucose are associated with several harmful effects, dysfunction and failure of distinct organs such as kidneys, nerves, eyes, heart and blood vessels[86]. People with diabetes have a higher risk of developing several health problems than people without diabetes. In fact, different long-term macrovascular (coronary artery disease, stroke and peripheral arterial disease) and microvascular complications (diabetic nephropathy, neuropathy and retinopathy) have been associated with the disease[87].

Diabetes is classified in 4 different categories according to the American Diabetes Association[88]:

Type 1 diabetes (T1D): This form is presented only in 5-10% of all the individuals with diabetes. It is characterized by an autoimmune destruction of the pancreatic β-cells leading to absolute insulin deficiency. Although it is mainly presented in childhood or adolescence, it can also occur at any age. People with T1D need

insulin administration every day to control their levels of glucose and survive.

Type 2 diabetes: This is the most frequent type of diabetes accounting for approximately 90-95% of all diabetes cases. It is characterized by the presence of IR and usually by a relative (not absolute as in T1D) insulin deficiency. Although not all, the vast majority of patients with T2D are obese or overweight, and they have a greater accumulation of body fat at the abdominal level. This form of diabetes can be undiagnosed for many years because the hyperglycemia status is gradually developed, and at early stages, patients do not have any of the classic symptoms of the disease. In these patients, the secretion of insulin is defective and insufficient to compensate for IR status. Importantly, although it usually occurs in adults, nowadays its prevalence is increasing in children and adolescents. Unlike people with T1D, most of patients with T2D do not require the administration of daily insulin to survive. However, if blood glucose levels continue rising despite using oral antidiabetic medication, insulin administration may be prescribed. Obesity, increased age, and lack of physical activity are considered as the main risk factors for developing T2D.

Specific types of diabetes due to other causes: For instance, diseases of the exocrine pancreas (cystic fibrosis), monogenic diabetes syndromes (neonatal diabetes and maturity-onset diabetes of the young), drug or chemical induced diabetes (such as after organ transplantation or use of drugs for the treatment of HIV/AIDS), and diabetes secondary to metabolic stress.

Gestational diabetes mellitus: Diabetes that appears for the first time during the second or third trimester of pregnancy, but only when it is clear that T1D or T2D were not preexisting.

Diabetes diagnosis is based on plasma glucose levels measured by either fasting plasma glucose (FPG) or the 2-h plasma glucose concentrations (2-h PG) after a 75g OGTT[86], or glycosylated hemoglobin (A1C) levels[89]. **Table 2** summarizes the criteria proposed by the American Diabetes Association according to the method used.

Table 2. Criteria for the diagnosis of diabetes[88]
FPG ≥126 mg/dL (7.0 mmol/L) Fasting is defined as no caloric intake for at least 8 h*
OR
2-h PG ≥200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water*
OR
A1C ≥6.5% (48 mmol/mol). The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay*
OR
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).

NGSP, National Glycohemoglobin Standardization Program; DCCT, Diabetes Control and Complications Trial
*In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.

It is important to highlight that although FPG, OGTT and A1C are similarly adequate for the diagnosis of diabetes, the concordance between these tests is imperfect, and more people with diabetes have been diagnosed with 2-h plasma glucose levels than with FPG or A1C.

A1C has certain advantages over FPG and OGTT. For instance, fasting is not required for its analysis, it has a higher stability, and it is less susceptible to disturbances under stress and illness conditions. Nonetheless, it also has some disadvantages, such as a greater cost or a limited availability of A1C testing in certain areas of the developing word. Moreover, it cannot be ignored that A1C is an indirect measurement of plasma glucose levels[88].

Unless the diagnosis of diabetes is totally clear, such as in the case of patients presenting classic symptoms of hyperglycemia, a second test using the same methodology should be repeated during the 3-4 months following the first test in order to confirm the diagnosis and minimize possible analytic bias from laboratories.

2.2. EPIDEMIOLOGY OF DIABETES

T2D is an important health problem worldwide. According to the IDF is one of the largest health emergencies of the 21st century.

In 2015, the IDF published the seventh edition of the diabetes atlas, where worldwide prevalence of diabetes in 2015 were described (**Figure 2**). After analyzing data from 220 countries, results revealed that 415 million adults aged 20-79 years suffered from diabetes, which represents an 8.8% of this total adult population. More alarming is the figure estimated for 2040. It is expected that the number of individuals with diabetes will be continue growing to 642 million people, which is the same as one adult in ten[90]. It is noteworthy that IDF has estimated that nearly 46.5% of all individuals with diabetes (193 million people) are unaware of their disease.

The prevalence of diabetes varies by age, gender and urban and rural environments. 320.5 million people aged 20-64 suffered from diabetes, whereas 94.2 million adults aged 65-79 presented the disease. Although differences are small, a higher prevalence has also been observed in men (215.2 million) than in women (199.5 million). According to the area of residence, the prevalence of diabetes was almost double in urban (269.7 million) than in rural areas (145 million). Following the increased estimation of worldwide diabetes prevalence by 2040, the number of individuals with the disease is also expected to be higher in both areas (477.9 and 163.9 million people in urban and rural areas, respectively).

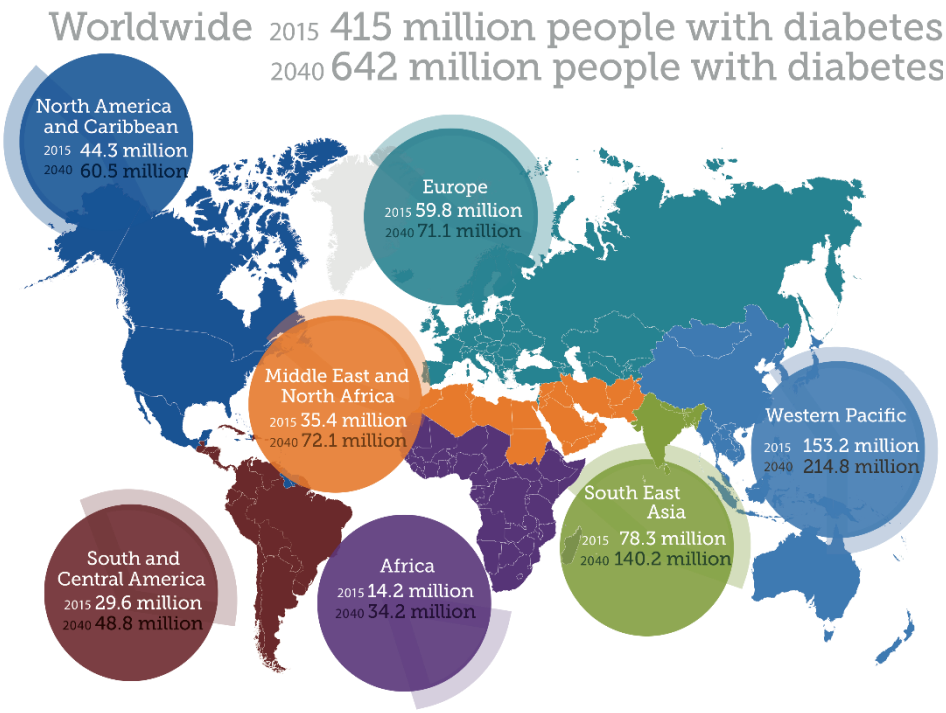

Figure 2. Estimated number of people with diabetes worldwide and per region in 2015 and 2040 in adults aged 20-79 years old. International Diabetes Federation. IDF Diabetes, 7 ed. Brussels, Belgium: International Diabetes Federation, 2015. http://www.diabetesatlas.org

Regarding different regions in the world, the highest prevalence (11.5%) of diabetes was observed in the North America and Caribbean Region whereas the lowest was observed in the Africa Region (3.8%). Europe is the second region with the lowest age-adjusted prevalence of diabetes with 59.8 million people suffering from the disease (approximately 7.3% of the population aged 20-79). However, Europe has the highest prevalence of T1D in children.

Spain is one of the 47 countries that reported information about diabetes to the IDF. According to the seventh atlas edition, there were over 3.5 million cases of diabetes in Spain in 2015, which represents a 10.4% of the total adult population. **Figure 3** represents the prevalence of diabetes in Spanish adults by age. It is important to note that as age increases, the prevalence of diabetes in Spain is higher than that reported in Europe or worldwide.

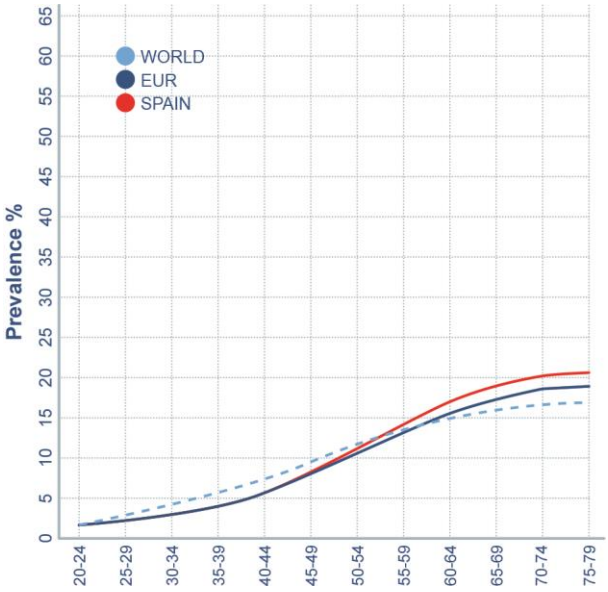

Figure 3. Prevalence of diabetes in adults by age, Spain 2015. Figure describes which age groups in the population have the highest proportions of diabetes. The dotted line is the distribution of diabetes prevalence by age for the world; the black line is the distribution for the Europe region; and the Spain distribution is plotted in the red line[91].

As the IDF figures have shown, diabetes is an important health issue whose prevalence is increasing dramatically worldwide, being T2D the most common type. Therefore, it is important to develop efficient prevention strategies to slow its progression.

2.3. PHYSIOPATHOLOGY OF TYPE 2 DIABETES

There is widespread agreement that both insulin resistance and β-cell dysfunction are main responsible factors for the hyperglycemic status presented in T2D. Genetics and environmental elements are thought to be the main causes of these alterations[92]. In order to understand all cellular and molecular mechanisms implicated in the development of T2D, it is imperative to contextualize the picture frame under which glycaemia is controlled.

Pancreas is the organ responsible for maintaining glucose levels constant. Its endocrine cells represent a 1% of the total pancreas mass and are grouped into populations of 1000-3000 cells forming what is known as islets of Langerhans. These cells are responsible for the production and secretion of insulin (β-cells) and glucagon (α-cells), which are implicated in the regulation of glucose homeostasis.

Under normal physiological conditions, the release of insulin from pancreatic β-cells, after meal consumption, maintains glucose levels within a narrow physiological range through different pathways. On the one hand, insulin increases glucose transport and the rate of glycolysis in muscle and adipose tissue by the activation of hexokinase and 6-phosphofructokinase enzymes. On the other hand, it inhibits the production and release of glucose in the liver. Moreover, insulin also reduces HSL activity in adipocytes inhibiting lipolysis[93,94].

Insulin exerts its metabolic effects by binding to the insulin receptor with tyrosine kinase activity, which stimulates its autophosporylation and, consequently, its activation. This process leads to phosporilation on tyrosine residues of different proteins, including members of the insulin substrate family (IRS), starting the insulin-signaling cascade. This process ends up promoting the translocation of glucose transporter 4 (from intracellular vesicles to the plasma membrane), the synthesis of glycogen, protein, mRNAs and DNA, affecting eventually cell proliferation and survival[93,95].

Insulin resistance

As has been commented in the MetS section of the present doctoral thesis, IR is considered as a condition where normal levels of insulin are not capable to produce a normal insulin response in its target tissues such as adipose, muscle and liver. Thus, insulin-mediated glucose uptake by adipose and muscle tissues is reduced and the suppression of glucose production in liver is insufficient. Under this condition, levels of glucose cannot be maintained within the normal range and hyperglycaemic status appears (**Figure 4**)[95].

The molecular mechanisms of IR in humans are currently not well understood, but changes in insulin receptor isoform expression and defects in the signalling cascade may play an important role in its development. The increased action of different phosphatases and the enhanced production of several inflammatory molecules (i.e. IL-6 and TNF-α) play an important role in the negative regulation of insulin signalling[93,95].

Although mechanisms implicated in insulin resistance are not well elucidated, it seems that insulin sensitivity is influenced by several factors, such as exercise[96], genetics, dietary components[97–99] and obesity[100], being body fat distribution[101–103] one of the main determinants.

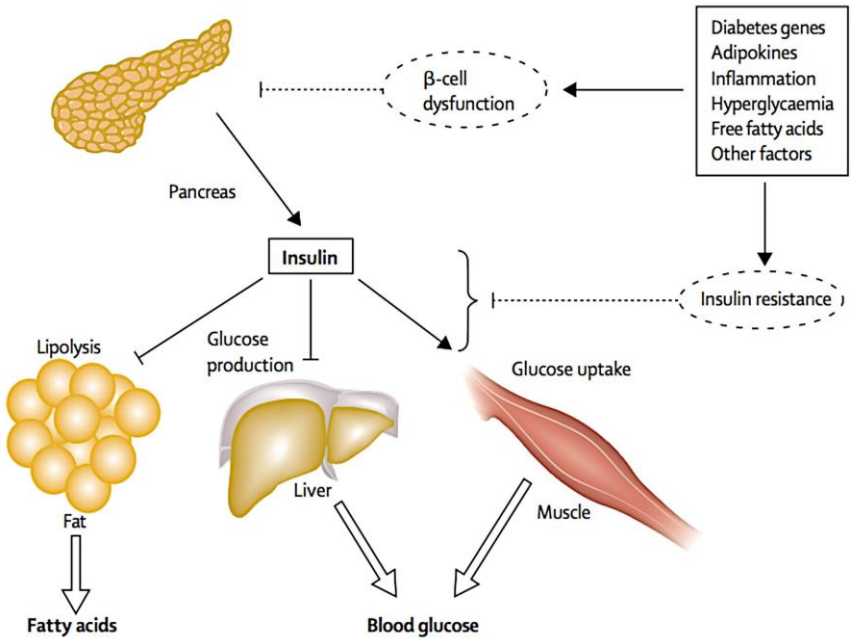

Figure 4. Pathophysiology of hyperglycemia in type 2 diabetes[95].

Beta-cells dysfunction

β-cells play a crucial role ensuring that glucose levels are maintained within narrow physiological range in healthy subjects. The amount of insulin release by β-cells varies according to different factors, such as the nature, quantity and administration route of the stimulus, and the prevailing glucose concentration[104].

In healthy individuals, there is a feedback loop between insulin sensitivity and insulin release. Therefore, in order to maintain glucose levels constant when there is a change in insulin sensitivity, an equivalent and complementary variation in insulin release takes place. A failure in this process leads to glucose abnormal levels and

underlies diabetes development[104,105].

It is well known that β-cells dysfunction is present at a time of T2D diagnosis. However, the hypothesis that this dysfunction exists many years before its development[106] is gaining support. A major effort has been made to understand the pathogenesis of β-cell dysfunction in T2D, but the mechanism remains unclear and current evidence appoints towards a multifactorial cause. It could be because of a β-cell exhaustion as consequence of a higher demand of insulin secretion due to IR[107], glucotoxicity due to a chronic hyperglycemic status[108], lipotoxicity[109] and a reduction in β-cell mass possibly due to the deposition of amyloid[110].

In the presence of both β-cells dysfunction and IR, glucose levels cannot be maintained within a physiological range because release of insulin is insufficient to compensate for IR status observed in its target tissues (**Figure 4**).

2.4. TYPE 2 DIABETES AS A RISK FACTOR FOR CARDIOVASCULAR DISEASE

CVD is the first cause mortality worldwide accounting for a 31% of all global deaths in 2012[111]. The WHO global report on diabetes stated that heart attack and stroke are among one of the many complications associated with diabetes[112]. In fact, evidence so far shows that diabetes is a well-established risk factor for CVD. According to the Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and he European Association for the Study of Diabetes (EASD), more than a half of the mortality and morbidity in patients with diabetes is related to CVD[113].

In 2010, it was published a collaborative meta-analysis of 97 prospective studies analyzing the associations between diabetes and fatal or non-fatal ischemic vascular disease. In total, data from 592,830 participants was analyzed[114]. The study revealed that, in comparison with participants without diabetes, individuals with the disease had a higher risk of coronary heart disease (HRs: 2,00; 95%CI 1.83-2.19), ischemic stroke (HRs: 2,27; 95%CI 1.95-2.65), hemorrhagic stroke (HRs: 1.56; 95%CI 1.19-2.05), unclassified stroke (HRs: 1.84; 95%CI 1.59-2.13) and vascular death (HRs: 1.73; 95%CI 1.51-1.98). Moreover, it is important to highlight that sex differences

were observed. Compared to those without diabetes, the risk of coronary heart disease and ischemic stroke disease was higher in women with diabetes than in diabetic men. The main limitation of this meta-analysis was the lack of information about the prevalence of T1D or T2D in their populations, although the age distribution suggested that the vast majority of participants would have T2D.

This gender differences in the impact of T2D on coronary heart disease risk were also observed in another study including individuals aged 45-64 years without CVD at baseline. After adjusting for different CVD risk factors, the risk of coronary heart disease was higher in diabetic women than in diabetic men (HRs: 2.8; 95%CI 2.0–3.7 and HRs: 9.5; 95%CI 5.5–16.9, respectively) compared with their counterparts without the disease[115].

More recently, in 2015, a cohort study including 1.9 million people also observed similar trends in the risk of different CVD events associated with the risk of T2D. The study included 1,887,062 individuals without diabetes and 34,198 individuals with T2D. After a median follow-up of 5.5 years, 6,137 new CVD events were developed among participants with T2D, being peripheral arterial disease and heart failure the most common events. The analysis showed a higher risk of peripheral arterial disease, ischemic stroke, stable angina, heart failure and non-fatal myocardial infarction in those individuals with T2D compared to those without this condition.

3. Risk factors for metabolic syndrome and type 2 diabetes

MetS and T2D are considered as multifactorial diseases resulting from a complex interplay between genetics and environmental elements[92,116]. Due to their etiology, factors that increase the risk of MetS also increase the risk of T2D. Different risk factors, namely modifiable and non-modifiable risk factors, have been identified and associated with MetS and T2D risk. In the following section of the present doctoral thesis, a detailed description of both types of risk factors will be carried out.

3.1. NON-MODIFIABLE RISK FACTORS

3.1.1 RACE AND GENETIC FACTORS

It is well known that T2D and MetS are, in part, inherited. Evidence from family and twin-based studies supports the role of genetic factors in the development of both diseases in conjunction with modifying effects of the environment.

Family history of T2D has been associated with a higher risk to develop this condition in comparison with individuals without family history of the disease. The risk of T2D incidence in those individuals with an affected parent or sibling, compared to the general population, is approximately 3 times greater, whereas, if both parents are affected this risk increases up to 6[117]. It has also been observed that the concordance rate of T2D in monozygotic twins is significantly higher than those for dizygotic twins[118,119]. Regarding MetS, the vast majority of genetic studies have assessed the individual components or different combinations of those, rather than the MetS as an entity itself[120–122]. The few studies focused on evaluating MetS heritability, have reported that this is around 25-30%. In the Northern Manhattan Family study the heritability was 24%[123] and in the Linosa Study was 29.9%[124]. Similarly to those studies with T2D, data from twin studies assessing MetS have shown a concordance of 31.6% in monozygotic twins compared to 6.3% in dizygotic twins for the combination of 3 components of the Mets (hypertension, obesity and diabetes)[125].

Since 2007, thanks to the genome-wide association studies (GWAS), different genetic

variants have been associated with T2D and MetS. In the case of T2D, approximately 88 genetic loci have been associated with its incidence risk[126]. It is important to highlight that most of these loci are mainly associated with β-cell function and insulin release, rather than insulin resistance. This could be probably because obesity and insulin resistance shares genetic variants and it is difficult to dissect out their role[127]. In the case of MetS, few studies using the condition as binary trait or pairs of its components have been published. All of them agreed that gene variants are mostly linked to genes that play a role in lipid metabolism or are associated with lipid levels[127,128]. Regarding MetS components, beyond the loci associated with T2D, 56 loci have been associated with obesity, 157 with lipids and approximately 90 with hypertension[128].

In the last years, a growing body of evidence suggested that, beyond genetic variants itself, epigenetic mechanisms may play an important role in the pathogenesis of T2D and MetS[128,129]. Epigenetic modifications change gene expression through DNA methylation, histones modification or activation of microRNAs, yet DNA sequence remains unchanged[130]. Although it is too early to elucidate their degree of involvement in the physiopathology of both diseases, epigenetic changes should also be considered.

It is noteworthy that there are differences between ethnicity in the MetS and T2D prevalence and/or incidence, which emphasize the role of genetic in its physiopathology. In a random sample of medicare fee-for-service beneficiaries, the lowest prevalence of T2D was observed among Whites (184 cases per 1000 individuals), whereas Hispanics (334 cases/1000 individuals), Blacks (296 cases/1000 individuals) and Asians (243 cases/1000 individuals) had the highest prevalence[131]. In the same line, higher incidence of T2D has been observed in African American women (about 2.4-fold greater) and men (about 1.5-fold greater) than their White counterparts. These results are confirmed by the Nurses' Health Study, where after 20 years of follow-up, a higher risk of T2D was observed among Asian, Hispanic and Black women than Whites even after adjusting for differences in BMI[132]. Whereas in T2D seems that Black individuals present a higher risk of suffer the

disease, paradoxically for MetS appears to be the opposite. In spite of the fact that Blacks have higher prevalence of insulin resistance or T2D, a lower prevalence of MetS has been observed in this group[133–135]. It has been hypothesized that the low rates of dyslipidemia observed among blacks could be responsible for this ethnic differences[134,135]. However, it is important to point out that further studies are needed in this field due to some inconsistencies between published results.

3.1.2. SEX

Data from epidemiological studies have shown that sex-differences may play a role in the epidemiology and pathophysiology of T2D and MetS, suggesting that, the risk of suffering from these diseases could be different depending on the sex. In the 7th edition of the IDF atlas, slightly differences between women and men were observed in diabetes prevalence in 2015 and the estimated prevalence for 2040. Nearly, there were 15.6 million more men with diabetes than women in 2015. But this difference is expected to be reduced to 15.1 million by 2040[90]. However, it is noteworthy that others studies have shown that T2D affects women disproportionately. Furthermore, in general, they also have worse glycemic control and more difficulties to reach the goals for A1C than men[136,137].

As stated in the MetS section of the present doctoral thesis, it is difficult to elucidate trends in MetS due to the existence of several definitions over time. However, studies agreed that the prevalence of the MetS is increasing year-by-year. In a study including individuals from Member States of the Gulf Cooperative Council (GCC; Bahrain, Kuwait, Oman, Qatar, Saudi Arabia and the United Arab Emirates) a higher prevalence of MetS in women (ranging from 32.1 to 45,9%) than men (ranging from 20.7 to 37.2%) was observed, regardless of the definition used (NCEP/ATP III or IDF)[138]. This figure was also observed in the NHANES sample, where prevalence of MetS differed between women and men, being the highest percentage in women[7]. Nevertheless, in this cross-sectional study, data showed that prevalence of MetS remained stable in men from 2007-2008 to 2011-2012, whereas decreased in women from 39.4% to 36.6% during the same period.

Despite all of these figures suggest that sex-differences in the risk of T2D and MetS maybe exist, it is certainly that there is a lack of knowledge on the true impact of gender in the pathophysiology of both diseases. There is a great need for large prospective studies designed specifically to evaluate, if any, the impact of sex in T2D and MetS risk.

3.1.3. AGE

Controversies exist about the fact that advanced age may confer higher risk of T2D and MetS. Different epidemiological studies support this notion. The 2015 prevalence of T2D was higher as the age increased in men and women (**Figure 5**). Moreover, in the first national study conducted in Spain to examine the prevalence of diabetes, a significant increase in the percentage of individuals suffering from this disease was observed with age[139].

Evidence surrounding the MetS is on the same line. A cross-sectional analysis carried out on more than 10,000 participants in the Nord-Trøndelag Health Study observed an increased prevalence of MetS in men from 11.0% in those aged between 20-29 years to 47.22% in those aged between 80-89 years. Regarding women, the percentage increased from 9.2% to 64.4% in the corresponding age ranges[140]. Similar results were reported in the NHANES study. Among those individuals aged 20-39, the prevalence of the MetS was 18.3%, increasing to 46.7% among those individuals aged 60 years or above[7].

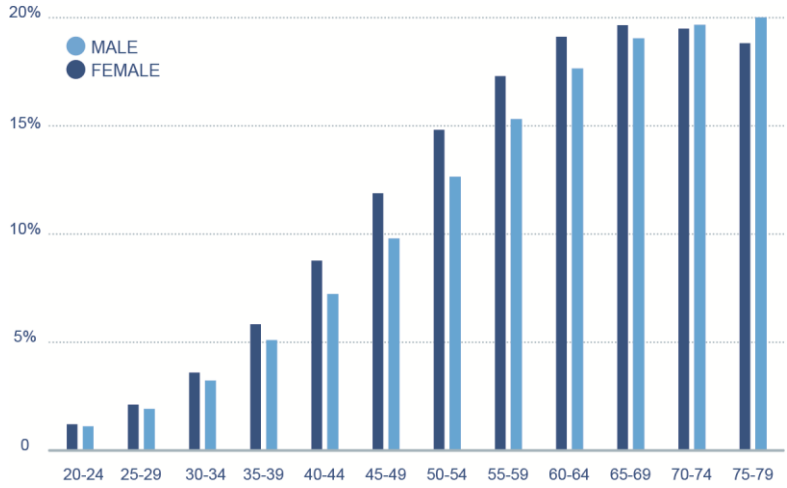

Figure 5. Worldwide prevalence of diabetes stratified by sex and age in 2015. Adapted from: International Diabetes Federation. IDF Diabetes, 7 ed. Brussels, Belgium: International Diabetes Federation, 2015. http://www.diabetesatlas.org

3.2. MODIFIABLE RISK FACTORS

3.2.1. SMOKING HABIT

The fact that active smoking increases the risk of T2D and MetS is well documented. A meta-analysis including 88 prospective cohorts studies with 5,898,795 participants, reported 37% higher risk of T2D in current smokers compared with nonsmokers. The risk was also higher when former smokers were compared with never smokers (HRs: 1.14; 95%CI: 1.10-1.18). Importantly, authors also reported a dose-response relationship between smoking and T2Drisk. The highest risk (57%) was reported for heavy smokers compared with never smokers, whereas moderate smokers had a 34% risk and light smokers a 21% risk[141]. Another important aspect to highlight is that, compared to never smokers with no passive smoke exposure, passive smokers had a 22% higher risk of T2D. Finally, the risk of T2D was also high in new quitters, but it decreases as the time since quitting increases.

Another meta-analysis also explored the association between smoking and MetS risk[142]. After analyzed data from 13 prospective cohort studies involving 56,691 participants, a 26% higher risk of MetS was observed in active smokers. In the dose

response analysis, results followed the same line as in the T2D meta-analysis. The risk of MetS was stronger in heavy smokers (RR: 1.42; 95%CI: 1.27-1.59) than in light smokers (RR: 1.10; 95%CI: 0.90-1.35).

Different mechanisms have been proposed to explain the association between smoking and T2D and MetS. Tobacco consumption has been associated with IR, higher triglycerides and lower HDL levels. This could be partly explained by cigarette smoking stimulation of sympathetic nervous system and the increase of different hormones with insulin antagonistic activity such as cortisol and growth hormone. Moreover, smoking has also been associated with endothelial dysfunction[143].

3.2.2. OVERWEIGHT AND OBESITY

Obesity is defined as an excess of body fat. In clinical practice, BMI as determined by weight in kilograms divided by height squared in meters, is used as a tool to identify overweight and obese individuals. Briefly, BMI ranging 25-29 kg/m^2 indicates overweight and BMI ≥ $30kg/m^2$ indicates obesity[144]. It has been observed a worldwide increase in BMI by 0.4 kg/m^2 per decade in men and $0.5kg/m^2$ per decade in women from 1980 to 2008[145]. Data reported from the WHO state that approximately 1.9 billion adults worldwide were overweight in 2014, and more than 600 million were obese[144]. Nowadays, as all of the aforementioned figures corroborate, obesity can be considered as an epidemic condition and, importantly, it has important implications in the development of numerous chronic diseases, including T2D and MetS.

In US adults the prevalence of T2D increased parallel to an increment in obesity prevalence from 1990 to 2001[146]. A meta-analysis including 18 prospective cohort studies showed that overweight and obesity were associated with a higher risk of T2D development. Specifically, obese and overweight participants had nearly 7-fold and 3-fold higher risk of T2D, respectively, in comparison with their normal weight counterparts[147]. Obesity itself can increase the risk of T2D even when other metabolic alterations are not present. In fact, in a recent published study, although those metabolically unhealthy obese presented more than 3-fold high risk of T2D

compared with those metabolically healthy normal weight participants, those metabolically healthy obese still had a higher risk of T2D (OR: 2.96;95%CI: 1.07-8.24) followed by the metabolically unhealthy overweight (OR: 2.75; 95% CI: 1.17-6.4) and the metabolically unhealthy normal weight individuals (OR: 1.79; 95% CI: 0.71-4.49)[148].

Results regarding MetS risk are in the same direction. A study conducted in Finland showed that the risk of MetS was lower in those obese adults who were not obese in their childhood than those obese adults who had also been obese as children. Nevertheless, the risk of MetS development was lower in non-obese subjects, independent of the childhood obesity than those obese individuals[149]. In this line, a recent systematic review and meta-analysis has reported a higher risk of MetS in adults, associated with overweight or obesity in Asian and European children aged from birth to 6 years[150]. Another recent study has shown that higher baseline BMI was associated with a higher risk of MetS development or with a higher risk to develop more MetS features during aging in the PIVUS and ULSAM cohorts. Importantly, in the PIVUS study, the risk of MetS was also high in those individuals who gained weight during the follow-up[151].

Despite the fact that overweight and obesity, defined using BMI, have been associated with a higher risk of T2D and MetS, it is important to point out that BMI does not consider body fat distribution. Excessive fat accumulation within visceral depots may not be associated with high BMI, whereas it has been associated with different metabolic disturbances. As evidence from different studies suggests, abdominal obesity measured with waist circumference would be a better predictor of T2D and MetS development than BMI[152–154].

3.2.3. PHYSICAL ACTIVITY AND SEDENTARY LIFESTYLE

In the last decades numerous changes in our life behaviors, e.g. changes of occupations, emergence of new technologies, accelerated pace of life in urban areas, have resulted in the acquisition of more sedentary work and less energy expenditure. Moreover, leisure-time activities have also undergone a shift from outdoor games to

indoor entertainment, including specially playing video games, watching television and using computer.

In order to maintain general health, public health guidelines recommend at least 150 minutes of moderate to vigorous physical activity or 75 minutes of vigorous physical activity per week[155,156]. However, some epidemiological data suggests that, worldwide, a 30% of adults do not meet these targets[157].

Several prospective cohort studies and clinical trials have analyzed the association between physical activity and the risk of T2D and MetS. In the last decade an effort to summarize the evidence between physical activity and the risk of T2D have resulted in the publication of 3 meta-analyses[158–160]. All of them agreed that high levels of total physical activity or leisure-time physical activity (including activities such as sport, exercise or recreational activity excluding occupational activity) are associated with a lower risk of T2D development when compared with low-levels of physical activity[158,159]. Regarding the type of physical activity, results showed that not only vigorous activity (RR: 0.61; 95%CI 0.51–0.74) was associated with a lower risk of T2D incidence, but also moderate activity (RR: 0.68; 95%CI 0.52–0.90), low intensity activity (RR: 0.66; 95%CI 0.47–0.94) and walking (RR: 0.85; 95%CI 0.79–0.91) when high versus low activity categories were compared in each physical activity type[160]. Another meta-analysis including 17 prospective cohort studies with more than 64,000 individuals and 11,271 new cases of MetS showed a 20% lower risk of MetS development when compared high versus low levels of leisure time physical activity. The protection was weakly when moderate versus low levels were compared (RR:0.95; 95%CI 0.91-1.00)[161].

Therefore, all these findings points physical activity as one of the main modifiable risk factors associated with the risk of T2D and MetS development. Strategies focused on incrementing the time devoted to physical activity are needed in order to prevent greater incidence rates of both diseases.

3.2.4. DIET

In the last decades, important changes in dietary patterns have been observed, mainly due to the globalization process. As a result of this phenomenon, also known as nutritional transition, an impairment of the nutritional and health status of the general population has been detected. Some of the important observed changes across the globe includes an increase in the consumption of sugar, salt, highly processed grains and animal food sources, whereas the consumption of legumes, vegetables and fruits has been reduced[162,163]. These patterns usually are accompanied by long time spent in sedentary behaviors. The combination of both diet and physical inactivity have largely promoted the so-called non-communicable diseases, such as T2D, hypertension, MetS, obesity and cancer.

There is increasing scientific evidence that supports the important role of nutrition on population health, showing both positive and negative effects. The importance lies in the fact that diet not only influence current health, but can also help preventing multiple diseases, including T2D and MetS[164,165]. As a consequence, diet is considered as an important modifiable risk factor.

In 2002, the joint WHO/FAO expert consultation on diet, nutrition and the prevention of chronic diseases took place in Geneva. The main objective was to review and update international recommendations on diet, nutrition and the prevention of chronic diseases. Different nutrient intake goals were derived in order to establish scientific basis to develop and implement strategies to improve population health. Hereby, this consultation highlights again the importance that diet plays in the preservation of the overall health[166].

Although data from epidemiological studies have shown shifting in dietary patterns associated with an increased development of chronic diseases, results of a recent study invite us to be optimistic. After analyze trends in dietary quality of individuals from 187 countries between 1990-2010, a consumption improved of healthy items was observed, whereas the consumption of unhealthy items worsened across the globe[167]. These results suggest that international, national and regional strategies focused on diet changes are effective.

In conclusion, diet plays an important role in the prevention of several chronic diseases including T2D and MetS. In the next section of the present doctoral thesis, the scientific evidence regarding nutrients, foods, and dietary patterns associated with T2D and MetS risk will be described in more detail.

4. Dietary components affecting metabolic syndrome and type 2 diabetes

This section comprises an extended literature review on T2D and MetS risk factors related to dietary components. All these factors have been associated to an increased or decreased risk of T2D and MetS development and it can be modified by intensive lifestyle changes.

4.1 NUTRIENTS

4.1.1 MACRONUTRIENTS

From a traditional point of view, science has been focused on the total amount of macronutrients consumed (carbohydrates, proteins and fats) and their associations with T2D and MetS risk. Nevertheless, recent evidence has shown different effects on the risk of both metabolic conditions depending on the type of macronutrient consumed, suggesting that the quality itself would be more important than the quantity consumed.

4.1.1.1 CARBOHYDRATES

Carbohydrates are the dietary components that have the highest influence on postprandial blood glucose, as they rise considerably glucose levels with the consequence stimulation of insulin release. As has been discussed throughout the present doctoral thesis, sustained hyperglycemia for long time has detrimental effects on human health, thus leading to an increased risk of T2D and MetS development. Therefore, different studies aimed to analyze whether higher amounts of carbohydrates consumption predispose individuals to develop T2D and MetS. Some controversy surrounds in this field, especially because the metabolic effects of carbohydrates consumption not only depend on their quantity but also on their

quality. In this regard, it has been shown that the increase of blood glucose levels after a certain amount of carbohydrates consumption depends on the food sources. From this observation appeared as the so-called glycemic index [168]. This concept is defined as the incremental area under the blood glucose response curve after the ingestion of 50g of carbohydrates of a test food expressed as a % of the response obtained after the ingestion of 50gr of glucose or bread in the same subject. Glycemic index is a characteristic of the food and it is considered a valid marker of carbohydrate quality[169]. This index only takes into account the type of carbohydrates of the food based on an equal amount of available carbohydrate, and therefore, not the total quantity of carbohydrates consumed. Because the amounts of total available carbohydrates can vary in a food or in the overall diet, another concept, the glycemic load, was developed in order to easily overcome this issue. The glycemic load is calculated as the product of glycemic index and the total available carbohydrates in a given amounts of foods[170]. Since the development of both concepts, a grown body of evidence has been published in relation of its association with T2D and MetS risk. However, its utility and validity have been extensively criticized, arguing that the total whole grain and fiber content are best markers of carbohydrate quality.

Carbohydrates and T2D

Some evidence supports the notion that high amounts of carbohydrate consumption increase the risk of T2D[171–174]. However, other studies could not observe any significant association between high amounts of total carbohydrates consumption and T2D incidence[170,175,176]. In 2012 a meta-analysis evaluating the association between different macronutrients intake and the risk of T2D observed a positive association between total carbohydrate consumption and the risk of development this disease[173]. Nevertheless, authors did not analyze the dose-response relationship. One year later, this issue was addressed in another meta-analysis[177] where non-linear association was observed between total carbohydrate consumption and the risk of T2D. Recently, a meta-analysis of observational studies reported no differences in the risk of T2D between those participants adhering to a high-carbohydrate diet compared to those following a low-carbohydrate diet in the long

term.[178]. The combined results of these meta-analyses reinforce the fact that further research is needed in this field in order to clarify the role of total carbohydrate consumption on the risk of T2D.

Prospective studies evaluating the association between carbohydrate quality and the risk of T2D development have also yielded inconsistent results. Although some of them observed a direct association between glycemic index, glycemic load and T2D[170–172,176,179,180], others did not observe any significant association [175,181–183]. However, whereas data for the relation between carbohydrates quality and risk of T2D from prospective studies has been mixed, results coming from meta-analyses are more consistent. All the meta-analyses published up to date suggested that diets with a high glycemic index or a high glycemic load were associated with an increased risk of T2D development[177,180,184]. The most recent showed a 19% higher risk of T2D in those individuals in the highest category of glycemic index and a 13% in those participants in the top category of glycemic load, compared to their counterparts in the lowest category[180].

Taking into account all discussed above, it seems that quality rather than quantity of carbohydrate consumption maybe associated with the risk of development T2D.

Carbohydrates and MetS

Limited evidence exists regarding carbohydrates quantity and quality consumption and the risk of MetS. The vast majority of studies presented a cross-sectional design and showed inconsistent results. Three studies, all conducted in Korea, observed a higher increase of MetS prevalence with increased total carbohydrate consumption[185–187]. Conversely, a non-significant association was observed in a study conducted in Massachusetts[188]. In terms of carbohydrate quality, a similar situation exists. While three studies did not observe any association between the glycemic index and/or the glycemic load of the diet and the prevalence of the MetS[186,188,189], others observed an inverse significant association[185,190,191]. However, it is important to highlight that cross-sectional studies do not allow establishing cause-effect associations. To date, only one prospective study, conducted in the framework of the PREDIMED (PREvención con DIeta MEDiterránea) study, has

analyzed the association between glycemic index, glycemic load and the risk of MetS development. After a mean of 4.8 years of follow-up an increase in glycemic index was associated with a 3% higher risk of MetS development in those individuals aged 65-74. No association was observed for the youngest and oldest age groups. Similarly, changes in glycemic load were only associated with a higher risk of MetS incidence in the middle age category (HR: 1.005; 95% CI: 1.002–1.009)[192].

Further studies are required in order to clarify the effect, if any, of carbohydrate quality and quantity and the risk of MetS.

4.1.1.2 PROTEINS

In recent years, protein-rich diets have become trendy for the treatment of obesity. Because obesity is one of the main modifiable risk factors for MetS and T2D, it has been hypothesized that a high protein consumption may also have a beneficial role in the prevention of both diseases. However, it is important to point out that most of clinical trials have evaluated the effect of high-protein diets, compared to low-protein diets, on weight loss and metabolic disturbances in the short-term[193,194] and these benefits seems to be not apparent in long-term trials[193,195].

Proteins and T2D

Several prospective studies have observed a direct association between high-protein diets and the risk of T2D development[196–201]. However, some did not find any significant association[174,202]. Similar to the observations with carbohydrate consumption, strong evidence suggests that different protein sources have different impact on T2D incidence. While animal protein consumption has been associated with a higher risk[174,196,197,199,201], a null[174,196,197,201] or even an inverse association[199] has been observed with vegetable protein intake .

In 2016, a meta-analysis of 11 prospective studies showed a modest inverse association between total protein consumption and the risk of T2D (HR: 1.09; 95%CI: 1.06-1.13). In the same line, animal protein consumption was associated with a 19% increased risk of T2D (HR:1.19;95%CI: 1.11-1.28), whereas plant protein intake was not associated (HR:0.95;95%CI:0.89-1.02)[201]. Guo-Chong et al, attempted to

characterize the shape of the association regarding animal protein consumption and re-analyzed data from the aforementioned meta-analysis. A nonlinear relation between animal protein consumption and the risk of T2D was observed. Results showed that dietary animal protein was only associated with an increased risk when the percentage of energy from animal protein was up to 12%[203].

Proteins and MetS

Several studies have analyzed the association between protein consumption and the incidence of different components of the MetS, such as blood pressure, HDL-c and triglycerides, showing a beneficial effect for plant-sourced proteins compared to animal-sourced proteins[204]. However, limited evidence is available on the association between protein consumption and the risk of MetS as an entity. A cross-sectional study conducted in more than 38,000 adults observed a significant inverse association between total protein intake and MetS prevalence[187]. Similar results were observed only for vegetable protein consumption in another cross-sectional study including 149 individuals, whereas animal proteins intake was not associated[205]. To date, only one prospective study has evaluated the effect of protein consumption on the risk of MetS development. After a mean of 11.3 years of follow-up, participants in the highest quartile of total and animal proteins consumption had a higher risk of MetS, whereas those with a higher consumption of vegetable proteins presented a lower risk[206].

4.1.1.3. FATTY ACIDS

Dietary fat, especially the type of fat consumed, has become the focus of scientific community attention because of the important effects that exerts on human health. During long time, low-fat diets have been recommended in order to prevent CVD. Consequently, the consumption of total fat was reduced whereas the consumption of total carbohydrate (independently of the quality) was increased. However, this fact did not decrease the incidence of chronic diseases, probably because the consumption of healthy fats was also reduced along with the consumption of harmful ones. With the emergence of large nutritional clinical trials, which have demonstrated that the quality of the fat content of the diet is more important that the

amount in the prevention of several chronic diseases[207,208], a shift in this paradigm has been occurred.

Previous research has focused on assessing the association between the quantity and quality of fats and the risk of CVD development[209,210]. Nevertheless, little is known about their role in the prevention of T2D and MetS.

Fatty acids and T2D

While total fat intake has not been considered a risk factor for the development of T2D[211–213], evidence for specific types of fat remains unclear. Some studies have observed a reduced risk of T2D associated to a higher consumption of extra virgin olive oil[214], which is an important food source of monounsaturated fatty acids (MUFAs). However, prospective cohort studies have not reported any association between MUFAs intake and the risk of T2D development[211,212,215]. Findings from polyunsaturated fatty acids (PUFAs) are also contradictory. Whereas some studies observed an increased risk with higher intakes of PUFAs[215], other studies observed a decreased risk[211] or non-association with T2D[212]. PUFAs are fats seeming to have different effect on T2D depending on the type[215]. However, previous meta-analyses have observed non-harmful/beneficial associations between different types of PUFAs (ω-6 or ω-3 fatty acids and its different subtypes) and the risk of T2D[173,216]. It is important to remark that all the analyses yielded evident heterogeneity between studies (I-squared >50% in all of them) and the majority of the analysis included less than 10 studies making difficult the exploration of heterogeneity sources.

Saturated fatty acids (SFAs) are another subtype of fat that have received especial attention in the last years. It has been suggested that the replacement of SFA with MUFAs or PUFAs has beneficial effects on insulin sensitivity[217]. However, a recent meta-analysis concluded that SFAs are not associated with the risk of T2D with the limitation of high heterogeneity between studies[218]. Different SFAs exist, according to the chain lengths (ranging from 6 to more than 22 carbon atoms), therefore their biological effects are expected to be different[219]. For instance, plasma 15:0 and 17:0 SFAs, which are considered biomarkers of fat dairy consumption, have been associated with lower risk of T2D development[220]. SFAs are present in foods along

with other nutritional components that may modify its health effects. Moreover, SFAs can be found in wide range of different foods such as red meat and processed meat, butter, dairy products or vegetable oils, which have been associated with different effects on T2D risk.

Finally, limited evidence exists for the association of trans-unsaturated fatty acids with the risk of T2D. Although a recent meta-analysis observed no association between its consumption and the risk of T2D, only 6 studies were included in the analysis and, again, substantial heterogeneity was presented, which made difficult the interpretation of the results[218]. Furthermore, trans-unsaturated fatty acids are also a heterogeneous group of fat, which can exerts different biological effects. Whereas plasma levels of t-16:1n9 and t-18:1 have positively been associated with the risk of T2D[221], circulating levels of trans-palmitoleic (produced by ruminant stomach bacteria and consumed mainly through dairy and meats products) have been associated with a lower risk[218,222].

Fatty acids and MetS

Several studies have been conducted in order to better understand the role of different fatty acids on the progression of the features of the MetS, particularly blood pressure and plasma lipids, suggesting that more attention should be paid not only to the quantity but also to the quality of dietary fatty acids intake[223].

MUFAs-rich diets seems to have health attributes, as have demonstrated in different research. Its consumption improves lipid profile and blood pressure[224]. Moreover, it seems not affect waist circumference, as have been observed in the Health Professional Study. The replacement of 2% of energy from PUFAs or carbohydrates with MUFAs was not associated with changes in waist circumference, whereas an increase was observed in the replacement with trans-unsaturated fatty acids [225]. Similarly, in the PREDIMED study a less gain in abdominal adiposity was observed in those individuals following a high MUFAs Mediterranean diet compare to those following a low-fat control diet[226]. PUFAs, especially ω-3 fatty acids, have a considerable potential to decreased the risk of MetS because its consumption has been associated with beneficial effects on different components, reducing

triglycerides[227] and blood pressure levels[228,229]. Trans-unsaturated fatty acids seems to play adversely physiological effects, beyond those affecting T2D risk, impairing lipid profile, producing endothelial dysfunction and increasing body weight and visceral adiposity [230], which is one of the main underlying causes of MetS. However, all these studies have been only focused in one component of the MetS, and little it is known about their association with the MetS as an entity. To date, few studies have analyzed the association between fatty acids consumption and the risk of MetS, which have been included relatively small samples, have been conducted in different populations and have shown inconsistent results. Total fat consumption has been associated with a higher risk of MetS prevalence in Iranian[231] and Japanese Brazilian [232] populations, but not in Spanish adults[233]. MUFAs consumption have not been associated with MetS risk[231–233]. Whereas some have associated SFAs with a higher prevalence of MetS[231], others have not been observed any association[233]. Regarding PUFAs consumption, some studies[232,233], but not all [231], observed a reduced risk of MetS prevalence with higher intakes. The only prospective study conducted to date, observed a lower risk of MetS development, after 20.7 months of follow-up, with higher intakes of vegetable fats. However, non-association between animal, SFAs, PUFAs or MUFAs and MetS incidence was observed[234].

4.1.2 OTHER COMPONENTS

4.1.2.1 ANTIOXIDANT VITAMINS AND MINERALS

It has been postulated that an excess of reactive oxygen species may play an important role in the pathogenesis of T2D and MetS[235,236]. Vitamins and minerals with antioxidant capacities inhibit the oxidation of other molecules and, therefore, it seems biological plausible that sufficient amounts of antioxidant may prevent and delay the development of both diseases, as it has been proposed by different authors[237]. However, the evidence in this field is limited for supporting this hypothesis.

A meta-analysis of prospective cohort studies, published in 2007, observed an inverse association between vitamin E, carotenoids and T2D development, whereas non-significant association was observed with vitamin C[238]. However, the meta-

analysis included few studies ranging from 2 to 5 in each individual antioxidant analysis, highlighting the need for further studies to draw conclusions. On the other hand, in a cohort of male smokers, no association was observed between vitamin E and C and the risk of T2D[239]. In a recent study conducted in 2 different Chinese cohorts, an inverse association between vitamin C consumption and the risk of T2D has been observed, whereas the consumption of vitamin E has not been associated[240]. Similarly, in a cross-sectional study of 22.672 Korean adults, dietary vitamin C has been associated with a lower risk of MetS development (OR: 0.89; 95%CI: 0.80–0.99)[241]. Another cross-sectional study concluded that low vitamin C consumption predisposed to MetS risk, while vitamin E was not associated[242]. Large and well-conducted randomized clinical trials are needed in order to clarify the role of antioxidant vitamins in the prevention of MetS and T2D.

Selenium and magnesium are two essential minerals with a recognized antioxidant capacity. In a meta-analysis of 22 prospective cohort studies, 100mg/day increment of dietary magnesium consumption was associated with a 10% lower risk of T2D (RR:0.90; 95%CI:0.81-0.99)[243]. Similarly, a recent meta-analysis of 9 studies found an inverse association between high dietary magnesium intake and the risk of MetS (OR: 0.73; 95%CI: 0.62-0.86)[244]. The association between selenium and T2D and MetS remains inconclusive. The majority of studies have been focused on serum selenium status rather on selenium consumption. A recent study found a negative correlation between selenium intake and insulin resistance at intakes below 1.6 μg/kg/day, whereas the benefit disappeared with consumption above this value [245]. However, results from epidemiological studies evaluating its association with T2D suggest a detrimental effect on this disease. Cross-sectional[246] and prospective[247] studies reported an increased risk of T2D in those individuals in the highest categories of dietary selenium intake compared with those in the lowest categories. Results from randomized clinical selenium supplementation trials also corroborate this concern. In the Nutritional Prevention of Cancer Study Group, supplementation of 200 μg/d was associated with a 55% higher risk of T2D development compared to the placebo group[248]. In the Selenium and Vitamin E Cancer Prevention Trial study a non-significant increased risk of T2D was found in those individuals with

supplementation of 200 µg/d of selenium compared with the placebo group[249]. Only one study has specifically evaluated the association between dietary selenium intake and the risk of MetS, showing a non-significant inverse association in those individuals with a higher consumption compared to those with a lower intake[242].

4.1.2.2 POLYPHENOLS

Polyphenols are organic compounds with more than one phenol ring, which come from secondary metabolites of plants. The main food sources of polyphenols are fruits, nuts, tea, red wine, coffee, chocolate and dry legumes[250]. Polyphenols have antioxidant properties and its consumption improves some oxidative stress related parameters that could be linked to different diseases[251]. However, although in recent years a lot of research has been focused on health effects of dietary polyphenols, the true impact of these plants metabolites on T2D and MetS needs further investigation. Higher consumption of polyphenol has been associated with a reduced incidence of T2D in several studies[252–254]. Recently, the association between different subtypes of polyphenols and T2D incidence has been evaluated in the PREDIMED study[255]. Results revealed that not only total polyphenols (HRs: 0.72; 95%CI: 0.52-0.99), but also total flavonoids (HRs: 0.67; 95%CI: 0.48-0.93), stilbenes (HRs: 0.57; 95%CI: 0.38-0.84), dihyroflavonols (HRs: 0.59; 95%CI: 0.40-0.88) and flavanones (HRs: 0.69; 95%CI: 0.49-0.97) consumption was associated with a lower risk of T2D development.

High amounts of polyphenols intake may also have beneficial effects on different MetS components[256], with the corresponding possible reduction on the risk of suffering this syndrome. However, analysis evaluating the association between polyphenol and MetS are scarce and controversial. In a cross-sectional study conducted in Iranian adults, only flavonoids consumption was associated with a lower risk of MetS prevalence, whereas total polyphenols, phenolic acid, stilbenes and lignans were not associated[257]. Contrary, in Polish adults, total polyphenols intake was inversely associated with a lower risk of MetS prevalence. In the subgroup analysis, only phenolic acids intake was associated with the MetS risk[258].

4.2. FOOD GROUPS

4.2.1. FRUITS AND VEGETABLES

The consumption of high amounts of fruits and vegetables is a main characteristic of the most of healthy diets, such as the Mediterranean diet, vegetarian diet or DASH diet. This food group contains sizeable amounts of fiber, vitamins with antioxidant capacity (vitamin C, E), minerals (mainly potassium and magnesium) and polyphenols, such as flavonoids. This nutritional value may explain the protective effects that have been attributed to fruits and vegetables consumption. It seems that their capacity to reduce antioxidant stress and inflammation[259], to lower blood pressure[260] and to prevent weight gain and the risk of adiposity[261] would be the major contributors to its healthy effects. However, their association with the risk of T2D and MetS it is not totally clear.

Up to date, several prospective cohort studies have been published in relation to fruits and vegetables consumption and the risk of T2D incidence, which is reflected in the publication of several meta-analyses in this field[238,262–264]. In the most recent meta-analysis, neither fruit nor vegetable consumption was associated with the risk of T2D[265]. However, a non-linear dose response association was observed, with a 9% reduced risk with increasing intake up to 300gr/day of vegetables and a 10% lower risk increasing intake of fruits up to 200-300gr/day. The increase of the intake above these values showed no benefit on T2D risk.

The evidence regarding fruits and vegetables consumption and the risk of MetS is scarce. Results from epidemiological studies also shown a trend toward to a lower risk of MetS with higher consumption of fruits and vegetables, but results are still inconsistent[266–268]. In fact, a meta-analysis of eight randomized clinical trials revealed that fruits and vegetables consumption reduced diastolic blood pressure, but had non-effect on other MetS components, such as fasting glucose, HDL-cl, waist circumference or triglycerides levels in individuals with MetS[260].

4.2.2. LEGUMES

Legumes are a food group rich in plant proteins, fiber, vitamins, minerals and polyphenols[269]. Moreover, are considered as a low glycemic index food[270]. This nutritional composition confers legumes excellent properties to improve cardiometabolic health. Indeed, its consumption has been associated with lower systolic blood pressure, body weight, fasting glucose, LDL-clevels and glycosylated blood proteins[271]. Therefore, taking into consideration these beneficial health effects, it would be expected that legumes intake protects against T2D and MetS. However, evidence to date does not support this notion, and the scarcity of epidemiological studies (especially regarding non-soy legumes consumption) highlight the need of further research in this field.

The majority of studies published so far have analyzed the association between soy-legumes and the risk of T2D, being scarce the studies focused on non-soy legumes consumption, which could exert different effects on T2D risk due to their differences in nutritional composition[272]. Five previous prospective studies have analyzed the association between non-soy legumes and the risk of T2D showing inconsistent results. Whereas some of them did not show any significant association between legume consumption and T2D incidence[175,198,273], others have shown a reduced[274] or an increased risk of T2D development[275].

Few studies have analyzed the association between legumes consumption and the risk of MetS. In a case-control study, individuals in the highest quartile of legumes consumption had lower odds of having MetS compared to those in the lowest quartile[276]. Studies that reported results stratified by sex, showed inconsistencies between them. In the Isfahan Healthy Heart Program study, compared to non-consumption, the intake of ≥3 servings/week was associated with a lower risk MetS prevalence in women, but not in men[277]. Contrary, in the DR's EXTRA Study, the consumption of 10g/day of legumes plus nuts was associated with a lower risk of MetS prevalence in men, but not in women[278].

4.2.3. CEREALS

Cereals can be divided in two groups: refined or whole grains. The main difference between both types remains in the preservation of the bran and germ in whole grains[279]. The majority of nutrients and phytochemicals are present in the out part of the grain; therefore, during the refined process there is a reduction in the nutrient content. As consequence, whole grains contain more fiber, starch, protein, vitamins and minerals than refined grains[279]. Due to these differences in the nutritional composition, whole and refined grains may play a different role in human health. Whereas refined grains are associated with a higher risk of developing chronic diseases, whole-grains seems to be protective[280,281]. Based on this, dietary guidelines recommend the consumption of whole grains as a part of healthy diets.

In 2013, the Food and Drug Administration (FDA) concluded that limited scientific evidence existed for qualified a health claim for whole grains and T2D risk[282]. However, in 2015 a systematic review and meta-analysis, of 8 observational studies including 316,051 participants, reported that consuming 3 servings/day of whole grain foods would reduce the risk of T2D by a 20% compared to consuming a half serving/day[283]. More recently, another meta-analysis that included 13 studies with 29,633 T2D cases, revealed a non-linear dose response relationship between whole grains consumption and the risk of T2D. More specifically, increasing the consumption up to 50g/day was associated with a 25% lower risk of T2D. However, small benefits were observed when its consumption was increase above this amount. It is important to note that non-significant association was observed between refined grains consumption and T2D risk[265].

Although dietary recommendations based on increasing the consumption of whole grains are generally accepted, the fact is that few studies have focused on evaluating the physiological effects of a diet high in whole grains on MetS. The Framingham Offspring Cohort reported lower odds (ORs: 0.67; 95%CI: 0.48-0.91) in those individuals in the highest quintile of whole grain consumption compared to those in the first quintile. Refined grains were not associated with the risk of MetS prevalence[188]. In the Tehran Lipids and Glucose Study, whole grains consumption

was also associated with a lower risk of MetS prevalence (ORs: 0.68; 95%CI: 0.60-0.78 for highest *vs.* lowest quartile). Nevertheless, the consumption of refined grains was associated with a higher risk (ORs: 2.25; 95%CI: 1.80-2.84 for highest *vs.* lowest quartile)[284].

4.2.4. VEGETABLE OILS

Vegetable oils can be obtained from different food sources, such as oilseeds (sunflower), nuts (almond, walnuts), legumes (soybean), or the flesh of fruits (olives). The composition of fatty acids can vary widely from one to another one. Although vegetable oils contain different fatty acids, always one type predominates over the others. For instance, the main fatty acids in olive oil are MUFAs (oleic acid), whereas in sunflower are PUFAs (linoleic acid) and in palm oils are SFAs (palmitic acid)[285]. The presence of fatty acids along with other minor components with bioactive properties (plant sterols and antioxidant vitamins) determines the physiological effects of vegetable oils.

There is a big gap in current scientific knowledge on the association between vegetable oils *per se* and the risk of T2D and MetS. The majority of studies have been focused only on the main fatty acids effects on human health, rather than vegetable oils as a whole. For instance, unhealthy effects have been attributed to palm oil because of its high content in SFAs, mainly palmitic acid[286]. Therefore, it seems that not all vegetable oils have the same metabolically effects. A review concluded that high-oleic acid soybean oil consumption might improve plasma lipids when replaces fats and oils high in SFAs or TFAs. Moreover, authors also suggested favorable effects on CHD risk factors when diets high in SFAs or trans-unsaturated fatty acids are replaced by high-oleic acid soybean oil or vegetable oils with high PUFAs content (sunflower, soybean or corn oil)[287]. Vegetable oils are a complex alimentary matrix and fatty acids are only one of its components. Therefore, its effects on human health cannot be attributed only to their fatty acid composition. Further research is needed in this field in order to better understand the role that vegetable oils can play in T2D and MetS.

One of the most studied vegetable oils, maybe because is one of the major components of the Mediterranean diet, is olive oil. A meta-analysis of 5 prospective cohort studies observed a lower risk of T2D incidence when highest versus lowest categories of olive oil consumption were compared (RRs: 0.84; 95%CI: 0.77-0.92). The dose-response meta-analysis showed a 9% reduced risk for each 10g/day increase in olive oil consumption. However, authors reported a non-linear relationship. No benefits of intakes above 15-20g/day were observed[214]. Although non previous studies have directly analyze the association of olive oil consumption and the risk of MetS, it seems that its intake could lower the risk of suffer the syndrome, beyond those beneficial effects on T2D, through benefits on blood pressure[288], plasma lipids levels and oxidative damage[289].

4.2.5. NUTS

Nuts are considered as energy-dense food due to its low water and high fatty acids content. Nevertheless, they are rich in many other healthy nutrients such as unsaturated fatty acids, minerals, vitamins, phytosterols and polyphenols that have been related with an improve in human health[290]. For instance, nuts intake has been associated with benefits in lipid profile (in a dose-related manner)[291], inflammation[292] and blood pressure[293]. However, the effect of nuts consumption on T2D and MetS remains inconclusive.

A recent meta-analysis that included eight studies with 27,016 cases of T2D concluded that nuts consumption is not associated with T2D risk (RRs: 0.95; 95%CI: 0.85-1.05 for highest versus lowest categories of nuts intake). It is important no point out that in the subgroup analysis, an inverse association was observed in those studies conducted in Asian population and those with a short-term follow-up[265]. Another meta-analysis that included 49 randomized clinical trials concluded that tree nuts consumption produces a modest diminution in triglycerides and fasting blood glucose levels, while does not affect other MetS components[294]. Besides, nuts consumption has been associated to a lower risk of MetS prevalence in the PREDIMED study [295] and NHANES (National Health and Nutrition Examination Survey) study[296]. Similarly, in the SUN (Seguimiento Universidad de Navarra) study,

a lower incidence of MetS was observed in those individuals with a higher consumption compared to those with a lower intake[297]. However, a review focused on nuts in the prevention and treatment of MetS suggested that large clinical trials are needed to clarify the role of nuts on this condition[298].

4.2.6. FISH

Fish is an important food source of unsaturated fatty acids (mainly ω-3 fatty acids), proteins, selenium and vitamin D, which could contribute to its positive benefits on human health[299].

In the last decades, several prospective studies have evaluated the association between fish intake and T2D risk, but findings were conflicting. A recent meta-analysis of 16 prospective studies and 45,029 T2D cases, reported a non-significant association between highest versus lowest fish consumption categories (RRs: 1.04; 95%CI: 0.95-1.13) with a high heterogeneity between studies (I^2=76%)[265].

In 2014, a systematic review suggested that fish intake may play an important role in MetS prevention. Of the seven included studies, four studies (3 cross-sectional and 1 prospective) observed an inverse association between fish consumption and MetS risk[300]. Recently, in the Tromsø Study 4, consumption of one serving/week of lean fish has been associated with decreased triglycerides and increased high-density lipoprotein HDL-c levels, whereas fatty fish has positively been associated with increased waist circumference in both sexes[301].

Further studies are required to explore the ability of fish consumption on MetS and T2D risk, especially because high heterogeneity have been observed in those studies published up to date in relation to T2D and few studies have assessed the association between fish intake and MetS risk as a whole.

4.2.7. RED MEAT AND PROCESSED MEAT

Meat is a food group with a high nutritional value due to its protein, minerals and vitamin (mainly B12) content. However, despite this nutritional characteristics, several studies have argue that plausible mechanisms exist linking its consumption, especially regarding red meat and processed meat, with the risk of different chronic

diseases[302]. This harmful effects could be attributed to its high SFAs, sodium, iron and nitrate content. It is important to point out that fat content of meats varies depending on the animal species[303], as well as sodium and nitrite and nitrate content, which are higher in processed than unprocessed red meat[304]. Therefore, it is expected that different types of meat exert different health effects.

A meta-analysis that included 15 prospective studies with 45,702 T2D cases observed a positive association between red meat consumption and the risk of T2D (RRs: 1.21; 95%CI: 1.13-1.30 for high versus low categories of red meat consumption). Similarly, a 27% higher risk of T2D was observed in those individuals in the highest category compared to those in the lowest category of processed meat consumption (RRs: 1.27; 95%CI: 1.20-1.35) after analyze 14 prospective cohort studies. In both cases a high heterogeneity was observed (I^2>55%)[265].

Red meat and processed meat consumption has also been associated with other components of the MetS such as hypertension[305,306] and abdominal obesity[307]. However, limited evidence exists in relation to its association with MetS risk and, importantly, findings from published studies so far are inconsistent. Whereas some prospective studies observed a positive association between red meat consumption and the risk of MetS incidence[308,309], others did not observed any association[310]. Besides, few studies have analyzed the association between poultry consumption and the risk of MetS[310,311], showing a non-significant association. Hence, these results suggest that meat from different animal species could exert different effects on MetS risk.

4.2.8. DAIRY PRODUCTS

Until recently, dairy products have been considered to be harmful to health because of its high fat content, especially in SFAs. However, recent evidence suggested that not all SFAs have the same health effects, as has been discussed in the macronutrient section of the present doctoral thesis. Moreover, dairy products are also good sources of vitamin D, calcium, magnesium and potassium. Therefore, this unique nutrient package could explain the beneficial health effects that have been associated with dairy products consumption in recent years[312].

Different meta-analyses have been published up to date evaluating the association between dairy products consumption and the risk of T2D[265,313–315]. The last dose-response meta-analysis that analyzed the association between total dairy products, different subtypes and the risk of T2D development, included 22 cohort studies and 579,832 individuals. Results revealed a lower risk of T2D per each 200g/day increment of total dairy consumption (RR: 0.97; 95%CI: 0.95-1.00). Yogurt and ice-cream intake showed a non-linear association with T2D risk (RRs: 0.86; 95%CI: 0.83-0.90 for 80g/day compared to 0g/day and RRs: 0.81; 95%CI: 0.78-0.85 for 10g/day compared to 0g/day, respectively). However, no incremental benefits were found at higher intakes. The other types of dairy products (low-fat products, high-fat products, milk and cheese) were not associated with T2D risk[313].

A meta-analysis of observational studies analyzed the association between dairy consumption and the risk of MetS. The final analyses included 15 cross-sectional studies, one case-control study and 7 cohort studies. Results revealed an inverse association between higher dairy consumption and the risk of MetS in the cross-sectional/case-cohort studies (ORs: 0.83; 95%CI: 0.73-0.94) and prospective cohort studies (RRs: 0.86; 95%CI: 0.79-0.92) analyses[316]. However, the meta-analysis only evaluated the association between total dairy products consumption and did not report results from different dairy subtypes, which seems to have different effects on MetS risk depending on the type consumed[317,318].

4.2.9. ALCOHOL

Alcohol consumption is thought to play an important role in T2D and MetS development. Evidence evaluating the association between alcohol consumption and both conditions is inconsistent and controversial, with results suggesting protective, detrimental or J-shape associations. In the last years, different meta-analyses have attempted to solve these discrepancies. In a meta-analysis that included 38 studies with 1,902,605 participants and 125,926 cases of T2D, a lower risk of T2D was observed at alcohol intake levels <63g/day, with increasing risk above this threshold compared to abstainers. The highest risk reduction (18%) was observed in consumptions ranging 10-14g/day. The stratification analyses showed that the

reduction risk may be specific only to women[319]. Similar results have been observed in another meta-analysis that has evaluated the association between alcohol consumption and the risk of MetS development. After including in the analyses 6 prospective studies involving 28,862 participants and 3,305 cases of MetS, a lower risk of MetS was observed in light drinkers compared with non-drinkers (RRs:0.86; 95%CI:0.75-0.99). Nevertheless, heavy drinkers showed a higher risk of MetS compared with non-drinkers (RRs: 1.84; 95%CI: 1.34-2.52)[320].

The results of these meta-analyses reinforce the fact that a J-shape association exists between alcohol consumption and T2D and MetS.

4.3. DIETARY PATTERNS

In the last decades, an alternative and complementary approach, the dietary pattern analysis, has gained strength to the detriment of the reductionist approach emphasizing the role of isolated nutrients or food groups on health. This new approach takes into account the synergy between nutrients and foods, which are consumed combined. The dietary approach is important not only because allows us to remove the confounding effects attributed to the interaction of one nutrient with another, but also because it facilities the interpretation of overall dietary patterns from a public point of view and it is easy to translate to diets[321]. However, it is important to highlight that it is crucial to explore the role of nutrients and food groups on health in order to establish dietary patterns and to better understand their possible beneficial or detrimental effects on health.

As consequence of several analyses, different dietary patterns have been identified and different health effects have been attributed to each one.

4.3.1. MEDITERRANEAN DIET

The Mediterranean diet (MedDiet) is characterized by a higher consumption of vegetables, fruits, legumes, whole grains, nuts, olive oil and fish alongside with a moderate consumption of dairy products and wine and a low intake of red and processed red meats[322]. MedDiet differs from other healthy dietary patterns due to the presence of high amounts of olive oil, nuts, sauces with tomato basis, onions,

garlic and spices, and the consumption of moderate wine[323]. It has been suggested that the synergetic effect of different bioactive components of the MedDiet in conjunction with the abundance of healthy foods, beneficially influence several metabolic pathways that causes T2D and MetS[323].

A meta-analysis of 6 prospective cohort studies showed a 13% lower risk of T2D development when highest versus lowest categories of adherence to MedDiet were compared, showing a low heterogeneity between studies (I^2=26%)[324]. In the same line, a meta-analysis including 8 cross-sectional and 4 prospective cohort studies showed a 19% lower risk of MetS in those individuals with a higher adherence to the MedDiet (RR: 0.81, 95%CI: 0.71-0.92). When the evaluation was focused on the individual components of the MetS, an inverse association was observed between MedDiet and waist circumference, blood pressure and low HDL-c levels[325].

Few randomized clinical trials have analyzed the effect of the MedDiet in the prevention of T2D and MetS. Results from the PREDIMED study demonstrated for the first time that, after a mean follow-up of 4.1 years, participants following a MedDiet enriched with extra virgin olive oil or nuts had a 40% and 18% lower risk of T2D incidence, respectively, compared with those individuals that followed a low-fat control diet[326]. The PREDIMED study did not demonstrate that a MedDiet reduces the risk of MetS development compared with the low-fat control group. However, participants in either MedDiet enriched with extra virgin oil or nuts were more likely to reverse the MetS during 4.8 years of follow-up (HRs: 1.35; 95%CI: 1.15-1.58 and HRs: 1.28; 95%CI: 1.08-1.51, for olive oil versus control group and nuts versus control group, respectively)[327].

4.3.2. PLANT-BASED DIETARY PATTERN

Plant-based diets, also widely known as vegetarian diets, are defined by the absence of meat products, seafood or products containing those foods. To date, different alternative/variations exist, such as the lacto-vegetarian diets (that consider the inclusion of dairy products) or the lacto-ovo-vegetarian diets (that not only consider the inclusion of dairy products, but also eggs). As a consequence, this dietary pattern is characterized by the consumption of high amounts of vegetables, fruits, legumes,

nuts and grains. Therefore, it is not surprising that the adoption of plant-based diets have been associated with a lower incidence of chronic diseases[328].

In the Adventist Health Study-2, vegans, lacto-ovo-vegetarians, and semi-vegetarians participants had a 62%, 38% and 51% lower risk of T2D, respectively, compared to those non-vegetarian individuals[329].

A recent meta-analysis including 2 prospective cohort and 12 cross-sectional studies, evaluated the association between vegetarian diets and the risk of T2D. The results showed an inverse significant association between vegetarian diets and the incidence/prevalence of T2D (ORs: 0.73; 95%CI: 0.61-0.87) compared to the omnivorous diets[330]. According to a systematic review, eight previous observational studies have evaluated the association between vegetarian or vegan diets and the risk of MetS. Of them, only four reported a beneficial effect of vegetarian diets on MetS risk, whereas three did not observe any association. Only one of them reported a lower risk of having MetS in those participants following an omnivorous diet compared to those following a vegan diet[331]. This review highlighted that no randomized clinical trials have been conducted up to date evaluating the effect of plant-based diets on the risk of MetS development.

4.3.3. DIETARY APPROACHES TO STOP HYPERTENSION (DASH) DIET

The DASH diet is characterized by a high consumption of fruits, vegetables, whole grains and low-fat dairy products along with a sodium restriction. This dietary pattern was originally developed in order to prevent hypertension[332]. Nowadays, due to its different healthy effects, it is widely recommended as a healthy dietary pattern. Because of its food composition, the DASH diet is rich in dietary fiber, unsaturated fatty acids and antioxidant components.

A meta-analysis of controlled trials reported beneficial effects of DASH diet reducing fasting insulin concentration (mean difference −0.16; 95% CI, −0.23 to −0.08). However, non- beneficial effects on fasting blood glucose or HOMA-IR was observed[333]. Another meta-analysis, including 6 studies, has observed a reduced risk

of T2D (RRs: 0.82; 95%CI: 0.74-0.92) when highest versus lowest quantiles were compared[324].

Few studies have analyzed the association between DASH diet and MetS. Cross-sectionally, higher adherence to DASH diet has been associated with a lower prevalence of MetS in Brazilian[334] and Isfahan population[335]. Although no previous clinical trials have evaluated the effect of DASH diet on the prevention of MetS, a randomized controlled trial conducted in 116 patients with MetS, demonstrated that DASH diet with caloric restriction increases HDL-c levels, decreases triglycerides, systolic and diastolic blood pressure, as well as reduces fasting blood glucose levels and weight, after 6 months of intervention compared to a control diet[336].

4.3.4. WESTERN PATTERN

Western type diets are mainly characterized by a high consumption of red meat and processed meat, refined grains, refined vegetable oils and refined sugar[337]. Moreover, this dietary pattern contains high amounts of SFAs and trans-unsaturated fatty acids. Contrary to the other types of dietary patterns described in this section, the wester pattern has been associated with a higher risk of several chronic diseases.

A meta-analysis of prospective cohort studies analyzing the association between a pattern derived from exploratory factor and principal component analyses, resembling the western diet (characterized by a higher consumption of red and processed meat, refined grains, high-fat dairy, eggs and fried products), has shown a significantly positive association with T2D (RRs: 1.44;95%CI: 1.27-1.62)[324].

Studies regarding MetS present mixed results. A higher prevalence of MetS has been observed in Iranian female teachers[338], whereas no association has been observed in community-dwelling older adults of Pennsylvania[339] and among Korean women[340]. Prospectively, results from the ARIC study showed a higher risk of MetS incidence in those individuals in the highest quintile of western dietary pattern scores compared with those in the lowest quintile after 9 years of follow-up (HRs: 1.18; 95%CI: 1.03-1.37)[308].

II. Justification

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DIETARY FACTORS ASSOCIATED WITH METABOLIC SYNDROME AND TYPE 2 DIABETES RISK
Nerea Becerra Tomás

MetS is considered a risk factor for T2D and CVD. It affects approximately a quarter of the adult population, increasing its prevalence rates with age. In turn, T2D is a major public health issue worldwide because of its high prevalence and its premature associated morbi-mortality. Likewise, both metabolic disorders are linked to a reduced quality and life expectancy. Therefore, to address this health problem, efficient and effective strategies focused on changes in lifestyle are critically required.

It has been observed that diet plays a crucial role in the prevention and management of MetS and T2D. Shift in dietary patterns, characterized by adoption towards a more westernized diet due to the nutrition transition process, has been one of the responsible factors for the high prevalence of different chronic diseases, including MetS and T2D. Contrary, adherence to healthy dietary patterns, such as the Mediterranean, DASH and vegetarian diets, have been associated with lower incidence of these chronic age-related diseases. Current nutritional epidemiology has centered in analyzing dietary patterns, which describe the overall diet and take into account the combination and synergic effects of foods and nutrients. In order to understand the observed differences in disease risk among dietary patterns, it is of great importance to evaluate the specific role that play particular nutrients and foods.

Some of the main differences between Western and plant-based dietary patterns are the frequency of consumption of dairy products, meat and legumes.
Dairy consumption has been considered as harmful for health because of its high-fat content. However, accumulating evidence suggests that may actually protect against T2D. Regarding MetS, few studies have investigated its association and most of them have been conducted in healthy adult population without evaluating different subtypes of dairy products.
Research also suggested that the consumption of meat, particularly red meat and processed red meat, may increase the risk of development some components of the MetS. However, the evidence regarding MetS as a whole entity is scarce and inconsistent. Previously, our research group observed a higher risk of MetS incidence

associated with red and processed red meat consumption in the PREDIMED Reus-Tarragona cohort after one year of follow-up. However, this association has not been previously evaluated in the full cohort.

Legumes consumption is widely recommended because of its beneficial effects on health, however, little it is known regarding its association with T2D risk. The majority of studies have been focused on evaluate the association between soy-legumes consumption and the risk of T2D and have been conducted in Asian, American and north Europe populations, but not in Mediterranean populations where legume consumption is relatively high.

Therefore, taking into account the big gap of knowledge regarding the consumption of the aforementioned foods and the risk the MetS and T2D incidence, with the present doctoral thesis we investigated in deeper manner the association between meat and dairy products consumption and the risk of MetS incidence, as well as the association between legume consumption and the risk of T2D using data from the PREDIMED trial as an observational prospective cohort. The PREDIMED study provides with a unique opportunity to evaluate these associations in adults at high CVD risk in the context of the MedDiet.

III. Hypothesis and aims

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Hypothesis 1: A greater intake of dairy products is associated with a reduced risk of MetS or any of its components incidence.

- **Objective 1:** To evaluate the association between total dairy products and its different subtypes (whole-fat dairy, low-fat dairy, yogurt, milk and cheese) consumption and the risk of MetS and its components incidence.

Hypothesis 2: Higher consumption of meat, and particularly red meat and processed red meat, is associated with an increased risk of MetS or any of its features development.

- **Objective 2.1:** To analyze the association between total meat and its different subtypes (red meat, processed meat and poultry) consumption and the risk of MetS and its features development.
- **Objective 2.2:** To assess the replacement effects of red meat and processed red meat with other protein-rich foods on the risk of MetS development.

Hypothesis 3: Higher consumption of legumes is inversely associated with the risk of T2D incidence.

- **Objective 3.1:** To evaluate the association between total legume and its different subtypes consumption (lentils, chickpeas, dry beans and fresh peas) and the risk of T2D incidence.
- **Objective 3.2:** To estimate the theoretical substitution effect of legumes for other protein- or carbohydrate-rich foods on the risk of T2D incidence.

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IV. Material and methods

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1. PREDIMED (prevención con dieta mediterránea) study

1.1. MAIN OBJECTIVES OF THE PREDIMED STUDY

The PREDIMED study is a randomized multicenter parallel clinical trial conducted in Spain between 2003 and 2011 with the objective to evaluate the effectiveness of the MedDiet (enriched with extra virgin olive oil or nuts) in the primary prevention of CVD, compared to a low-fat control diet in elderly individuals at high CVD risk.

The primary outcome comprised a composite of myocardial infarction, stroke and CVD mortality. Secondary endpoints included death from any cause, heart failure, T2D, MetS, dementia and cancer. In order to understand the role of dietary changes on clinical events, the study also included changes in intermediate outcomes such as fasting blood glucose, blood pressure, lipid profile and markers of inflammation and oxidation.

The main results of the study were published in 2013 in the New England Journal Medicine[207].

1.2. STUDY DESIGN

1.2.1. PARTICIPANTS SELECTION

PREDIMED participants were recruited between October 2003 and June 2009. Eleven centers from 9 cities in Spain participated in the recruitment process: Sevilla, Málaga, Reus-Tarragona, Barcelona, Islas Baleares, Pamplona, País Vasco, Valencia and Gran Canaria.

1.2.1.1. ELIGIBILITY CRITERIA

Inclusion criteria for the PREDIMED study were as follows: men (aged 55-80 years) and women (aged 60-80 years) who were free from CVD at baseline (Ischemic heart disease including angina pectoris or heart attack; stroke and peripheral artheriopathy), but who were at high CVD risk. Moreover, in order to be included in

the study, participants had to have either T2D or at least three of the following risk factors: Family history of premature CVD

- BMI ≥ 25 kg/m2
- 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 medication
- HDL cholesterol ≤ 40 mg/dL in men or ≤ 50 mg/dL in women

Besides, participants were excluded if they met one of the following criteria:

- Previous history of CVD: coronary heart disease (angina, myocardial infarction, coronary revascularization procedures or existence of abnormal Q waves in the electrocardiogram, stroke (ischemic or hemorrhagic, including transient ischemic attacks) and peripheral artery disease
- Presence of medical conditions that could impair the ability of the person to participate in the study or to attend visits
- Life expectancy less than 1 year
- Immunodeficiency or HIV-positive status
- Illegal drug use or chronic alcoholism or total daily consumption of 50g of alcohol
- BMI >40kg/m2
- Difficulties or major inconvenience to change dietary habits
- Impossibility to follow a Mediterranean diet due to religious reasons or presence of disorders that affect chewing or swallowing
- Low predicted likelihood to change dietary habits according to Prochaska and Diclemente transtheoretical model (or stages of change)
- Food allergy with hypersensitivity to any olive oil or nuts components
- 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 Centers every 3 months

- Illiteracy
- Patients with an acute infection or inflammation were allowed to participate in the study after three months from the resolution of their condition

The recruitment process started with the review of clinical records by medical doctors in order to extract the name of potential participants that met the eligibility criteria and exclude participants who met any of the exclusion criteria. After participants gave their consent, doctors sent to PREDIMED investigators the telephone number and, after a brief phone call and if they agreed, an appointment for a face-to face screening visit in their primary care center was scheduled.

1.2.1.2. SCREENING VISIT

This visit was performed at each primary care center in order to identify unequivocally the inclusion and exclusion criteria. At first, general characteristics and objectives of the study were explained and the informed consent for the trial participation and another for DNA collection for genetic analyses were obtained. This visit lasted ≈30min and encompassed an inclusion case report form and a general questionnaire of 26-items about medical conditions and risk factors for study purpose that were filled by PREDIMED investigators. Moreover, the last electrocardiogram available in the clinical history were revised or a new one was performed if the last one was not available; a validated semi-quantitative food frequency questionnaire (FFQ) of 137-items was administered to each participant as well as a validated Spanish version of the Minnesota Physical Activity questionnaire.

After this first screening visit, if participants met the eligibility criteria, they were randomized to one of the three possible intervention groups and a baseline visit was scheduled (see **figure 6**) asking to all participants to attend it after an overnight fast for blood extraction.

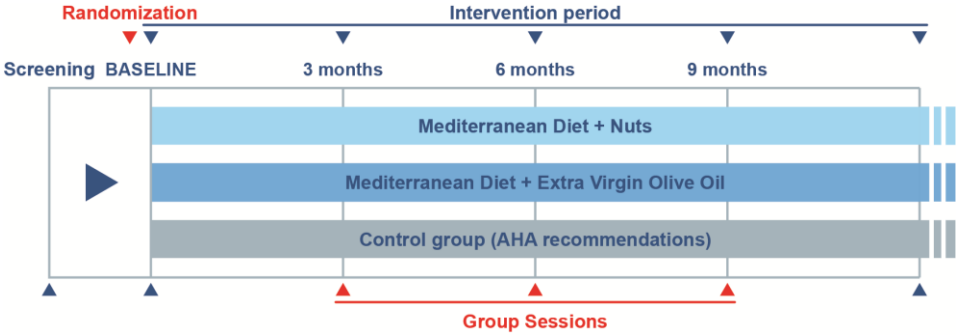

Figure 6. Design of the PREDIMED Study

1.2.1.3. RANDOMIZATION

A total of 7.447 participants met the eligibility criteria and were randomized to one of the three possible intervention groups in a 1:1:1 rate following tables of random allocation according to the recruitment order in blocks of 50 participants that were balanced by sex and age group. These tables were centrally elaborated by a coordinating group (IDIBAPS), and provided a stratified random sequence of allocation for each field center.

Intervention groups were as follows:

a) Low-fat control diet following the recommendations of the American Heart Association
b) Mediterranean type diet enriched with extra virgin olive oil
c) Mediterranean type diet enriched with mixed nuts (walnuts, almonds and hazelnuts)

1.2.2. INTERVENTION

PREDIMED dietitians were the responsible for all the dietary intervention and they received specific training sessions before and during the trial in order to ensure a

good implementation of the intervention. The main aim was to promote a Mediterranean dietary pattern in both intervention groups of MedDiet, without energy restriction or modifications in the physical activity, and to promote a low-fat diet in the control group. The delivery for free of extra virgin olive oil and nuts to both intervention arms increased the compliance and the adherence to the intervention. Free small gifts (e.g., kitchenware, aprons or shopping bags) were also given to participants in the control group in order to increase their compliance.

1.2.2.1. MEDITERRANEAN DIET GROUPS

Participants enrolled to the MedDiet enriched with extra virgin olive oil and MedDiet enriched with nuts, received ≈ 1L of olive oil per week and 30g per day of mixed nuts (15g of walnuts, 7,5g of almonds and 7,5g of hazelnuts), respectively.

Dietitians promoted a MedDiet giving the following specific advice to increase the frequency consumption of typical healthy foods of the MedDiet and to decrease the frequency of non-healthy foods: a) Use of extra virgin olive oil as main source of fat for cooking (≥ 4 tablespoon per day); b) Consumption of ≥ 2 daily servings of vegetables (one of them as fresh vegetables); c) Consumption of ≥ 2-3 daily servings of fresh fruits (including natural juices); d) Consumption of ≥ 3 servings/week of legumes; e) Consumption of ≥ 3 servings/week of fish or seafood; f) Consumption of ≥ 1 servings/week of nuts or seeds; g) Preferably consumption of poultry (without skin) instead of red meats or processed meat; h) Use of ≥ 2 times/week of *sofrito* sauce (tomato, garlic and onion adding or not aromatic herbs); i) Moderate consumption of wine within meals (optionally, only for habitual drinkers) (≥7 glasses/week); j) Avoid the consumption of industrial bakery products, sugar, butter, sugar sweetened beverages, French fries and fast food.

A leaflet on health benefits, use and conservation of olive oil was given to those participants randomized to the MedDiet + extra virgin olive oil, whereas those in the MedDiet + nuts received a similar leaflet with information regarding nuts.

Consumption of total fat was *ad libitum* as long as it was mainly from olive oil, nuts, vegetables and fish. A 14-item questionnaire (**Table 3**) was administered in order to

assess the adherence to the MedDiet and to personalize the intervention to achieve individual goals. The score ranged from 0 to 14 (high scores indicating greater adherence). Dietitians focused the intervention on the points that participants were not following.

Table 3. 14-item questionnaire of adherence to the Mediterranean Diet

Foods and frequency 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 use for frying, salads, out of house meals, etc.)?	≥ 4 tablespoons
3. How many vegetable servings do you consume per day? (1 serving = 200g – consider side dishes as ½ serving)	≥ 2 (at least 1 portion raw or as salad)
4. How many fruit units (including natural fruit juices) do you consume per day?	≥ 3
5. How many servings of red meat, hamburger, or meat products do you consume per day? (1 serving = 100-150g)	< 1
6. How many servings of butter, margarine, or cream do you consume per day? (1 serving = 12g)	< 1
7. How many sweet/carbonated beverages do you drink per day?	< 1
8. How much wine do you drink per week?	≥ 7 glasses
9. How many servings of legumes do you consume per week? (1 serving = 150)	≥ 3
10. How many servings of fish or shellfish do you consume per week?	≥ 3
11. How many times per week do you consume commercial sweets or pastries (not homemade), such as cakes, cookies, biscuits or custards?	≥ 3
12. How many servings of nuts (including peanuts) do you consume per week? (1 serving = 30g)	≥ 3
13. Do you preferentially consume chicken, turkey or rabbit meat instead of veal, pork, hamburger or sausage?	Yes
14. How many times per week do you consume vegetables, pasta, rice, or other dishes seasoned with sofrito (sauce made with tomato and onion, leek, or garlic, simmered with olive oil)?	≥ 2

*0 points if these criteria are not met

1.2.2.2. CONTROL GROUP

Participants enrolled to the control group did not receive information about MedDiet. Instead, information on how to follow a low-fat control diet following the recommendations of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adults Treatment Panel III)[341] was given.

Dietitians promoted the reduction of high-fat foods consumption advising to avoid the intake of red meat or processed meats, sweets and pastries, fatty fish, as well as the use of olive oil and nuts, as long as it have high amounts of fat. On the contrary, dietitians encouraged the consumption of low-fat dairy products, cereals, potatoes, pasta, rice, fruits, vegetables and lean meats. The dietary recommendations given by dietitians were as follows:

a) Consumption of ≥ 3 daily servings of low-fat dairy products
b) Consumption of ≥ 3 daily servings of bread, potatoes, pasta and rice
c) Consumption of ≥ 3 daily servings of fresh fruits
d) Consumption of ≥ 2 daily servings of vegetables
e) Consumption of ≥ 3 servings/week of lean fish and seafood
f) Consumption of ≤ 2 daily tablespoon of vegetable oils (including olive oil)Consumption of ≤ 1 serving/week of commercial bakery goods, sweets and pastries
g) Consumption of ≤ 1 serving/week of nuts and fried snacks
h) Consumption of ≤ 1 serving/week of red and processed fatty meats
i) Remove of visible fat in meats and soups
j) Consumption of ≤ 1 serving/week of fatty fish and seafood canned in oil
k) Consumption of ≤ 2 serving/week of *sofrito*

In order to evaluate the adherence to the low-fat intervention, a 9-point score was used (see **Table 4**).

Table 4. 9-item questionnaire of adherence to the low-fat control diet	
Foods and frequency 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 hose meals, etc.)?	≤ 2 tablespoons (1 tablespoon = 10ml)
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 = 100g; salami or bacon = 30g)	≤ 1
4. How many servings of butter, margarine, lard, mayonnaise, milk cream or milk-based ice-cream do you consume per week? (spread fat serving = 12g; ice-cream = 100g)	≤ 1
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, bacon, salami or fatty meats such as pork or lamb ribs?	≤ 2
7. How many times per week do you consume fatty fish or fish or seafood canned in oil?	≤ 1
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 = 80g; 6 biscuits = 40g)	≤ 1
9. How many times per week do you consume nuts (including peanuts), potato chips, French fries or commercial snacks?	≤ 1

*0 points if these criteria are not met

1.2.2.3. GROUP SESSIONS

Every three months participants attended to group sessions, with no more than 20 individuals, which were run by PREDIMED dietitians. These sessions were scheduled separated for each of the 3 intervention arms including:

- A reminder of the 14-item MedDiet score to those participants enrolled in the two MedDiet groups and a reminder of the 9-item low-fat diet score for those randomized to the low-fat control group
- Resolution of intervention-related problems about face-to-face counseling

- Delivery of written material, adapted to the season of the year, with description of 4-5 foods typical of the MedDiet or the low-fat diet; a quantitative shopping list of foods for 1-week along with a weekly plan of meals and cooking recipes according to the suggested menus.
- Delivery for free of either extra virgin olive oil (15L) or nuts (1.350g) for the MedDiet groups or gifts such as books, dispensers of olive oil, etc, for participants in the control group.

1.2.3. VARIABLES: DATA COLLECTION

At baseline, 6 months and yearly during the follow-up, participants of all intervention arms attended to a face-to-face visit in their primary care centers in which different questionnaires were administered and several anthropometric and biochemical measurements were collected (see **Table 5**).

Table 5. Overview of measurement scheduled in the PREDIMED study

	Baseline	1 year	2 years	3 years	4 years	5 years	6 years
Eligibility questionnaire	X						
General questionnaire	X						
FFQ	X	X	X	X	X	X	X
Physical activity questionnaire	X	X	X	X	X	X	X
Follow-up questionnaire*		X	X	X	X	X	X
Tolerance questionnaire		X	X	X	X	X	X
Abandonment questionnaire**		X	X	X	X	X	X
Electrocardiogram	X	X	X	X	X	X	X
Blood sample	X	X		X		X	X
Urine Sample	X	X		X		X	X
Toenail Sample	X						

*Includes measurements of weight, height, waist circumference, blood pressure and ankle-brachial blood pressure index.

** Only if applicable.

Abbreviations: FFQ, food frequency questionnaire

1.2.3.1. QUESTIONNAIRES

General and follow-up questionnaire: These questionnaires included different items related to CVD risk factors, drug treatment, medical history; family history of disease; smoking status; alcohol consumption (type and frequency); data on employment and socio-economic status; new onset of cardiovascular events; other medical conditions.

14-item questionnaire of adherence to the MedDiet: as explained before a validated short questionnaire was used to assess the adherence to the MedDiet diet[342].

9-item questionnaire of adherence to the low-fat diet: as stated before a specific questionnaire to assess the adherence to the low-fat diet was administered to those participants in the control group.

Leisure time physical activity questionnaire: a validated Spanish version of the Minnesota Leisure-time Physical Activity Questionnaire was administered to each participant. This questionnaire included information about the type and frequency of several physical activities during the previous month and year[343].

Food frequency questionnaire: a validated semi-quantitative FFQ of 137-items was administered in order to assess the dietary intake[344]. Participants were asked to report their frequency consumption, on an incremental scale (from never or almost never to more than six times per day) during the previous year, in a face-to-face interview with experimented dietitians. Nutrient and energy consumption were derived from these responses using Spanish food composition tables[345,346].

Tolerance and side effects questionnaire: In order to assess the side effects derived from the three interventions, participants completed a specific questionnaire at the first year of intervention and thereafter yearly.

1.2.3.2. ANTHROPOMETRIC AND BLOOD PRESSURE MEASUREMENTS

PREDIMED nurses were the responsible to measure blood pressure, body weight, height and waist circumference.

- Blood pressure: was measured in triplicate with participants in seated position and after 5min rest using a validated oscillometer (Omron

HEM705CP, Hoofddorp, The Netherlans). The average of the three measurements was written in the data collection form.

- Body weight: was measured using a calibrated balance beam scale with participants wearing light clothes and no shoes.
- Height: was measured using a wall-mounted calibrated stadiometer.
- Waist circumference: was measured using an anthropometric measuring tape in the midway between the lowest rib and the iliac crest.

1.2.3.3. BIOLOGICAL SAMPLES AND BIOCHEMICAL DETERMINATIONS

PREDIMED nurses were the responsible for the collection, processing and storage of all the biological samples. As indicated in **Table 5**, blood samples were collected at baseline and at years 1, 3, 5 and 6, following a standardized protocol and after an overnight fast. All biochemical measurements were performed blindly and in the same batch for consecutive samples of each participant. In the PREDIMED study urine sample was also collected at the time of blood extraction and stored at -80ºC.

Moreover, in the primary care centers, a complete blood count and routine biochemical measurements were also performed, which included: fasting blood glucose, uric acid, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltranspeptidase, total cholesterol, HDL-cholesterol and LDL-cholesterol, triglycerides, total protein and albumin.

1.2.4. ASCERTAINMENT OF METABOLIC SYNDROME AND TYPE 2 DIABETES

T2D and MetS were considered as secondary end-points in the PREDIMED study. In the following section, a description of the criteria used for its diagnosis is presented.

1.2.4.1. METABOLIC SYNDROME

For the present doctoral thesis, MetS was defined in accordance with the updated harmonized IDF and AHA/NHLBI criteria[6]. Participants were considered to have MetS if they had at least three of the following risk factors: a) abdominal obesity (≥88cm in men and ≥ 102cm in women), b) hypertriglyceridemia (≥150mg/dL) or drug treatment, c) low HDL-cholesterol levels (<40mg/dL in men and <50mg/dL in

women) or drug treatment, d) high blood pressure (systolic blood pressure ≥130mmHg and/or diastolic blood pressure ≥85mmHg) or drug treatment, e) high fasting glucose levels (≥100mg/dL) or drug treatment for diabetes.

This criteria was used to identify both prevalent and incidence cases during the follow-up.

1.2.4.2. TYPE 2 DIABETES

Prevalent cases of T2D were determined when clinical diagnosis was reported at the beginning of the study and/or when participants were taking oral hypoglycemic drugs or insulin.

New cases of T2D during the follow-up were diagnosed according to the criteria stablished by the American Diabetes Association[88], namely fasting plasma glucose ≥126mg/dL (7.0 mmol/L) in fasting conditions or ≥200mg/dL (11.1 mmol/L) after a 75g oral glucose load.

Physician investigators affiliated to each recruitment center, who were blinded to the intervention group, reviewed yearly all medical records of the participants. When a new-onset T2D was identified, the information was sent to the PREDIMED Clinical Events Committee who was also blinded to treatment allocation. The adjudication committee confirmed definitely the end-point only when a second test, repeated within the following 3 months, verify the new T2D case. Only those cases of T2D confirmed between 1 October 2003 and 1 December 2010 were included in the analyses.

2. Study population for the analyses

For the present doctoral thesis, data from the PREDIMED study was used as if it was an observational prospective cohort study.

Sample for analyses considering MetS as an outcome: From all the participants randomized to the PREDIMED study, we selected 5801 individuals with biochemical determinations available for a follow-up of ≥2 years. Due to MetS incidence was considered as an outcome, we excluded 3707 participants with the syndrome at baseline. Moreover, 226 participants with lack of information regarding FFQ at baseline or because they reported implausible total energy intake (≤500 and ≥3500 kcal/d in women and ≤800 and ≥4000 kcal/d in men) and individuals with missing data to determine the incidence of MetS, were also excluded. Therefore, the final sample for these analyses included 1868 individuals.

Sample for analyses considering T2D as an outcome: For these analyses, 3614 participants who had T2D at baseline, 292 with lack measures of blood glucose control, 98 individuals who had total energy consumption outside the pre-specified limits described before or with missing information regarding baseline FFQ and 94 individuals who were not followed up, were excluded from the original sample enrolled to the PREDIMED study (n=7447). The final available biological sample for these analyses consisted in 3349 participants.

3. Statistical analyses

A detailed description of the statistical analyses used for this doctoral thesis is explained in each publication presented in the results section. Briefly, all the analyses were performed using the statistical software SPSS versions 19.0 and 22.0 (SPSS Inc, Chicago, Illinois) or Stata versions 12.1 and 14.0 (StataCorp LP). The level of significance for all statistical tests was set at $P < 0.05$ for bilateral contrast.

All dietetic variables (exposures and co-variables) were energy-adjusted using the residual method described by Walter Willet. This approach allows us to represent nutrient and food consumption uncorrelated with total energy intake[347]. An exception was made in the analyses of fat intake, where all macronutrients consumption was expressed as percentage of total energy intake.

In order to compare baseline differences between categories of exposures, an ANOVA or Pearson chi-squared tests were used for continuous and categorical variables, respectively.

To assess the risk (Hazard Ratio) of the different outcomes according to categories of exposures, a Cox regression model adjusted for several potential confounders, was fitted. The follow-up time was calculated as the interval between the randomization and the date of the diagnosis of the outcome of interest or the date of the last visit or death for those participants who did not develop the disease. The linear trend was assessed through the assignment of the median intake within each category of exposure and modeled it as a continuous variable.

V. Results

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Table 6 presents the list of the 3 publications included in the present doctoral thesis.

Table 6. Reference, impact factor, category and journal rank of the publications presented in this thesis[1]

Reference	Impact factor	Category	Journal rank
Babio N, **Becerra-Tomás N**, Martínez-González MÁ, et al. Consumption of Yogurt, Low-Fat Milk, and Other Low-Fat Dairy Products Is Associated with Lower Risk of Metabolic Syndrome Incidence in an Elderly Mediterranean Population. J Nutr. 2015;145(10):2308-16. doi:10.3945/jn.115.214593	4.145	Nutrition & dietetics	Q1 16/81
Becerra-Tomás N, Babio N, Martínez-González MÁ, et al. Replacing red meat and processed red meat for white meat, fish, legumes or eggs is associated with lower risk of incidence of metabolic syndrome. Clin Nutr. 2016;35(6):1442-1449. doi:10.1016/j.clnu.2016.03.017	4.548	Nutrition & dietetics	Q1 9/81
Becerra-Tomás N, Díaz-López A, Rosique-Esteban N et al. Legume consumption is inversely associated with type 2 diabetes incidence in adults: A prospective assessment from the PREDIMED study. Clin Nutr. 2017. doi: 10.1016/j.clnu.2017.03.015. [Epub ahead of print]	4.548	Nutrition & dietetics	Q1 9/81

Q, Quartile

[1]Accessed date: July 05, 2017 (Journal Citation Reports of the ISI web of Knowledge, Thompson Reuters).

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1. Consumption of yogurt, low-fat milk, and other low-fat dairy products is associated with lower risk of metabolic syndrome incidence in an elderly Mediterranean population.

Babio N, Becerra-Tomás N, Martínez-González MÁ, Corella D, Estruch R, Ros E, Sayón-Orea C, Fitó M, Serra-Majem L, Arós F, Lamuela-Raventós RM, Lapetra J, Gómez-Gracia E, Fiol M, Díaz-López A, Sorlí JV, Martínez JA, Salas-Salvadó J; PREDIMED Investigators.

Key teaching points:

- Little it is known about the association between dairy products consumption and MetS risk.

- For the first time, the present study analyzed the association between the consumption of dairy products and its different subtypes and the risk of MetS and its components in a Mediterranean population at high CVD risk.

- A prospective assessment with 1868 elderly individuals at high CVD risk was conducted in the framework of the PREDIMED study.

- Results revealed an inverse association between low-fat dairy products, yogurt (total, low-fat and whole-fat yogurt) and low-fat milk consumption and the incidence of MetS. Cheese consumption was positively associated with the risk of MetS incidence.

- Whole-fat yogurt was associated with a lower risk of all MetS components, whereas low-fat yogurt related inversely to high triglycerides, low HDL-cholesterol and elevated fasting glucose.

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Supplemental Material can be found at:
http://jn.nutrition.org/content/suppl/2015/08/19/jn.115.214593.DCSupplemental.html

The Journal of Nutrition
Nutrition and Disease

Consumption of Yogurt, Low-Fat Milk, and Other Low-Fat Dairy Products Is Associated with Lower Risk of Metabolic Syndrome Incidence in an Elderly Mediterranean Population[1–3]

Nancy Babio,[4–6]* Nerea Becerra-Tomás,[4–6] Miguel Ángel Martínez-González,[5–7] Dolores Corella,[5,8] Ramon Estruch,[5,6,9] Emilio Ros,[5,10] Carmen Sayón-Orea,[6,7] Montserrat Fitó,[5,11] Lluís Serra-Majem,[5,6,12] Fernando Arós,[6,13] Rosa M Lamuela-Raventós,[5,6,14] José Lapetra,[6,15] Enrique Gómez-Gracia,[6,16] Miguel Fiol,[5,17] Andrés Díaz-López,[4–6] José V Sorlí,[5,8] J Alfredo Martínez,[6,18] and Jordi Salas-Salvadó,[4–6]* on behalf of the PREDIMED Investigators

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Abstract

Background: The association between consumption of dairy products and the risk of developing metabolic syndrome (MetS) is unclear.

Objective: The purpose of this study was to evaluate the associations between consumption of dairy products (total and different subtypes) and incident MetS in a Mediterranean population at high cardiovascular disease risk.

Methods: We prospectively analyzed 1868 men and women (55–80 y old) without MetS at baseline, recruited from different PREDIMED (Prevención con Dieta Mediterránea) centers between October 2003 and June 2009 and followed up until December 2010. MetS was defined according to updated, harmonized criteria. At baseline and yearly thereafter, we determined anthropometric variables, dietary habits by a 137-item validated food-frequency questionnaire, and blood biochemistry. Multivariable-adjusted HRs of MetS or its components were estimated for each of the 2 upper tertiles (vs. the lowest one) of mean consumption of dairy products during the follow-up.

Results: During a median follow-up of 3.2 y, we documented 930 incident MetS cases. In the multivariable-adjusted model, HRs (95% CIs) of MetS for the comparison of extreme tertiles of dairy product consumption were 0.72 (0.61, 0.86) for low-fat dairy, 0.73 (0.62, 0.86) for low-fat yogurt, 0.78 (0.66, 0.92) for whole-fat yogurt, and 0.80 (0.67, 0.95) for low-fat milk. The respective HR for cheese was 1.31 (1.10, 1.56).

Conclusions: Higher consumption of low-fat dairy products, yogurt (total, low-fat, and whole-fat yogurt) and low-fat milk was associated with a reduced risk of MetS in individuals at high cardiovascular disease risk from a Mediterranean population. Conversely, higher consumption of cheese was related to a higher risk of MetS. This trial was registered at controlled-trials.com as ISRCTN35739639. *J Nutr* 2015;145:2308–16.

Keywords: dairy products, metabolic syndrome, milk, yogurt, metabolic syndrome components, PREDIMED study

Introduction

Metabolic syndrome (MetS)[19] comprises a cluster of risk factors, including abnormal obesity, dyslipidemia, increased blood pressure, and high fasting plasma glucose, which markedly increase the risk of type 2 diabetes (T2D) and cardiovascular disease (CVD) (1).

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Diet and lifestyle are recognized as key elements in the prevention (2) and treatment of MetS (3). In recent years, a growing body of evidence has shown that the consumption of dairy products may have beneficial effects on risk factors defining MetS, including atherogenic dyslipidemia (4), hyperglycemia (5), insulin resistance (6) or T2D (7–9), blood pressure (10), and abdominal obesity (11).

In epidemiologic studies, the association between the total consumption of dairy products and the risk of MetS has been controversial. Some cross-sectional (4, 12–15) and prospective studies (5, 16, 17) have shown an inverse association, whereas others (18–20) have shown no association. Results by sex have also been inconsistent (18, 19).

Although most studies suggest that total dairy consumption could provide protection against the development of MetS, methodologic biases or multiple combinations of different types of dairy product with varying nutrient content may have contributed to the controversial results reported. Few studies have analyzed these associations across different dairy product subtypes. Some cross-sectional studies have reported an inverse association (15, 19) that was not found when data were analyzed prospectively (5). For example, in the case of cheese consumption, cross-sectional studies showed a positive association with MetS prevalence (19), whereas prospective studies showed a negative one (5, 17). Pereira et al. (5) showed an inverse association with total consumption of dairy products regardless of their fat content, whereas Louie et al. (20) found this inverse association only for whole-fat dairy products.

To the best of our knowledge, of the 4 prospective studies published to date on the relation between dairy product consumption and MetS incidence, 3 were conducted in healthy adult populations (5, 17, 20) and only 1 in older individuals (21), in whom MetS is more prevalent and potentially has more repercussions on health (22). In addition, only one study explored the associations for different dairy subtype products (5). Therefore, the aim of the present study was to examine the relation between the consumption of dairy products (whole or low-fat options) and risk of MetS in an older Mediterranean population in the frame of the PREDIMED (Prevención con Dieta Mediterránea) study.

Methods

Study design, participants, and outcome. The present study was conducted within the framework of the PREDIMED trial, the design of which has been described in detail elsewhere (23, 24). The PREDIMED study is a large, parallel-group, multicenter, randomized, controlled field trial aimed at assessing the effects of the Mediterranean diet on the primary prevention of CVD (25). The main results of the trial with respect to the primary endpoint have been published recently (26).

Briefly, 7447 community-dwelling men (aged 55–80 y) and women (aged 60–80 y) with no previously documented CVD were recruited. They were eligible if they had either T2D or ≥3 of the following CVD risk factors: hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or on antihypertensive medication), high plasma LDL cholesterol (≥160 mg/dL), low plasma HDL cholesterol (<40 mg/dL in men; <50mg/dL in women), overweight or obesity (BMI ≥25 kg/m^2), current smoking, or a family history of premature coronary heart disease (23). From October 2003 to June 2009, participants were randomly assigned to 3 intervention groups (2 of which were advised to follow a Mediterranean diet supplemented with either 1 L/wk extra-virgin olive oil or 30 g/d mixed nuts, and were compared with the third group, which was advised to follow a control low-fat diet). The study follow-up ended in December 2010. All participants provided their informed consent and the protocol was approved by the institutional review boards of each recruitment center.

In the present report, the data were analyzed assuming the design of an observational prospective cohort whose members were selected from all the PREDIMED recruiting centers with biochemical determinations available for a follow-up of ≥2 y (n = 5801). Because our aim was to explore the associations between the consumption of dairy foods and incident MetS, we excluded participants who had diagnoses of MetS at baseline (63.9%; n = 3707). We also excluded participants who had not completed the baseline FFQ or who reported an extreme total energy intake with values outside the prespecified limits (500–3500 kcal/d in women and 800–4000 kcal/d in men). A total of 2094 individuals were assessed. Of these, a total of 226 were excluded because of missing data that prevented the presence of MetS incidence from being determined. Thus, a total of 1868 participants were included in our longitudinal assessment for MetS incidence. The individual components of MetS—abdominal obesity, hypertriglyceridemia, low HDL cholesterol, high blood pressure, and high fasting glucose concentration—were analyzed for a total of 1386, 3539, 3745, 337, and 1844 participants (from 5801 participants initially considered with biochemical determinations available for a follow-up of ≥2 y), respectively.

The primary endpoint of the PREDIMED trial was a combination of several major cardiovascular clinical events (myocardial infarction, stroke, or CVD death). In the present analysis, incident MetS and its components were considered as the outcome.

MetS was defined in accordance with the updated harmonized criteria of the International Diabetes Federation and the AHA/National Heart, Lung, and Blood Institute (1). Individuals were diagnosed with MetS if they had ≥3 of the following components: hypertriglyceridemia

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[3] Supplemental Tables 1 and 2 are 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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[19] Abbreviations used: CARDIA, Coronary Artery Risk Development in Young Adults; CVD, cardiovascular disease; ICC, intraclass correlation coefficient; MetS, metabolic syndrome; PREDIMED, Prevención con Dieta Mediterránea; T2D, type 2 diabetes.

[≥150 mg/dL (≥1.7 mmol/L)] or drug treatment for elevated TGs; low concentrations of HDL cholesterol [<50 mg/dL (<1.3 mmol/L) and <40mg/dL (<1.03 mmol/L) in women and men, respectively] or drug treatment for low HDL cholesterol; elevated blood pressure (systolic ≥130 mmHg and/or diastolic ≥85 mmHg) or being treated for hypertension; high fasting plasma glucose [≥100 mg/dL (≥5.5 mmol/L)] or drug treatment for hyperglycemia; and elevated waist circumference for European individuals (≥88 cm in women and ≥102 cm in men.

Dietary assessment. At baseline and yearly during follow-up, dietary intake was quantified by trained dietitians with a 137-item semi-quantitative FFQ validated for the PREDIMED study (27). In the validation study, the FFQ was administered twice to explore reproducibility at 1 y; 4 3-d dietary records for the different seasons of the year were used as gold standard. The reproducibility of the FFQ used in PREDIMED for food groups and energy and nutrient intake, explored by the Pearson correlation coefficient, ranged from 0.50 to 0.82, and the intraclass correlation coefficient (ICC) ranged from 0.63 to 0.90. The validity indexes of the FFQ in relation to the dietary records for food groups and energy and nutrient intake ranged from 0.24 to 0.72, whereas the range of the ICC was between 0.40 and 0.84. Regarding dairy product consumption, the reproducibility and validity of FFQs were 0.81 (ICC 0.89), and 0.72 (ICC 0.84), respectively.

Dairy product consumption was assessed yearly with the use of 15 items from the FFQ. In order to assess habitual dietary intake over the previous year, frequencies of consumption were measured in 9 categories (ranging from never/almost never to >6 servings/d) for each food item. These responses to individual dairy items were then converted into mean daily consumption (grams per day) during the follow-up by multiplying the typical portion sizes (grams) by the consumption frequency for each food and making the appropriate division for the period assessed to obtain daily consumption. The total dairy foods category included low-fat/skim milk and skim yogurt, whole milk, condensed milk, whole yogurt, custard, and all types of cheeses, including petit Swiss, ricotta, cottage, and semicured/cured cheeses such as cheddar, Manchego, and Emmentaler). Low-fat dairy foods included low-fat/skim milk and skim yogurt, whole-fat dairy foods (whole milk and whole yogurt), and total dairy foods, including all of the above. Consumption of dairy products was also categorized by subtype, including milk (including total, low-fat and whole milk), yogurt (including total, low-fat and whole-fat yogurt), and cheese. Energy and nutrient intake and food groups were calculated from Spanish food composition tables (28, 29). We adjusted dairy consumption for total energy intake with the use of the nutrient residual method (30).

Measurements. Participants completed the following at baseline and yearly: *1*) a questionnaire about lifestyle variables, medical history, and medication use; *2*) a 14-item validated questionnaire (31) designed to assess adherence to the Mediterranean diet; *3*) a validated 137-item semiquantitative FFQ (27); and *4*) the validated Spanish version (32) of the Minnesota Leisure-Time Physical Activity Questionnaire. In addition, anthropometric variables and blood pressure were determined by trained staff. Blood pressure was measured in triplicate with the use of a validated semiautomatic oscillometer with a 5 min interval between measurements and the subject in a sitting position (Omron HEM-705CP).

Blood samples were collected after an overnight fast, coded, shipped to a central laboratory, and stored at −80°C until analysis. Biochemical analysis was performed in local laboratories. Glucose was measured by the glucose-oxidase method, cholesterol by esterase-oxidase-peroxidase, TGs by glycerol-phosphate oxidase-peroxidase, and HDL cholesterol by direct measurement. All local laboratories satisfied external quality-control requirements. When TGs were <300 mg/dL, LDL cholesterol was calculated with the use of the Friedewald formula so that the LDL cholesterol was not underestimated. A concordance study of 9 laboratories was conducted. From each study, a mean of 200 samples were analyzed for total cholesterol, HDL cholesterol, and TGs with the use of the Medical Research Institute of del Mar laboratory as reference. The Medical Research Institute of del Mar laboratory used ABX-Horiba commercial kits in a PENTRA-400 autoanalyzer (ABX-Horiba). One center was unable to provide samples for the concordance study.

The analysis of concordance of lipid measurements showed an r^2 and an ICC (95% CI) between 0.85 and 0.97, and 0.85 (0.77, 0.90) and 0.97 (0.95, 0.98) for total cholesterol, respectively; between 0.819 and 0.92, and 0.81 (0.78, 0.83) and 0.92 (0.89, 0.95) for HDL cholesterol, respectively; between 0.81 and 0.99, and 0.81 (0.73, 0.87) and 0.99 (0.99, 0.99) for triglycerides, respectively; and between 0.82 and 0.96, and 0.82 (0.74, 0.88) and 0.99 (0.99, 0.99) for glucose, respectively.

Statistical analysis. We averaged the intake reported during the baseline interview and the yearly consumption during the follow-up. Then, participants were categorized into tertiles of the mean consumption of total dairy products and different subtypes during the follow-up. To better represent the long-term consumption of dairy products and to minimize within-person variation, we used the mean energy-adjusted dairy consumption for all analyses based on assessments of items from all FFQs, which were administered at baseline and yearly during the follow-up for those participants who did not develop MetS. For those who did develop MetS, and given that participants can alter their dietary pattern after developing MetS, we only used data from all the available FFQs until the year before MetS was diagnosed. The baseline characteristics of the participants are expressed as mean ± SD or median (IQR) for continuous variables and number and percentages for categorical variables. Chi-square and 1-factor ANOVA tests were used to assess differences in the baseline characteristics of the study population.

Multivariable time-dependent Cox proportional regression models were fitted to assess the HRs of developing MetS and its components during follow-up according to tertiles of consumption of total, low-fat, and whole-fat dairy products; milk, low-fat milk, and whole milk; total yogurt, low-fat yogurt, and whole-fat yogurt; and cheese. Both upper tertiles were compared with the lowest tertile (reference). The assumption of proportional hazards was tested with the use of time-dependent covariates.

The time variable was the interval between random assignment and the date of the last follow-up, or the last recorded clinical event (MetS incidence) of participants who were still alive, whichever occurred first. Participants who were free of MetS or who were lost during follow-up were censored at the date of the last visit.

Three different Cox regression models were adjusted for potential confounding factors. Model 1 was adjusted for intervention group; sex; age (years); leisure time physical activity (metabolic equivalent task · d); BMI (kilograms per meter squared); current smoker (yes/no); former smoker (yes/no); and hypoglycemic, hypolipidemic, antihypertensive, and insulin treatment at baseline. Model 2 was additionally adjusted for mean consumption during follow-up of vegetables, fruit, legumes, cereals, fish, red meat, cookies, olive oil, and nuts (all grams per day), as well as alcohol (grams per day and quadratic term). Model 3 was adjusted for model 2 plus the prevalence of MetS components at baseline, including abdominal obesity (yes/no), hypertriglyceridemia (yes/no), low HDL cholesterol (yes/no), hypertension (yes/no), and high fasting plasma glucose (yes/no).

Statistical interaction between tertiles of total dairy consumption and its different subtypes, and potential effect-modifying variables, such as sex and intervention group, was assessed by including product terms in the models. To assess the linear trend, the median value of each tertile of dairy product and the different dairy subtypes was assigned and used as a continuous variable in the Cox regression models. The level of significance for all statistical tests was $P < 0.05$ for bilateral contrast. The Benjamini-Hochberg method was used to correct P values for multiple comparisons (33).

We also conducted a sensitivity analysis of a number of MetS components at baseline to test the robustness of our results. The main analysis was stratified by those individuals who did not have any components of MetS or had one, or who had 2 components. This was done to prevent possible bias effects, because it is easier for those who already have 2 criteria to meet the diagnostic definition than those with one or none.

All analyses were performed with SPSS software, version 19.0.

Results

After a median of 3.2 y of follow-up (IQR: 1.9, 5.8), 930 participants without MetS at baseline (53.8% women) developed new-onset MetS. Of those not showing the specific MetS components at baseline, 43.4% of 1040 participants developed abdominal obesity during follow-up, 27.7% of 1770 developed hypertriglyceridemia, 24.5% of 1810 developed low HDL

TABLE 1 Baseline characteristics of study individuals at high cardiovascular disease risk by tertiles of total dairy consumption[1]

	Total dairy consumption,[2] g/d			
	T1 (≤287; *n* = 622)	T2 (287–449; *n* = 623)	T3 (≥450; *n* = 623)	*P*[2]
Total dairy consumption, median (P25, P75)	207 (142, 250)	354 (322, 393)	577 (518, 661)	
Age, y	66.0 (6.0)	67.0 (5.9)	67.7 (6.2)	<0.001
Women, % (*n*)	38.7 (241)	52.6 (328)	66.0 (411)	<0.001
Waist circumference, cm				
Women	92.3 ± 10.6	92.43 ± 10.3	92.5 ± 10.8	0.84
Men	98.8 ± 7.9	97.4 ± 7.6	97.5 ± 7.3	0.042
BMI, kg/m^2	28.2 ± 3.4	28.3 ± 3.4	28.6 ± 3.7	0.08
Leisure time physical activity, MET · min/d	297 ± 260	273 ± 258	253 ± 242	0.010
Former smokers, % (*n*)	33.6 (209)	24.7 (154)	19.3 (120)	<0.001
Current smokers, % (*n*)	19.3 (120)	14.6 (91)	13.2 (82)	0.008
Blood pressure, mm Hg				
Systolic	147.6 ± 20.3	145.7 ± 19.6	145.7 ± 21.5	0.18
Diastolic	82.6 ± 10.9	81.9 (10.5)	81.6 ± 10.9	0.25
Biochemistry, mg/dL				
Plasma fasting blood glucose	101.7 ± 27.9	102.5 ± 32.1	107.5 ± 39.0	0.004
Serum HDL cholesterol	56.0 (49.0, 66.0)	58.0 (51.0, 68.0)	60.0 (53.0, 68.2)	0.001
Serum TGs	99.9 (77.0, 122)	95.0 (75.0, 118)	94.0 (73.0, 118)	0.022
Current medication use, % (*n*)				
Use of hypoglycemic agents	12.3 (76)	14.5 (90)	16.4 (102)	0.12
Use hypolipidemic agents	45.0 (280)	46.7 (291)	46.5 (290)	0.66
Use of antihypertensive agents	65.6 (408)	66.6 (415)	63.4 (395)	0.46
Insulin treatment	3.2 (20)	2.7 (17)	7.1 (44)	<0.001
MetS components, % (*n*)				
Abdominal obesity	41.2 (255)	41.4 (256)	49.1 (303)	0.006
Hypertriglyceridemia	6.9 (43)	5.3 (33)	3.4 (21)	0.019
Low HDL cholesterol	2.6 (16)	3.4 (21)	3.1 (19)	0.71
High blood pressure	88.9 (552)	87.3 (543)	85.1 (530)	0.13
High fasting plasma glucose	29.7 (184)	29.7 (183)	35.7 (222)	0.032
Intervention groups, % (*n*)				0.44
Mediterranean diet + EVOO	32.6 (203)	35.0 (218)	36.1 (225)	
Mediterrandean diet + nuts	36.8 (229)	34.5 (215)	31.8 (198)	
Control low-fat diet	30.5 (190)	30.5 (190)	32.1 (200)	
Energy intake, kcal/d	2368 (541)	2264 (527)	2336 (522)	0.002
Food consumption,[3] g/d				
Vegetables	336 ± 122	348 ± 120	343 ± 126	0.23
Fruits	383 ± 154	391 ± 135	397 ± 160	0.24
Legumes	22 ± 9	22 ± 10	23 ± 11	0.40
Meat	128 ± 43	125 ± 40	118 ± 42	<0.001
Fish	111 ± 39	105 ± 35	101 ± 37	<0.001
Cereals	238 ± 71	223 ± 60	210 ± 60	<0.001
Cookies	21 ± 24	21 ± 20	21 ± 22	0.94
Nuts	16 ± 14	16 ± 13	14 ± 13	0.015
Olive oil	46 ± 13	45 ± 13	43 ± 14	<0.001
Alcohol	14 ± 15	9 ± 10	6 ± 9	<0.001
Low-fat dairy	125 ± 88	270 ± 96	485 ± 191	<0.001
Whole-fat dairy	32 ± 58	51 ± 85	79 ± 161	<0.001
Total yogurt	41 ± 44	92 ± 61	122 ± 89	<0.001
Low-fat yogurt	29 ± 40	67 ± 60	95 ± 88	<0.001
Whole-fat yogurt	12 ± 25	25 ± 44	26 ± 51	<0.001
Cheese	27 ± 22	33 ± 23	37 ± 32	<0.001
Total milk	117 ± 82	229 ± 73	442 ± 131	<0.001
Low-fat milk	97 ± 85	203 ± 90	389 ± 176	<0.001
Whole milk	25 ± 68	30 ± 85	61 ± 170	<0.001

[1] Values are means ± SDs or medians (IQRs) unless otherwise indicated. Tertile cutoffs are based on the energy-adjusted mean of total dairy consumption during the follow-up. EVOO, extra virgin olive oil; MET, metabolic equivalent task; MetS, metabolic syndrome; P, percentile; T, tertile.
[2] *P* values for differences between tertiles were calculated by chi-square tests for categorical variables and ANOVA tests for continuous variables.
[3] All dietary variables were adjusted for energy.

cholesterol, 82.2% of 240 developed high blood pressure, and 41.4% of 1268 developed high fasting glucose concentration.

The median consumption during the follow-up of total dairy products in the whole study population was 363 g/d (IQR: 257, 525 g/d), low-fat dairy products being the largest contributors to total dairy consumption (72.5%). The median of consumption of milk, yogurt, and cheese was 207 g/d, 70 g/d, and 30 g/d, respectively.

The general characteristics of the study participants according to their mean consumption categories of total dairy products (tertiles) during the follow-up are shown in **Table 1**. Compared with those in the lowest tertile, participants in the top tertile were more likely to be older women and less likely to smoke, be physically active, and have lower serum concentrations of TGs and higher concentrations of HDL cholesterol. Participants in the highest tertile of dairy consumption also had lower total energy intake and consumed less red meat, fish, cereals, nuts, olive oil, and alcohol.

Consumption of total dairy products and incidence of MetS. The multivariable-adjusted HRs (95% CIs) for MetS incidence across tertiles of consumption of total dairy products are shown in **Table 2**. After adjusting for several potential confounders, subjects in the top tertile of low-fat dairy consumption, but not total dairy or high-fat dairy, had a lower risk of incident MetS [HR: 0.72; 95% CI: 0.61,0.86; *P*-trend = 0.001] compared with those in the bottom tertile.

Consumption of dairy product subtypes and incidence of MetS. The HRs of incident MetS across tertiles of consumption of specific subtypes of dairy products (yogurt, cheese, and milk) also adjusted for potential confounders are shown in **Table 3**. Among the subtypes of dairy products, consumers in tertile 3 of low-fat yogurt [HR: 0.73; 95% CI: 0.62, 0.86; *P*-trend = 0.004], whole-fat yogurt [HR: 0.78; 95% CI: 0.66, 0.92; *P*-trend = 0.003], and low-fat milk [HR: 0.80; 95% CI: 0.67, 0.95); *P*-trend = 0.007] had a lower risk of developing MetS than did participants in the lowest tertile of consumption. Compared with participants in the lowest tertile of consumption of cheese, those in the highest tertile had an increased risk of incident MetS [HR: 1.31; 95% CI: 1.10, 1.56; *P*-trend < 0.001]. No statistical interactions were found between the consumption of total dairy products or subtypes and sex or intervention group.

Yogurt consumption and MetS and its components. The multivariable-adjusted HR of each MetS component in subjects who were initially free of MetS in extreme categories of total, whole-fat, and low-fat yogurt consumption (tertile 3 vs. tertile 1) are shown in **Figure 1**. With the exception of high blood pressure, participants in the highest tertile of total yogurt consumption had a significantly lower risk of developing each of the MetS components than those in the lowest tertile. However, the linear trend was significant only for high fasting glucose (*P*-trend = 0.004). Compared with participants in the lowest tertile, participants in the highest tertile of whole-fat yogurt consumption had a lower risk of several components of MetS, including abdominal obesity [HR: 0.80; 95% CI: 0.65, 0.98; *P*-trend = 0.048], hypertriglyceridemia [HR: 0.74; 95% CI: 0.64, 0.86; *P*-trend < 0.001], low HDL cholesterol [HR: 0.73; 95% CI: 0.63, 0.85; *P*-trend < 0.001], high blood pressure [HR: 0.62; 95% CI: 0.44, 0.86; *P*-trend = 0.001], and high fasting plasma glucose [HR: 0.80; 95% CI: 0.66, 0.94; *P*-trend = 0.005]. The associations with low-fat yogurt were in the same direction as those with total and whole-fat yogurt, but inverse associations were limited to hypertriglyceridemia [HR: 0.73; 95% CI: 0.63, 0.85; *P*-trend = 0.18], low HDL cholesterol [HR: 0.76; 95% CI: 0.66, 0.88; *P*-trend = 0.35], and high fasting plasma glucose [HR: 0.81; 95% CI: 0.68, 0.96; *P*-trend = 0.004].

TABLE 2 HRs (95% CIs) of MetS incidence across energy-adjusted tertiles of consumption of total, low-fat, and whole-fat dairy products in elderly individuals at high cardiovascular disease risk[1]

	Total dairy consumption, g/d			
	T1	T2	T3	*P*-trend
Total dairy consumption, median (P25, P75)	207 (142, 250)	354 (322, 393)	577 (518, 661)	
MetS incidence, *n* (%)	319 (51.3)	293 (47.0)	318 (51.0)	0.24
Crude model	1.00 (ref.)	0.84 (0.72, 0.99)	1.02 (0.87, 1.20)	0.60
Multivariate model 1	1.00 (ref.)	0.82 (0.70, 0.97)	0.93 (0.78, 1.10)	0.54
Multivariate model 2	1.00 (ref.)	0.83 (0.70, 0.98)	0.89 (0.73, 1.07)	0.30
Multivariate model 3	1.00 (ref.)	0.80 (0.68, 0.95)	0.83 (0.69, 1.01)	0.11
Whole-fat dairy, median (P25, P75)	0	18 (12, 25)	94 (53, 179)	
MetS incidence, *n* (%)	327 (47.4)	289 (46.4)	314 (50.4)	0.09
Crude model	1.00 (ref.)	0.88 (0.75, 1.03)	0.95 (0.81, 1.11)	0.85
Multivariate model 1	1.00 (ref.)	0.82 (0.69, 0.96)	0.93 (0.80, 1.09)	0.88
Multivariate model 2	1.00 (ref.)	0.90 (0.76, 1.06)	0.96 (0.81, 1.13)	0.87
Multivariate model 3	1.00 (ref.)	0.92 (0.78, 1.10)	0.99 (0.84, 1.16)	0.92
Low-fat dairy, median (P25, P75)	87 (7, 163)	263 (227, 316)	503 (429, 587)	
MetS incidence, *n* (%)	325 (52.3)	310 (49.8)	295 (47.4)	0.22
Crude model	1.00 (ref.)	0.90 (0.77, 1.06)	0.87 (0.74, 1.02)	0.18
Multivariate model 1	1.00 (ref.)	0.87 (0.74, 1.02)	0.79 (0.67, 0.93)	0.005
Multivariate model 2	1.00 (ref.)	0.90 (0.76, 1.05)	0.78 (0.66, 0.93)	0.005
Multivariate model 3	1.00 (ref.)	0.88 (0.75, 1.03)	0.72 (0.61, 0.86)	0.001

[1] Tertile cutoffs are based on energy-adjusted mean total, low-fat, or whole-fat dairy consumption during the follow-up. Cox regression model 1 adjusted for intervention group; sex; age (year); leisure time physical activity (metabolic equivalent task · day); BMI (kilograms per meter squared); current smoker (yes/no); former smoker (yes/no); and use of hypoglycemic, hypolipidemic, antihypertensive, and insulin treatment at baseline. Cox regression model 2 additionally adjusted for mean consumption during the follow-up of vegetables, fruit, legumes, cereals, fish, red meat, cookies, olive oil, and nuts (all grams per day), as well as alcohol (grams per day and quadratic term). Cox regression model 3 additionally adjusted for prevalence of MetS components at baseline, including abdominal obesity (yes/no), hypertriglyceridemia (yes/no), low HDL cholesterol (yes/no), hypertension (yes/no), and high fasting plasma glucose (yes/no). All models were stratified by recruitment center. MetS, metabolic syndrome; P, percentile; ref., reference; T, tertile.

TABLE 3 HRs (95% CIs) of MetS incidence across energy-adjusted tertiles of consumption of specific dairy products (yogurt, cheese, and milk) in elderly individuals at high cardiovascular disease risk[1]

	Tertiles of specific dairy consumption			
	T1	T2	T3	*P*-trend
Total yogurt,[2] g/d	7 (1, 24)	70 (54,94)	127 (125, 189)	
MetS incidence, *n* (%)	318 (51.1)	283 (45.4)	329 (52.8)	
Crude model	1.00 (ref.)	0.82 (0.69, 0.97)	1.10 (0.95, 1.29)	0.26
Multivariate model 1	1.00 (ref.)	0.81 (0.69, 0.96)	1.10 (0.93, 1.29)	0.31
Multivariate model 2	1.00 (ref.)	0.88 (0.74, 1.03)	0.75 (0.64, 0.89)	0.15
Multivariate model 3	1.00 (ref.)	0.88 (0.74, 1.04)	0.77 (0.65, 0.91)	0.14
Low-fat yogurt,[2] g/d	1 (0, 5)	46 (27, 60)	124 (107, 159)	
MetS incidence, *n* (%)	366 (58.8)	260 (41.7)	304 (48.8)	<0.001
Crude model	1.00 (ref.)	0.56 (0.47, 0.65)	0.74 (0.64, 0.87)	0.004
Multivariate model 1	1.00 (ref.)	0.53 (0.47, 0.64)	0.72 (0.62, 0.85)	0.002
Multivariate model 2	1.00 (ref.)	0.57 (0.49, 0.67)	0.76 (0.65, 0.90)	0.016
Multivariate model 3	1.00 (ref.)	0.56 (0.47, 0.66)	0.73 (0.62, 0.86)	0.004
Whole-fat yogurt,[2] g/d	0	6 (4, 9)	46 (24, 78)	
MetS incidence, *n* (%)	346 (55.6)	310 (49.8)	274 (44.0)	<0.001
Crude model	1.00 (ref.)	0.88 (0.75, 1.03)	0.71 (0.61, 0.84)	<0.001
Multivariate model 1	1.00 (ref.)	0.83 (0.71, 0.98)	0.71 (0.60, 0.83)	<0.001
Multivariate model 2	1.00 (ref.)	0.91 (0.77, 1.07)	0.74 (0.63, 0.87)	<0.001
Multivariate model 3	1.00 (ref.)	0.93 (0.79, 1.10)	0.78 (0.66, 0.92)	0.003
Cheese,[2] g/d	11 (6, 15)	28 (23, 33)	51 (44, 66)	
MetS incidence, *n* (%)	293 (47.1)	280 (44.9)	357 (57.3)	
Crude model	1.00 (ref.)	0.94 (0.79, 1.11)	1.41 (1.20, 1.66)	<0.001
Multivariate model 1	1.00 (ref.)	0.90 (0.76, 1.07)	1.29 (1.10, 1.52)	0.001
Multivariate model 2	1.00 (ref.)	0.94 (0.79, 1.12)	1.34 (1.13, 1.58)	<0.001
Multivariate model 3	1.00 (ref.)	0.93 (0.79, 1.11)	1.31 (1.10, 1.56)	<0.001
Total milk,[2] g/d	120 (35, 162)	222 (205, 250)	462 (380, 504)	
MetS incidence, *n* (%)	313 (50.3)	303 (48.6)	314 (50.4)	0.78
Crude model	1.00 (ref.)	0.97 (0.83, 1.14)	1.04 (0.88, 1.22)	0.57
Multivariate model 1	1.00 (ref.)	0.90 (0.76, 1.06)	0.93 (0.78, 1.10)	0.58
Multivariate model 2	1.00 (ref.)	0.93 (0.79, 1.10)	0.90 (0.75, 1.78)	0.29
Multivariate model 3	1.00 (ref.)	0.90 (0.77, 1.07)	0.85 (0.70, 1.02)	0.11
Low-fat milk,[2] g/d	28 (0, 107)	204 (193, 216)	433 (345, 499)	
MetS incidence, *n* (%)	306 (49.2)	331 (53.1)	293 (47.0)	0.09
Crude model	1.00 (ref.)	1.17 (1.00, 1.37)	0.94 (0.80, 1.11)	0.38
Multivariate model 1	1.00 (ref.)	1.14 (0.98, 1.34)	0.86 (0.73, 1.02)	0.06
Multivariate model 2	1.00 (ref.)	1.16 (0.99, 1.37)	0.85 (0.71, 1.01)	0.040
Multivariate model 3	1.00 (ref.)	1.16 (0.98, 1.36)	0.80 (0.67, 0.95)	0.007
Whole-fat milk,[2] g/d	0	5 (2, 8)	31 (18, 136)	
MetS incidence, *n* (%)	314 (50.5)	288 (462)	328 (52.6)	0.07
Crude model	1.00 (ref.)	0.92 (0.78, 1.08)	1.12 (0.96, 1.31)	0.08
Multivariate model 1	1.00 (ref.)	0.89 (0.75, 1.05)	1.02 (0.87, 1.21)	0.50
Multivariate model 2	1.00 (ref.)	1.00 (0.85, 1.19)	1.10 (0.93, 1.30)	0.21
Multivariate model 3	1.00 (ref.)	1.02 (0.86, 1.21)	1.12 (0.95, 1.33)	0.16

[1] The MetS components were defined with updated harmonizing criteria. Tertile cutoffs are based on energy-adjusted mean dairy product consumption during the follow-up. Cox regression model 1 adjusted for intervention group; sex; age (year); leisure time physical activity (metabolic equivalent task · day); BMI (kilograms per meter squared); current smoker (yes/no); former smoker (yes/no); and use of hypoglycemic, hypolipidemic, antihypertensive, and insulin treatment at baseline. Cox regression model 2 additionally adjusted for mean consumption during the follow-up of vegetables, fruit, legumes, cereals, fish, red meat, cookies, olive oil, and nuts (all grams per day), as well as alcohol (grams per day and quadratic term). Cox regression model 3 additionally adjusted for prevalence of MetS components at baseline, including abdominal obesity (yes/no), hypertriglyceridemia (yes/no), low HDL cholesterol (yes/no), hypertension (yes/no), and high fasting plasma glucose (yes/no). All models were stratified by recruitment center. MetS, metabolic syndrome; ref., reference; T, tertile.
[2] Values are medians; 25th, 75th percentiles in parentheses.

Consumption of dairy products, dairy product subtypes, and MetS components. HRs for the components of incident MetS across tertiles of consumption of total dairy products and subtypes are shown in **Supplemental Table 1.** An increased consumption of total milk and low-fat milk was significantly associated with a lower incidence of low HDL cholesterol and high fasting glucose. Low-fat dairy consumption was inversely associated with high fasting glucose, hypertriglyceridemia, and low HDL cholesterol (Supplemental Table 1).

Consumption of dairy products, subtypes of dairy products, and incidence of MetS based on the number of MetS components at baseline. A sensitivity analysis based on the number of MetS components at baseline found that in those

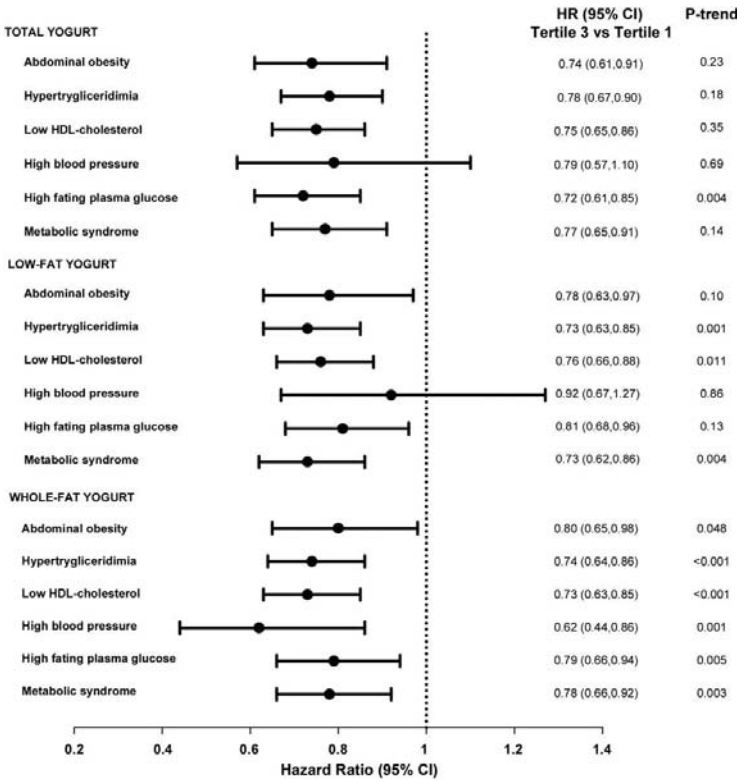

FIGURE 1 HRs (95% CIs) of metabolic syndrome and its components [abdominal obesity (n = 1386), hypertriglyceridemia (n = 3539), low HDL cholesterol (n = 3745), high blood pressure (n = 337), and high fasting plasma glucose (n = 1844)], comparing tertile 3 and tertile 1 of yogurt consumption in elderly individuals at high cardiovascular disease risk. Tertile cutoffs are based on energy-adjusted mean yogurt consumption during the follow-up. Cox regression models adjusted for intervention group; sex; age (years); leisure time physical activity (metabolic equivalent task · day); BMI (kilograms per meter squared); current smoker (yes/no); former smoker (yes/no); and use of hypoglycemic, hypolipidemic, antihypertensive, and insulin treatment at baseline plus mean consumption during follow-up of vegetables, fruit, legumes, cereals, fish, red meat, cookies, olive oil, and nuts (all grams per day), as well as alcohol (grams per day and quadratic term). All models were stratified by recruitment center.

individuals who had only one component or none at all, there was no significant association between total dairy or its subtypes and MetS incidence, except for cheese (P-trend < 0.05). In those individuals with 2 MetS components at baseline, there were still significant inverse associations between low-fat dairy, low-fat yogurt, and whole-fat yogurt consumption and MetS incidence. A positive association was observed between cheese consumption and MetS development (**Supplemental Table 2**).

Discussion

In this longitudinal assessment of the PREDIMED cohort, an older Mediterranean population at high CVD risk, we evaluated the consumption of total and specific dairy products in relation to the risk of developing MetS. The results show that the consumption of low-fat dairy products, yogurt (total, low-fat, and whole yogurt), and low-fat milk is associated with a lower incidence of MetS. These results remained even after we used the Benjamini-Hochberg method to correct P values for multiple comparisons. The association between total dairy consumption and MetS remained in the same direction, although it was not significant (P = 0.11). In contrast, increased consumption of total cheese was directly associated with a higher risk of MetS. Likewise, increased consumption of whole yogurt was also inversely associated with all MetS components, whereas consumption of low-fat yogurt related inversely to high TGs, low HDL cholesterol, and elevated fasting glucose.

Our results are in line with those of other prospective studies showing an inverse association between total dairy product consumption and MetS (5, 16, 17). The results of other cross-sectional (19, 34) and prospective studies (20, 21), however, are not fully consistent. These mixed results can be partially explained by the heterogeneity of dairy products included in the total dairy category. Further reasons for discordant results could relate to the design of the studies, because, unlike in the present study, most previous studies did not use repeated measurements of consumption, and to inherent differences in the characteristics of the population studied.

Our results for the type of product and fat content are discordant with those reported by the prospective CARDIA (Coronary Artery Risk Development in Young Adults) study (5), in which an inverse association between the consumption of whole-fat dairy products and cheese and MetS was observed in individuals above 18 y of age. We observed a direct association between the consumption of cheese and incident MetS. Unlike in our findings, individuals in the Blue Mountain Eyes Study (20) who consumed more whole-fat dairy or low-fat dairy products showed a decreased or an increased risk of developing MetS, respectively. The population in our study consisted of older individuals at high CVD risk, whereas the study subjects were younger in both the CARDIA and the Blue Mountain Eyes Studies. This may partly explain the contradictory results. It should be pointed out that only the CARDIA study analyzed the associations between MetS and dairy product subtypes, although

they were classified differently from in our study. This may help explain the divergent results.

In support of our findings, the protective role of yogurt consumption on MetS has been noted previously in cross-sectional studies (15, 19), but the prospective CARDIA study (5) found no association between yogurt consumption and MetS development.

Numerous biological mechanisms may mediate the relation between dairy consumption and risk of MetS.

Dairy products are an important source of calcium. The calcium in milk products interacts with SFAs to form calcium–FA soaps, thereby increasing fecal fat excretion (35) and, thus, improving the HDL-to-LDL cholesterol ratio. Lorenzen et al. (36) also showed that, unlike calcium from supplements, calcium from milk and low-fat yogurt reduced the TG content of chylomicrons postprandially (36). Intervention studies have also shown that calcium intake decreases blood pressure (37, 38), and that milk-derived bioactive peptides have antihypertensive properties (39). Milk-derived bioactive peptides increasingly have been shown to play an important role in preventing MetS by regulating insulinemia, blood pressure, dyslipidemia, and central fat accumulation (39–41). Nutrients from dairy products may act synergistically on metabolic pathways that have a beneficial impact on MetS. It has been reported that insulin concentrations are lower in those subjects consuming diets high in dairy products than in subjects consuming diets low in dairy products (41), which suggests that calcium or other nutrients that make up dairy products have beneficial effects on glucose metabolism. Although some studies (40) have suggested that there are beneficial associations between dairy consumption and body weight or body composition, clinical trials data are not supportive (42). Recently, however, a high consumption of total and whole-fat yogurt was associated with a lower risk of being overweight/obese (43). It has also been suggested that probiotics from yogurt beneficially influence the inflammatory/anti-inflammatory balance of the microbiota, which might mediate the lower risk of presenting overweight/obesity (44).

In our study, whole-milk yogurt protected against all MetS components. Although nutritionally yogurt is comparable to milk, added ingredients and fermentation may improve its nutritional value (45) and provide it with unique properties that enhance the bioavailability of some nutrients (46, 47). As far as fat is concerned, dairy products contain mostly SFAs, in addition to high proportions of oleic, stearic, rumenic and trans-palmitoleic acids. The results of recent meta-analyses have questioned the role of SFAs on CVD risk (48). Likewise, there is meta-analytical evidence that a high intake of total dairy products and most dairy subtypes do not increase the risk of CVD (49). In our study, cheese was directly associated with an increased risk of MetS, which may be partly explained by the fact that it is rich in sodium and has a higher energy density and phosphorus content than other dairy products (19).

In elderly individuals, MetS is an important health problem with potentially greater repercussions on health than in other population groups (22). According to our results, dairy products are a food group with a high nutritional value that could prevent MetS development. Consequently, the consumption of this food group may be promoted in elderly individuals in order to try to attempt to reduce the incidence of this disease.

Our study has several strengths, including the use of yearly measurements of diet, a relatively long follow-up period, the analysis of dairy subtypes with different fat contents, and adjustment for a large number of potential confounders for which multiple testing corrections minimized small differences among individuals and potential confounders.

The study also has limitations. First, incident MetS was a secondary endpoint of the PREDIMED trial, which make our analyses exploratory in nature. Second, our cohort was made up of elderly participants at high risk of CVD; thus, our findings cannot be generalized to other populations. Third, although diet was assessed by a validated FFQ, potential measurement errors are unavoidable. Nevertheless, to minimize the random measurement error caused by within-person variation and dietary changes during follow-up, we calculated the mean of consumption during the follow-up for dietary variables to better represent long-term habitual dietary consumption when these associations were explored (50). The present study suggests that consumption of low-fat dairy products, all types of yogurt, and low-fat milk is associated with a lower incidence of MetS in older individuals at high CVD risk. Furthermore, increased consumption of whole-fat yogurt is associated with a lower incidence of all MetS components. Conversely, cheese consumption is associated with an increased risk of MetS development.

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Online supporting material

Supplemental Table 1. Hazard ratios (95% CI)[1] of MetS components[2] across energy-adjusted tertiles of specific dairy consumption[3] in elderly individuals at high cardiovascular risk

	T1	T2	T3	*P*-trend
Total dairy				
Abdominal obesity	1.00 ref.	0.98 (0.79-1.21)	1.06 (0.83-1.36)	0.55
Hypertriglyceridemia	1.00 ref.	0.70 (0-60-0.81)	0.92 (0.78-1.08)	0.60
Low HDL-cholesterol	1.00 ref.	0.79 (0.68 -0.91)	0.87 (0.74-1.03)	0.19
High blood pressure	1.00 ref.	0.86 (0.62-1.19)	0.93 (0.65-1.33)	0.71
High fasting plasma glucose	1.00 ref.	0.88 (0.73-1.04)	0.97 (0.80-1.18)	0.94
Low-fat dairy				
Abdominal obesity	1.00 ref.	1.26 (1.04-1.56)	1.01 (0.81-1.26)	0.92
Hypertriglyceridemia	1.00 ref.	0.78 (0.68-0.91)	0.84 (0.73-0.98)	0.034
Low HDL-cholesterol	1.00 ref.	0.98 (0.85-1.13)	0.85 (0.73-0.99)	0.029
Hypertension	1.00 ref.	0.90 (0.65-1.25)	0.87 (0.63-1.20)	0.41
High fasting plasma glucose	1.00 ref.	0.88 (0.74-1.04)	0.82 (0.69-0.98)	0.033
Whole-fat dairy				
Abdominal obesity	1.00 ref.	0.90 (0.70-1.06)	0.90 (0.73-1.11)	0.59
Hypertriglyceridemia	1.00 ref.	0.81 (0.70-0.94)	0.85 (0.74-0.99)	0.16
Low HDL-cholesterol	1.00 ref.	0.88 (0.77-1.02)	0.87 (0.75-1.01)	0.14
High blood pressure	1.00 ref.	0.94 (0.68-1.32)	0.85 (0.61-1.19)	0.34
High fasting plasma glucose	1.00 ref.	0.89 (0.74-1.07)	1.05 (0.89-1.25)	0.28
Cheese				
Abdominal obesity	1.00 ref.	0.77 (0.62-0.95)	0.95 (0.77-1.18)	0.97
Hypertriglyceridemia	1.00 ref.	0.81 (0.70-0.94)	0.88 (0.76-1.03)	0.16
Low HDL-cholesterol	1.00 ref.	0.86 (0.74-1.00)	0.92 (0.79-1.07)	0.36
High blood pressure	1.00 ref.	0.76 (0.56-1.04)	0.95 (0.68-1.34)	0.86
High fasting plasma glucose	1.00 ref.	0.95 (0.80-1.14)	1.04 (0.87-1.25)	0.61
Total milk				
Abdominal obesity	1.00 ref.	1.02 (0.83-1.26)	1.08 (0.86-1.36)	0.49
Hypertriglyceridemia	1.00 ref.	0.79 (0.68-0.92)	0.92 (0.79-1.08)	0.77
Low HDL-cholesterol	1.00 ref.	0.92 (0.79-1.06)	0.84 (0.72-0.98)	0.035
High blood pressure	1.00 ref.	0.84 (0.60-1.18)	0.81 (0.57-1.15)	0.30
High fasting plasma glucose	1.00 ref.	1.01 (0.85-1.20)	0.84 (0.69-1.01)	0.039
Low-fat milk				
Abdominal obesity	1.00 ref.	1.11 (0.89-1.33)	0.96 (0.78-1.18)	0.66
Hypertriglyceridemia	1.00 ref.	1.00 (0.86-1.15)	0.91 (0.79-1.06)	0.22
Low HDL-cholesterol	1.00 ref.	1.14 (0.99-1.31)	0.81 (0.70-0.95)	0.007
High blood pressure	1.00 ref.	1.20 (0.87-1.65)	0.78 (0.56-1.08)	0.08
High fasting plasma glucose	1.00 ref.	1.13 (0.96-1.33)	0.79 (0.66-0.94)	0.009
Whole-fat milk				
Abdominal obesity	1.00 ref.	0.87 (0.71-1.08)	0.97 (0.78-1.19)	0.91
Hypertriglyceridemia	1.00 ref.	0.78 (0.67-0.90)	0.91 (0.79-1.06)	0.95
Low HDL-cholesterol	1.00 ref.	0.81 (0.69-.93)	0.90 (0.78-1.05)	0.66
High blood pressure	1.00 ref.	0.92 (0.66-1.30)	1.01 (0.72-1.42)	0.16
High fasting plasma glucose	1.00 ref.	0.88 (0.73-1.05)	1.16 (0.97-1.38)	0.028

Abbreviations: CI, confidence interval, MetS, metabolic syndrome; T, tertile.

[1] Cox regression models adjusted for intervention group, sex, age (year), leisure time physical activity (MET-day), BMI (kg/m^2), current smoker (yes/no), former smoker (yes/no) and use of hypoglycemic, hypolipidemic, antihypertensive and insulin treatment at baseline, and mean consumption of vegetables (g/d), fruit (g/d), legumes (g/d), cereals (g/d), fish (g/d), red meat (g/d), alcohol (g/d and quadratic term), biscuits (g/d), olive oil (g/d) and nuts (g/d) during the follow-up. All models were stratified by recruitment center.

[2] The MetS components (abdominal obesity, hypertriglyceridemia, low HDL-cholesterol, high blood pressure and high fasting plasma glucose) were defined according to updated harmonizing criteria.

[3] Tertile cut-offs are based on mean energy-adjusted dairy product consumption during the follow-up.

Online Supporting Material

Supplemental table 2. Hazard ratios[1] (95%CI) of metabolic syndrome incidence across energy-adjusted tertiles of total dairy consumption and its different subtypes, based on the number of metabolic syndrome components at baseline, in individuals at high cardiovascular risk.

	T3 vs T1[2]	
	0-1 MetS components n=512	**2 MetS components n=1356**
Total dairy	0.78 (0.48,1.27)	0.96 (0.78,1.18)
Low-fat dairy	0.77 (0.50,1.20)	0.77 (0.64,0.93)†
Whole-fat dairy	0.84 (0.61,1.45)	0.99 (0.83,1.18)
Total yogurt	0.89 (0.59,1.35)	0.77 (0.64,0.92)
Low-fat yogurt	0.79 (0.53,1.19)	0.73 (0.61,0.87)†
Whole-fat yogurt	0.77 (0.49,1.20)	0.77 (0.65,0.93)†
Cheese	1.79 (0.13,2.85)*	1.31 (1.09,1.57)*
Total milk	0.99 (0.63,1.54)	0.91 (0.74,1.10)
Low-fat milk	0.92 (0.60,1.43)	0.86 (0.71,1.04)
Whole-fat milk	1.06 (0.69,1.61)	1.12 (0.93,1.34)

[1]Cox regression model adjusted for intervention group, sex, age (year), leisure time physical activity (MET-day), BMI (kg/m^2), current smoker (yes/no), former smoker (yes/no), use of hypoglycemic, hypolipidemic, antihypertensive and insulin treatment at baseline, mean consumption during the follow-up of vegetables (g/d), fruit (g/d), legumes (g/d), cereals (g/d), fish (g/d), red meat (g/d), alcohol (g/d and quadratic term), biscuits (g/d), olive oil (g/d) and nuts (g/d). Stratified by recruitment center.
[2]Tertile cut-offs are based on energy-adjusted mean during the follow-up.
Abbreviations: T, Tertile
*P-trend <0.05
†P-trend <0.01

Online Supporting Material

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2. Replacing red meat and processed red meat for white meat, fish, legumes or eggs is associated with lower risk of incidence of metabolic syndrome.

Becerra-Tomás N, Babio N, Martínez-González MÁ, Corella D, Estruch R, Ros E, Fitó M, Serra-Majem L, Salaverria I, Lamuela-Raventós RM, Lapetra J, Gómez-Gracia E, Fiol M, Toledo E, Sorlí JV, Pedret-Llaberia MR, Salas-Salvadó J.

Key teaching points:

- Few prospective cohort studies have analyzed the association between red meat and processed red meat consumption and the risk of MetS showing inconsistent results.

- Previous results from the PREDIMED Reus-Tarragona center showed an increased risk of MetS with high consumption of red meat and processed red meat.

- The present study has gone beyond and has analyzed the association between meat (total meat, red meat, processed red meat and poultry) consumption and the risk of MetS incidence in the full PREDIMED cohort. Besides, the replacement effect of red meat and processed meat with other protein-rich foods on MetS risk was also analyzed.

- Total meat, red meat and processed red meat consumption was positively associated with a higher risk of MetS incidence, whereas poultry consumption was inversely associated.

- The replacement or red meat and processed red meat with other protein-rich foods were associated with a lower risk of MetS incidence.

UNIVERSITAT ROVIRA I VIRGILI
DIETARY FACTORS ASSOCIATED WITH METABOLIC SYNDROME AND TYPE 2 DIABETES RISK
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Original article

Replacing red meat and processed red meat for white meat, fish, legumes or eggs is associated with lower risk of incidence of metabolic syndrome

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SUMMARY

Background & aims: Few studies have assessed the association between consumption of red meat (RM) and processed red meats (PRM) and the incidence of metabolic syndrome (MetS) and results have been inconsistent. We investigated associations between total consumption of meat and its subtypes and incident MetS and estimated the effect of substituting RM or PRM for alternative protein-rich foods.
Methods: We analyzed 1868 participants (55–80 years-old) recruited into the PREDIMED study who had no MetS at baseline and were followed for a median of 3.2 years. MetS was defined using updated harmonized criteria. Anthropometric variables, dietary habits, and blood biochemistry were determined at baseline and yearly thereafter. Multivariable-adjusted hazard ratios (HRs) of MetS were estimated for the two upper tertiles (versus the lowest one) of mean consumption of meat and its subtypes during the follow-up as exposure.
Results: Comparing the highest vs the lowest tertile of consumption, we observed an increased risk of MetS incidence, with HRs of 1.23 (95% confidence interval [CI]: 1.03–1.45) and 1.46 (CI: 1.22–1.74) for total meat and pooled RM and PRM, respectively. Compared with participants in the lowest tertile, those in the highest tertile of poultry and rabbit consumption had a lower risk of MetS incidence. The risk of MetS was lower when one-serving/day of RM or PRM was replaced by legumes, poultry and rabbit, fish or eggs.

Abbreviations: CVD, cardiovascular disease; ICC, intra-class correlation coefficient; FFQ, food frequency questionnaire; HR, hazards ratios; MedDiet, Mediterranean diet; MetS, metabolic syndrome; PRM, processed red meat; RM, red meat; RM&PRM, red meat and processed red meat; SFAs, saturated fatty acids.

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Conclusion: RM and PRM consumption was associated with higher risk of MetS. Replacing RM or PRM with other protein-rich foods related to a lower risk of MetS and should, therefore, be encouraged. This trial was registered at controlled-trials.com as ISRCTN35739639.

1. Introduction

Metabolic syndrome (MetS) is a cluster of metabolic disorders associated with abdominal obesity that is associated with an increased risk of cardiovascular disease (CVD) and diabetes [1]. It has been suggested that adherence to the Mediterranean diet (MedDiet) and a healthy lifestyle are cornerstones in the prevention and treatment of MetS [2]. On the other hand, a Western dietary pattern, characterized by a high consumption of red meat, processed meat, butter and margarine and refined grain has been associated with an increased prevalence and incidence [3] of MetS.

Some studies have reported a positive association between meat consumption – mainly red meat and processed meat – and hypertension [4], abdominal obesity [5], and type 2 diabetes [6,7], all of which are MetS components. Cross-sectional [8–12] and prospective studies [3,9,13] have examined the association between red meat consumption and MetS, with controversial results. To our knowledge only three prospective studies have analyzed the association between red meat consumption and MetS [3,9,13]. In the Atherosclerosis Risk in Communities study, a direct association was observed between meat consumption (hamburger, hot dogs, processed meats, bacon, meat sandwiches or mixed dishes, meat as a main dish) and MetS incidence in middle-aged women and men [3]. Along the same lines, in a study limited to one of the centers of the PREDIMED trial we found an increased risk of MetS development in those individuals in the highest baseline quartile of red meat and processed red meat consumption compared to those in the first quartile after one year of follow-up [9]. Finally, in a cohort of Japanese ancestry a 4.7-fold increased risk of developing MetS was observed in those individuals in the top tertile of red meat consumption compared to those in the lower tertile, although the relationship was lost after adjustment for saturated fatty acid intake [13]. As far as we know, only two previous studies related exposure to poultry consumption with MetS prevalence [12] or incidence [13] and reported no associations.

In the present analysis we provide the results obtained in the full cohort of the PREDIMED study, a nutritional intervention trial for the primary prevention of cardiovascular disease [14] for the associations between total meat and specific types of meat consumption (especially red meat and processed red meat) and the incidence of MetS during the total study follow-up. We also estimated the effects on MetS incidence of replacing red meat and processed red meat with alternative protein-rich foods.

2. Material and methods

2.1. Study design and participants

This study is a secondary analysis of a previously published randomized clinical trial, the PREDIMED (PREvención con DIetaMEDiterránea, www.predimed.es) study. Briefly, PREDIMED is a randomized, multicentre, parallel-group field trial that was conducted in Spain between October 2003 and December 2010 to assess the effectiveness of the MedDiet on the primary prevention of CVD. The protocol and design have been described elsewhere [14]. The trial was registered at http://www.controlledtrials.com/ISRCTN35739639 and included 7444 men and women (aged 55–80 and 60–80 years, respectively), without previously documented cardiovascular disease. Participants were eligible if they had either type 2 diabetes or at least three of the following cardiovascular risk factors: hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or on antihypertensive medication), high plasma LDL-cholesterol (≥160 mg/dL), low plasma HDL-cholesterol (<40 mg/dL in men; <50 mg/dLin women), overweight or obesity (BMI ≥ 25 kg/m^2), current smoking, or a family history of premature coronary heart disease. Participants were randomized to one of three intervention groups: a MedDiet supplemented with 1 L/week of extra-virgin olive oil, a MedDiet supplemented with 30 g/day of mixed nuts, or a control diet (advice to follow a low fat-diet). The main results in relation to cardiovascular events have been published [15].

In the present report, data were analyzed considering the PREDIMED study as a observational cohort. We selected participants from all the PREDIMED recruiting centers with biochemical determinations available for at least 2 years of follow-up ($n = 5081$).

Because our main aim was to explore the associations between different types of meat consumption and the risk of MetS development, we excluded participants with MetSat baseline ($n = 3707$). We also excluded participants who had not completed a baseline food frequency questionnaire (FFQ) and those who reported total energy intake values outside the pre-specified limits (500–3500 kcal/d in women and 800–4000 kcal/d in men). Finally, 2094 individuals were available for evaluation. The protocol was approved by the institutional review boards of each recruitment center and all participants provided written informed consent.

2.2. Dietary assessment

Dietary intake was evaluated at baseline and yearly during follow-up using a previously validated FFQ [16]. The reproducibility of the FFQ used in the PREDIMED study for food groups, and energy and nutrient intake, explored by the Pearson correlation coefficient (r), ranged from 0.50 to 0.82, and the intra-class correlation coefficient (ICC) ranged from 0.63 to 0.90. The validity indices of the FFQ in relation to the dietary records for food groups, nutrient and energy intake ranged (r) from 0.24 to 0.72, while the ICC ranged from 0.40 to 0.84. The ICC was 0.75 for total meat/meat products, 0.59 for fish or seafood, 0.40 for legumes, and 0.58 for eggs. Information about meat consumption was assessed using 13 items included in the FFQ. Energy and nutrient intake were estimated using Spanish food composition tables [15].

Trained dieticians asked the participants about the frequency with which they consumed red meat, poultry or rabbit, processed meat products, fish, eggs and legumes: never, one to three times per month, once per week, two to four times per week, five to six times per week, once per day, two to three times per day, four to six times per day or more than six times per day. The responses were transformed to grams per day and then categorized into red meat (RM) including pork, veal, beef and lamb; processed red meat (PRM) including offal, ham, sausages, pâté, hamburgers and bacon. Red meat and processed red meat were merged into one category (RM&PRM) and poultry and rabbit, into another category, including

chicken, turkey and rabbit, while total meat included all of the above categories. All dietary variables at baseline and yearly during the follow-up were adjusted for total energy intake using the residuals method [17].

2.3. Ascertainment of metabolic syndrome

The primary endpoint of the PREDIMED trial was a composite of major cardiovascular clinical events (non-fatal myocardial infarction, non-fatal stroke or cardiovascular death). For the present study, we considered MetS incidence and its components to be the outcome. The definition of MetS we used was in accordance with the updated harmonized criteria of the International Diabetes Federation and the American Heart Association/National Heart, Lung, and Blood Institute [1]. Individuals were diagnosed with MetS if they had three or more of the following components: elevated waist circumference for European individuals ($\geq$88 cm in women and $\geq$102 cm in men), hypertriglyceridemia (>150 mg/dl) or drug treatment for elevated triglycerides, low concentrations of HDL-cholesterol (<50 mg/dl and <40 mg/dL in women and men, respectively) or drug treatment for low HDL-cholesterol, elevated blood pressure (systolic $\geq$130 mmHg and/or diastolic $\geq$ 85 mmHg) or taking antihypertensive medication; and high fasting plasma glucose ($\geq$100 mg/dl) or drug treatment for hyperglycemia.

2.4. Assessment of covariates

At baseline and yearly during follow-up, participants completed a 47-item questionnaire about lifestyle variables, medical history and medication use; a validated Spanish version of the Minnesota Leisure Time Physical Activity Questionnaire [18]; a 14-item validated questionnaire designed to assess adherence to the MedDiet [19]; and a validated semi-quantitative FFQ with 137items [16].

Trained personnel measured height in centimeters, weight in kilograms, and waist circumference by standard methods and blood pressure in triplicate with a 5-min interval between each measurement by using a validated oscillometer (Omron HEM705CP, Hoofddorp, The Netherlands). BMI was calculated by dividing weight in kilograms by the square of height in meters.

Fasting blood samples were collected from all participants. Total cholesterol, triglycerides and glucose concentrations were measured using standard methods. HDL-cholesterol was determined after precipitation with phosphotungstic acid and magnesium chloride. The laboratory technicians were blinded to the intervention group.

2.5. Statistical analyses

To take advantage of the yearly dietary assessments, we averaged the meat consumption from baseline to the end of the follow-up or from baseline to the last follow-up FFQ before the occurrence of MetS (if it ever occurred) as the relevant exposure. Because participants who developed MetS during follow-up might have changed their dietary habits after the diagnosis of MetS, their average consumption was calculated from baseline to the year before MetS diagnosis. Then, participants were categorized into tertiles of average daily consumption of total meat and its different subtypes during follow-up. The baseline characteristics of the study population are expressed as percentages and numbers for categorical variables and mean ± SD or median (IQR) for continuous variables. The Chi-square and one-way ANOVA tests were used to appraise differences in the baseline characteristics according to tertiles of the average energy-adjusted daily consumption of total meat. Multivariable Cox regression models were fitted to assess the hazards ratios (HR) of incident MetS and its components during follow-up for tertiles of total meat, RM, RM&PRM, PRM, and poultry and rabbit. The Cox regression models were adjusted for several potential confounders. Model 1 was adjusted for intervention group, sex, age, leisure time physical activity (METs/min-day), BMI (kg/m^2), smoking (current, former or never) at baseline; model 2 was additionally adjusted for quintiles of daily average consumption (g/d) during follow-up of vegetables, fruit, legumes, cereals, fish, dairy products, biscuits, olive oil, nuts and alcohol(continuous and adding the quadratic term); and model 3 was additionally adjusted for the prevalence of MetS components at baseline: abdominal obesity (yes/no), hypertriglyceridemia (yes/no), low HDL-cholesterol (yes/no), high blood pressure (yes/no), and high fasting plasma glucose (yes/no). The first tertile was used as the reference category in all models. The time variable was calculated as the difference between the date of death or end of follow-up (the date of the last visit or the last recorded clinical event [MetS incidence] of participants who were still alive) and the date of recruitment.

Statistical interaction between tertiles of total meat or its different subtypes and potential confounding variables such as sex, diabetes status and BMI were checked including product terms in the multivariable model. Because no significant interactions were observed with sex, age or BMI, the product terms were removed.

To assess the linear trend, the median value of each tertile of total meat and different subtypes of meat consumption was included in the Cox regression models as a continuous variable. We conducted subsequent multivariable analyses to examine the HRs for MetS of substituting RM and PRM with one portion/day of other protein-rich foods such as fish, poultry and rabbit, legumes and eggs. These dietary variables were included in the same fully adjusted model as continuous variables, and the differences in their β-coefficients, variances and covariance were used to calculate the β-coefficient ± SE for the substitution effect. Thereafter, these parameters were used to estimate the HR and 95% CI. The level of significance for all statistical tests was set at $P < 0.05$ for bilateral contrast. All analyses were performed with the SPSS software (version 22.0).

3. Results

A total of 1868 individuals free of MetS at baseline and without extreme total energy values in FFQ were included in the final longitudinal analyses after 226 individuals had been excluded because data on some of the MetS components during follow-up were missing. The mean daily consumption of total meat was 124 g, for which RM&PRM were the major contributors (55%).

After a median follow-up of 3.2 years (interquartile range 1.9–5.8), 980 participants without MetS at baseline (53.8% women) developed new-onset MetS. Table 1 depicts the baseline characteristics of the study subjects by tertiles of average daily consumption of total meat. Participants, in the top tertile were more likely than those in the bottom tertile to have abdominal obesity and use oral antidiabetic agents or insulin; they also consumed less fruit, legumes, dairy products, nuts, and olive oil.

The risk of MetS development across tertiles of total meat consumption and its different subtypes is presented in Table 2. Participants in the top tertile of total meat and RM&PRM consumption had a greater risk of incident MetS than those in the bottom tertile, with HRs of 1.23 (95%CI: 1.03–1.45) for total meat and 1.46 (95%CI: 1.22–1.74) for RM&PRM. When RM and PRM were analyzed separately, similar direct associations were observed, with HRs of 1.27 (95%CI: 1.06–1.52) and 1.37 (95%CI; 1.15–1.62), respectively. On the other hand, the consumption of poultry and rabbit was inversely associated with the risk of MetS [HR: 0.83 (95% CI: 0.70–0.99) for the upper tertile compared to the lowest tertile].

Table 1
Baseline characteristics of the study population according to tertiles of energy-adjusted average daily consumption of total meat.[a]

	Total meat consumption (g/day)			
	T1 ≤ 106.92 *n* = 622	T2 106.94–137.80 *n* = 623	T3 ≥ 137.82 *n* = 623	*P*-value[b]
Age, years	67.3 ± 6.0	66.9 ± 6.0	66.5 ± 6.2	0.06
Women, % (*n*)	54.0 (336)	52.5 (327)	50.9 (317)	0.54
Waist circumference, cm	95.5 ± 9.8	94.00 ± 9.5	95.7 ± 9.9	0.05
Women	93.2 ± 10.5	91.0 ± 10.9	92.8 ± 10.2	0.02
Men	98.1 ± 8.1	97.3 ± 6.1	98.6 ± 8.5	0.11
BMI, kg/m^2	28.4 ± 3.4	28.1 ± 3.5	28.5 ± 3.6	0.15
Leisure time physical activity, METs-min/d	272 ± 270	269 ± 244	282 ± 248	0.66
Former smokers, % (*n*)	24.8 (154)	25.7 (160)	27.1 (169)	0.63
Current smokers, % (*n*)	17.4 (108)	14.6 (91)	15.1 (94)	0.36
Blood pressure, mmHg				
Systolic	145.9 ± 20.0	147.3 ± 21.0	146.3 ± 20.5	0.35
Diastolic	81.4 ± 10.9	82.3 ± 10.7	82.3 ± 10.7	0.27
Biochemistry, mg/dL				
Fasting blood glucose	101.2 ± 37.1	99.0 ± 34.5	99.1 ± 34.6	0.01
HDL-cholesterol, median [IRQ]	59.0 [51.0–68.0]	58.7 [51.0–68.0]	57.0 [50.0–66.5]	0.41
Triglycerides, median [IRQ]	97.0 [75.0–120.0]	94.0 [76.0–116.0]	96.0 [73.0–121.0]	0.56
Current medication use, % (*n*)				
Use of hypoglycemic agents	13.2 (82)	12.7 (79)	17.2 (107)	0.04
Use hypolipidemic agents	46.9 (292)	47.2 (294)	44.1 (275)	0.31
Use of antihypertensive agents	65.3 (406)	66.5 (414)	63.9 (398)	0.59
Insulin treatment	2.3 (14)	4.7 (29)	6.1 (38)	<0.01
Metabolic syndrome components, % (*n*)				
Abdominal obesity	47.0 (289)	38.1 (237)	46.6 (288)	<0.01
Hypertriglyceridemia	5.6 (35)	5.1 (32)	4.8 (30)	0.81
Low HDL-cholesterol	2.6 (16)	4.2 (26)	2.2 (14)	0.10
High blood pressure	87.8 (545)	86.8 (541)	86.7 (539)	0.82
High fasting plasma glucose	28.6 (177)	31.7 (196)	34.8 (216)	0.07
Intervention group, % (*n*)				
MedDiet + EVOO	37.1 (231)	34.3 (214)	32.3 (201)	0.32
MedDiet + nuts	33.8 (210)	35.3 (220)	34.0 (212)	
Low-fat control diet	29.1 (181)	30.3 (189)	33.7 (210)	
Energy intake, kcal/day	2358 ± 534	2279 ± 521	2332 ± 538	0.03
Food consumption, g/day[c]				
Vegetables	335 ± 145	330 ± 133	348 ± 151	0.09
Fruits	392 ± 211	388 ± 202	366 ± 194	0.05
Eggs	19 ± 11	20 ± 10	21 ± 12	<0.01
Legumes	23 ± 17	21 ± 11	20 ± 10	<0.01
Dairy	421 ± 241	384 ± 216	360 ± 212	<0.01
Fish	100 ± 47	102 ± 43	105 ± 45	0.10
Cereals	232 ± 92	234 ± 82	225 ± 79	0.11
Biscuits	25 ± 30	24 ± 29	21 ± 24	0.07
Nuts	13 ± 16	12 ± 13	11 ± 14	0.01
Olive oil	43 ± 18	42 ± 16	40 ± 16	0.03
Alcohol	10 ± 16	10 ± 13	10 ± 14	0.61

Data are expressed as means (standard deviation) or medians [IRQ, interquartile range] for continuous variables and percentages and numbers (*n*) for categorical variables.
Abbreviations: T, tertile; BMI, body mass index; MedDiet, Mediterranean diet, EVOO, extra-virgin olive oil.
[a] Tertile cut-offs are based on energy-adjusted daily average of total meat intake.
[b] *P* values for differences between tertiles were calculated by chi-square or ANOVA tests for categorical and continuous variables, respectively.
[c] All dietary variables were adjusted for total energy intake.

Table 3 shows HR and 95% CI of the MetS components for the daily average tertiles of energy-adjusted total meat consumption and its different subtypes. An increased intake of total meat was associated with an increased risk in the incidence of all MetS components, except high blood pressure. Results were similar when RM and PRM were merged and when PRM was analyzed alone.

Individuals in the top tertile of RM consumption showed a 40%, 25% and 36% higher risk of abdominal obesity, hypertriglyceridemia and low HDL-cholesterol, respectively, compared to those in the bottom tertile. Conversely, compared with participants in the bottom tertile of poultry and rabbit consumption, those in the top tertile had a lower risk of all MetS components, except for abdominal obesity.

The risk of MetS was lower when one serving/day of legumes (150 g boiled), poultry and rabbit (150 g), fish (150 g) or eggs (60 g) were substituted for RM (150 g). The corresponding HR and 95%CI were 0.32 (0.09–0.60), 0.34 (0.20–0.66), 0.40 (0.24–0.87), 0.37 (0.19–0.76), respectively. Results were similar when one-serving/day of PRM (150 g) was replaced (Fig. 1).The replacement of one serving/day of RM for one serving/day of PRM was non-significantly associated with a lower risk of MetS development [HR:0.72 (95%CI; 0.34–2.92)].

4. Discussion

To the best of our knowledge, this is the first epidemiologic study that has evaluated the association between total meat and different subtypes of meat and the risk of MetS development in older individuals at high cardiovascular risk. The results showed that a high consumption of total meat (around more than one serving/day), especially RM&PRM, was associated with increased risk of MetS after adjusting for several potential confounders. In contrast, poultry and rabbit consumption was associated with a reduced risk of MetS and all its components except abdominal obesity. The consumption of total meat, RM&PRM and PRM was

Table 2
Hazard ratios (95% confidence intervals) of metabolic syndrome incidence across average energy-adjusted tertiles of total meat, red meat and processed red meat, red meat, processed red meat and poultry and rabbit consumption during the follow-up.[a]

	Meat consumption (g/day)			
	T1[a]	T2	T3	*P*-trend
Total meat, median g/day[b]	87.0	120.6	158.9	
Metabolic syndrome incidence, % (*n*)	49.2 (306)	42.1 (262)	58.1 (362)	<0.01
Crude model	1.00 ref.	0.82 (0.69–0.97)	1.31 (1.12–1.54)	<0.01
Multivariable model 1	1.00 ref.	0.83 (0.70–0.98)	1.32 (1.12–1.55)	<0.01
Multivariable model 2	1.00 ref.	0.95 (0.80–1.13)	1.29 (1.09–1.53)	0.01
Multivariable model 3	1.00 ref.	0.93 (0.78–1.11)	1.23 (1.03–1.45)	0.02
Red meat and processed red meat, median g/day[c]	38.4	62.9	96.4	
Metabolic syndrome incidence, % (*n*)	45.5 (283)	44.3 (276)	59.6 (371)	
Crude model	1.00 ref.	0.96 (0.81–1.14)	1.61 (1.37–1.89)	<0.01
Multivariable model 1	1.00 ref.	0.97 (0.82–1.15)	1.67 (1.41–1.97)	<0.01
Multivariable model 2	1.00 ref.	1.03 (0.87–1.23)	1.57 (1.32–1.86)	<0.01
Multivariable model 3	1.00 ref.	0.98 (0.82–1.17)	1.46 (1.22–1.74)	<0.01
Red meat, median g/day[d]	19.5	39.3	67.5	
Metabolic syndrome incidence, % (*n*)	47.9 (298)	44.1 (275)	57.3 (357)	<0.01
Crude model	1.00 ref.	0.89 (0.75–1.05)	1.38 (1.17–1.63)	<0.01
Multivariable model 1	1.00 ref.	0.89 (0.75–1.05)	1.43 (1.21–1.68)	<0.01
Multivariable model 2	1.00 ref.	0.91 (0.77–1.09)	1.32 (1.10–1.57)	<0.01
Multivariable model 3	1.00 ref.	0.86 (0.72–1.02)	1.27 (1.06–1.52)	<0.01
Processed red meat, median g/day[e]	12.3	22.4	35.3	
Metabolic syndrome incidence, % (*n*)	46.0 (286)	45.1 (281)	58.3 (363)	<0.01
Crude model	1.00 ref.	0.96 (0.81–1.14)	1.44 (1.22–1.69)	<0.01
Multivariable model 1	1.00 ref.	0.97 (0.82–1.14)	1.46 (1.24–1.72)	<0.01
Multivariable model 2	1.00 ref.	1.06 (0.89–1.26)	1.42 (1.20–1.68)	<0.01
Multivariable model 3	1.00 ref.	1.06 (0.89–1.26)	1.37 (1.15–1.62)	<0.01
Poultry and rabbit, median g/day[f]	28.9	58.6	79.4	
Metabolic syndrome incidence, % (*n*)	56.4 (351)	43.2 (269)	49.8 (310)	<0.01
Crude model	1.00 ref.	0.67 (0.57–0.79)	0.79 (0.67–0.93)	<0.01
Multivariable model 1	1.00 ref.	0.67 (0.57–0.78)	0.78 (0.66–0.92)	<0.01
Multivariable model 2	1.00 ref.	0.76 (0.64–0.90)	0.85 (0.72–1.01)	0.03
Multivariable model 3	1.00 ref.	0.74 (0.63–0.88)	0.83 (0.70–0.99)	0.02

Abbreviations: T, tertile.
Multivariable model 1 adjusted for intervention group, sex, age (years), leisure time physical activity (METs-min/day), BMI (kg/m^2), current smoker (yes/no), former smoker (yes/no). Multivariable model 2 additionally adjusted for average consumption quintiles of vegetables (g/d), fruit (g/d), legumes (g/d), cereals (g/d), fish (g/d), dairy products (g/d), alcohol (g/d and quadratic term), biscuits (g/d), olive oil (g/d) and nuts (g/d). Multivariable model 3 additionally adjusted for the prevalence of metabolic syndrome components at baseline: abdominal obesity (yes/no), hypertriglyceridemia (yes/no), low HDL-cholesterol (yes/no), hypertension (yes/no) and high fasting plasma glucose (yes/no). All models were stratified by recruitment center.
[a] Tertile cut-offs are based on energy-adjusted daily average of total meat, red meat and processed red meat, red meat, processed red meat and poultry and rabbit.
[b] Includes all meat products: chicken, turkey, rabbit, pork, beef, veal, lamb, several types of sausages and processed red meat.
[c] Includes pork, veal, lamb, several types of sausages and processed red meat.
[d] Includes pork, beef, veal and lamb.
[e] Includes several types of sausages and processed red meat.
[f] Includes chicken, turkey and rabbit.

also associated with components of the MetS such as abdominal obesity, hypertriglyceridemia, low HDL-cholesterol and high fasting glucose. In addition, the substitution of one serving/day of poultry and rabbit, legumes, fish or eggs for one serving/day of RM or PRM was associated with a significant lower risk of developing MetS.

Our results regarding RM, PRM and RM&PRM are in line with most of the previous cross-sectional [8–10,12] and prospective studies [3,9]. Although Damião and co-workers showed that individuals with a higher red meat consumption in a Japanese–-Brazilian population had an increased risk of developing MetS, this association disappeared after adjustment for saturated fatty acid (SFA) intake [13]. This discrepancy may be due to over-adjustment, because SFA may be mediators of the association rather than confounders.

Contrary to our results, two previous studies found no association between consumption of poultry and the risk of MetS [12,13]. This discrepancy may be due to differences in the meat subtypes included in the poultry category of these studies. Cocate et al., grouped poultry and fish in the same category [12], while Damião et al. did not mention which meats were included in their definition of poultry [13]. In our study, chicken, turkey and rabbit were included in the same category.

Various mechanisms can explain the associations observed between meat consumption and MetS incidence. For instance, red meat is a food group rich in compounds harmful for cardiometabolic risk, such as cholesterol, SFA and heme iron There is compelling evidence suggesting that SFA have a lower thermogenic effect and are more prone to oxidation than unsaturated fatty acids from plant sources [20], and this type of fat has been associated with a higher likelihood of weight gain in animals [21]. Indeed, in a recent meta-analysis [5], consumption of RM and PRM has been associated with higher waist circumference and BMI. Moreover, consumption of SFA from RM, but not from white meat, has also been associated with MetS, which suggests that this nutrient has an important role in the pathogenesis of metabolic disorders [12]. Heme iron from red meat, but not from other food sources, has also been associated with MetS [22]. Iron is

Table 3
Hazard ratios (95% CI) of metabolic syndrome components (abdominal obesity, hypertriglyceridemia, low HDL-cholesterol, high blood pressure and high fasting plasma glucose) across energy-adjusted tertiles of specific meat consumption.[a]

	T1	T2	T3	P-trend
Total meat[b]				
Abdominal obesity	1.00 ref.	0.87 (0.69–1.09)	1.34 (1.07–1.68)	0.01
Hypertriglyceridemia	1.00 ref.	0.94 (0.80–1.09)	1.21 (1.03–1.41)	0.01
Low HDL-cholesterol	1.00 ref.	0.90 (0.77–1.06)	1.29 (1.10–1.50)	<0.01
High blood pressure	1.00 ref.	0.76 (0.52–1.12)	0.88 (0.59–1.31)	0.64
High fasting plasma glucose	1.00 ref.	0.87 (0.72–1.05)	1.21 (1.00–1.46)	0.04
Red and processed red meat[c]				
Abdominal obesity	1.00 ref.	1.19 (0.96–1.49)	1.73 (1.36–2.18)	<0.01
Hypertriglyceridemia	1.00 ref.	1.02 (0.87–1.19)	1.47 (1.26–1.72)	<0.01
Low HDL-cholesterol	1.00 ref.	1.08 (0.92–1.26)	1.45 (1.24–1.70)	<0.01
High blood pressure	1.00 ref.	0.95 (0.66–1.37)	1.25 (0.84–1.88)	0.28
High fasting plasma glucose	1.00 ref.	0.99 (0.82–1.19)	1.28 (1.05–1.56)	0.01
Red meat[d]				
Abdominal obesity	1.00 ref.	1.07 (0.86–1.33)	1.4 (1.19–1.88)	<0.01
Hypertriglyceridemia	1.00 ref.	0.88 (0.76–1.03)	1.25 (1.08–1.46)	<0.01
Low HDL-cholesterol	1.00 ref.	0.99 (0.86–1.16)	1.36 (1.17–1.59)	<0.01
High blood pressure	1.00 ref.	0.78 (0.55–1.12)	1.05 (0.71–1.54)	0.69
High fasting plasma glucose	1.00 ref.	1.07 (0.89–1.29)	1.18 (0.97–1.43)	0.09
Processed red meat[e]				
Abdominal obesity	1.00 ref.	0.83 (0.66–1.03)	1.50 (1.21–1.86)	<0.01
Hypertriglyceridemia	1.00 ref.	0.89 (0.77–1.04)	1.26 (1.09–1.46)	<0.01
Low HDL-cholesterol	1.00 ref.	0.90 (0.77–1.04)	1.25 (1.08–1.45)	<0.01
High blood pressure	1.00 ref.	0.94 (0.66–1.34)	0.97 (0.66–1.41)	0.88
High fasting plasma glucose	1.00 ref.	0.96 (0.80–1.15)	1.23 (1.02–1.48)	0.02
Poultry and rabbit[f]				
Abdominal obesity	1.00 ref.	0.72 (0.59–0.89)	0.81 (0.65–1.01)	0.03
Hypertriglyceridemia	1.00 ref.	0.69 (0.59–0.80)	0.78 (0.67–0.91)	<0.01
Low HDL-cholesterol	1.00 ref.	0.70 (0.61–0.82)	0.83 (0.71–0.96)	<0.01
High blood pressure	1.00 ref.	0.69(0.48–0.99)	0.68 (0.47–0.97)	0.02
High fasting plasma glucose	1.00 ref.	0.74 (0.62–0.88)	0.83 (0.69–0.99)	0.01

Abbreviations: CI, confidence interval, T, tertile.
[a] Tertile cut-offs are based on energy-adjusted daily average meat intake. The metabolic syndrome components were defined according to updated harmonizing criteria. Cox regression models adjusted for intervention group, sex, age (year), leisure time physical activity (METs-min/day), BMI (kg/m^2), current smoker (yes/no), former smoker (yes/no), quintiles of average consumption of vegetables (g/d), fruit (g/d), legumes (g/d), cereals (g/d), fish (g/d) dairy (g/d), biscuits (g/d), olive oil (g/d) and nuts (g/d), and alcohol (g/d) (continuous and quadratic term). All models were stratified by recruitment center.
[b] Includes all meat products: chicken, turkey, rabbit, pork, beef, veal, lamb, several types of sausages and processed red meat.
[c] Includes pork, beef, veal, lamb, several types of sausages and processed red meat.
[d] Includes pork, beef, veal and lamb.
[e] Includes several types of sausages and processed red meat.
[f] Includes chicken, turkey and rabbit.

potentially harmful because it catalyses cellular reactions and produces reactive oxygen species that increase the oxidative stress. This has a particular effect on pancreatic beta cells, which can lead to insulin resistance [23].

Processed meat products are treated by salting, curing, or smoking, thus having high sodium content, besides harmful additives such as nitrites and nitrates, aromatic polycyclic hydrocarbons, and heterocyclic amines. Nitrites and nitrates can be converted into nitrosamines that have been associated with an increased risk of diabetes in experimental animal models [24]. Moreover, blood nitrites have been associated with endothelial dysfunction and impaired insulin response in adults [25], thus increasing the risk of MetS development. Finally, excessive sodium intake is clearly related to high blood pressure.

The mechanism by which poultry consumption may decrease MetS risk remains unclear. The substitution of poultry for RM and PRM entails a lower intake of SFA, heme iron, glycotoxins and sodium, which may be involved in the development of MetS through the aforementioned mechanisms. In fact, in observational studies the risk of type 2 diabetes was reduced when one serving of poultry/day was substituted for one serving of total red meat/day [6]. Our results also show that substituting a serving of poultry, fish, legumes or eggs for RM and PRM can protect against MetS development. A recent meta-analysis of prospective studies showed an inverse association between fish consumption and the risk of MetS incidence [26]. The mechanisms explaining this inverse association may be the high fish content of n-3 fatty acids, which have anti-inflammatory effects and may help reduce insulin resistance in muscle, improve the plasma lipoprotein profile and endothelial function, and control blood pressure [27]. In epidemiologic studies legume consumption has been associated with a reduced risk of MetS components such as increased waist circumference and high blood pressure [28]. Legumes have a high fiber and magnesium content, which has been associated with a better lipid profile and improved glucose and inflammatory responses [29] that may be responsible in part for these beneficial effects. The inverse association found with MetS when substituting eggs for RM and PRM may be explained in part because eggs are a good source of folate, B vitamins, and carotenoids and promote the absorption of other antioxidants present in vegetables [30]. Robust observational evidence suggests that high egg consumption is not associated with an increased risk of coronary heart disease or stroke, with the probable exception of high consumption levels among diabetic persons [31].

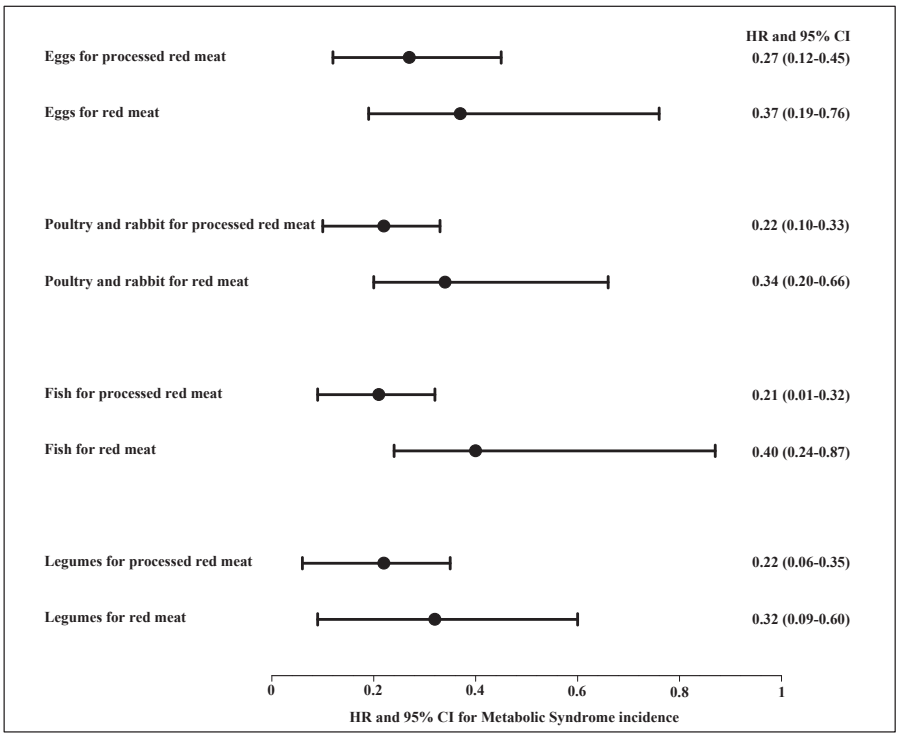

Fig. 1. HR and 95% CI of metabolic syndrome for replacing red meat and processed red meat with poultry and rabbit, fish, legumes and eggs. Cox regression model adjusted for age (years), sex, leisure time physical activity (METs min/day), BMI (kg/m^2), current smoker (yes/no), former smoker (yes/no) at baseline, daily average consumption quintiles of vegetables (g/d), fruit (g/d), legumes (g/d) (except when substitution with legumes was analyzed), cereals (g/d), fish (g/d) (except when substitution with fish was analyzed), dairy products (g/d), biscuits (g/d), olive oil (g/d) and nuts (g/d) and alcohol (as continuous variable in g/d and adding the quadratic term), and for the prevalence of metabolic syndrome components at baseline: abdominal obesity (yes/no), hypertriglyceridemia (yes/no), low HDL-cholesterol (yes/no), hypertension (yes/no) and high fasting plasma glucose (yes/no).

Although our study focuses on the risk of MetS attributable to exposure to a specific food group (meat and processed meat), it should be considered that the effect of the overall dietary pattern is likely to have a considerably greater effect than those of individual food groups or nutrients. For example, there is consistent evidence that some dietary patterns, such us the MedDiet, DASH and Nordic diet, have beneficial effects on MetS [32]. Probably, the joint effect of the whole dietary pattern is larger than the sum of its parts. Nevertheless, the associations we found remained significant after adjusting for other food groups within the background diet.

Our study has some limitations. First, the results cannot be generalized to other populations because study subjects are older individuals at high cardiovascular risk. Second, MetS was a secondary outcome of the PREDIMED study, hence the results are exploratory in nature. Third, our study has been conducted in the frame of a nutritional field trial with dietary patterns that might have a differential effect on the incidence of MetS or its components. However, this confounding effect was minimized by adjusting analyses for the intervention group. Fourth, as in any prospective study, there can be unknown or unmeasured confounding factors, such as the amounts of nitrates, nitrites and heterocyclic amines consumed, all of which have been related to the occurrence and progress of MetS and its components. This possibility may have introduced some degree of residual confounding.

Our study also has strengths, such as the relatively long follow-up, the control for a large number of potential confounders, the analysis of different meat subtypes and yearly repeated dietary assessments during follow-up, which allows updating the consumption of the foods under consideration and is rarely undertaken in large observational studies.

In conclusion, the present study suggests that total meat (when consumed to a level of around more than one serving/day), RM and PRM promote MetS development. In contrast, poultry consumption is associated with a lower risk of MetS. The substitution of other protein-rich foods for RM or PRM is also associated with a lower risk of MetS. Therefore, replacing RM and PRM by other healthy foods should be recommended to decrease the risk of MetS in individuals at high cardiovascular risk. Further studies are warranted to confirm these findings and elucidate the possible mechanisms involved.

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Conflict of interest

The authors disclose no conflict of interest related with the article.

Authors' responsibilities

M.A.M.-G., D.C, R.E, E.R, L.S-M, J.L, E.G.-G., M.F., and J.S.-S. designed the PREDIMED study; N.B.-T., N.B., M.A.M.-G., D.C., R.E., E.R., M.F., L.S.-M., I.S., R.M.L.-R., J.L., E.G.-G., M.F., E.T., J.V.-S., R.P. and J.S.-S. conducted the research; N.B.-T. and N.B. analyzed data; N.B.-T., N.B., and J.S.-S. wrote the manuscript; M.A.M.-G., D.C., R.E., E.R., L.S.-M., M.F., J.L., J.S.-S. were the coordinators of subject recruitment and follow-up at the outpatient clinics; N.B.-T., N.B. and J.S.-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 have read and approved the final manuscript.

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3. Legume consumption is inversely associated with type 2 diabetes incidence in adults: A prospective assessment from the PREDIMED study.

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Key teaching points:

- Legumes consumption is widely recommended for its healthy effects, but its association with T2D is scarce.

- Most of the previous studies have been focused on soy-legumes consumption and only few of them have analyzed the association between non-soy legumes and T2D risk.

- The present study analyzed for the first time the association between total non-soy legume and its different subtype consumption and the risk of T2D incidence in a Mediterranean population. Moreover, the theoretical substitution effect of legumes for other protein- or carbohydrate-rich foods on the risk of T2D was also assessed.

- Results suggest a beneficial effect of total legume, and particularly lentils, consumption on T2D risk incidence.

- The theoretically substitution of legumes for eggs, bread, rice and potato was associated with a lower risk of incidence of T2D.

UNIVERSITAT ROVIRA I VIRGILI
DIETARY FACTORS ASSOCIATED WITH METABOLIC SYNDROME AND TYPE 2 DIABETES RISK
Nerea Becerra Tomás

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Original article

Legume consumption is inversely associated with type 2 diabetes incidence in adults: A prospective assessment from the PREDIMED study

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SUMMARY

Background & aims: Legumes, a low-energy, nutrient-dense and low glycemic index food, have shown beneficial effects on glycemic control and adiposity. As such, legumes are widely recommended in diabetic diets, even though there is little evidence that their consumption protects against type 2 diabetes. Therefore the aim of the present study was to examine the associations between consumption of total legumes and specific subtypes, and type 2 diabetes risk. We also investigated the effect of theoretically substituting legumes for other protein- or carbohydrate-rich foods.
Methods: Prospective assessment of 3349 participants in the PREvención con DIeta MEDiterránea (PREDIMED) study without type 2 diabetes at baseline. Dietary information was assessed at baseline and yearly during follow-up. We used Cox regression models to estimate hazard ratios (HRs) and 95% confidence intervals (95% CIs) for type-2 diabetes incidence according to quartiles of cumulative average consumption of total legumes, lentils, chickpeas, dry beans and fresh peas.
Results: During a median follow-up of 4.3 years, 266 new cases of type 2 diabetes occurred. Individuals in the highest quartile of total legume and lentil consumption had a lower risk of diabetes than those in the lowest quartile (HR: 0.65; 95% CI: 0.43, 0.96; *P*-trend = 0.04; and HR: 0.67; 95% CI: 0.46–0.98; *P*-trend = 0.05, respectively). A borderline significant association was also observed for chickpeas consumption (HR 0.68; 95% CI: 0.46, 1.00; *P*-trend = 0.06). Substitutions of half a serving/day of legumes for similar servings of eggs, bread, rice or baked potato was associated with lower risk of diabetes incidence.

Abbreviations used: IDF, International Diabetes Federation; CVD, cardiovascular disease; MedDiet, Mediterranean diet; PREDIMED, PREvención con DIeta MEDiterránea; FFQ, Food Frequency Questionnaire; ICC, intraclass correlation coefficient; HRs, hazard ratios; Cis, Confidence intervals; METs, metabolic equivalent task.

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Conclusions: A frequent consumption of legumes, particularly lentils, in the context of a Mediterranean diet, may provide benefits on type 2 diabetes prevention in older adults at high cardiovascular risk.
Trial registration: The trial is registered at http://www.controlled-trials.com (ISRCTN35739639). Registration date: 5th October 2005.

Background

Type 2 diabetes is recognised as a major public health issue worldwide. According to the International Diabetes Federation (IDF), type 2 diabetes affected 415 million adults in 2015 and it is estimated that this figure will increase to 642 million in 2040 [1]. Type 2 diabetes is associated with significant systemic consequences, including microvascular and macrovascular complications affecting the quality of life and decreasing the life expectancy [2]. Therefore, it is imperative to identify strategies to prevent and manage this condition.

In recent years, accumulating evidence from prospective studies and randomized controlled trials indicates that changes in diet and lifestyle are critical for the prevention of type 2 diabetes [3]. Legumes have been proposed as one of the dietary factors that may offer protection against type 2 diabetes. However, the independent association between non-soy legume intake and type 2 diabetes has scarcely been studied.

Legumes, including green beans and peas, peanuts, soybeans, lupine, alfalfa, clover, dry beans, broad beans, dry peas, chickpeas and lentils [4], are protein- and fibre-rich foods, and have a low glycemic index [5]. In addition, legumes contain sizeable amounts of B vitamins, particularly folate, as well as beneficial minerals such as, calcium, magnesium and potassium [6]. As a consequence of this unique nutritional value, several diabetes guidelines recommend them [7,8]. Furthermore, legumes are importantly present in healthy plant-based dietary patterns as the Mediterranean diet (MedDiet), vegetarian diets and prudent diets, which have consistently been associated with a lower risk of chronic diseases and type 2 diabetes [9,10]. Legumes consumption has also demonstrated beneficial effects on obesity, abdominal adiposity and metabolic syndrome [11–14] which are well recognized risk factors for type 2 diabetes. In addition, the replacement of red meat by legume consumption decreased peripheral inflammation, glycaemia and insulinemia in diabetic individuals [15,16].

To date, the few epidemiological studies evaluating these associations show inconsistent results. According to India's Third National Family Health Survey, compared to non-consumers, women who consumed legumes daily or weekly, but not men, showed a significant reduced prevalence of type 2 diabetes [17]. In contrast, this association was not observed in the Indian Migration Study [18]. Results from prospective studies are also controversial and highlight the paucity of studies on legumes and diabetes. Whereas several studies did not show any significant association between legume consumption and type 2 diabetes development [19–21], in the Shanghai Women's Health Study [22] consumption of total legumes (including soybeans) was associated with a reduced risk of type 2 diabetes incidence. Contrary, in the Nurse's health study a higher risk of type 2 diabetes was observed in the highest categories of total legume consumption [23].

However, to the best of our knowledge, no previous prospective studies have been conducted in Mediterranean populations who customarily consume sizeable amounts of non-soy legumes, or in individuals at high cardiovascular risk. Moreover, the effect of substituting legumes for other food sources rich in proteins or carbohydrates, has not been previously assessed. Therefore, the aim of the current study was to examine the association between the consumption of total non-soy legumes and its different subtypes (dry beans, chickpeas, lentils, and fresh peas), and the risk of type 2 diabetes development in a Mediterranean population at high cardiovascular risk. We also investigated the effect of substituting legumes for other protein- and carbohydrate-rich foods.

Research design and methods

Study population

The present data was analysed using an observational prospective design conducted within the frame of the PREDIMED (PREvención con DIeta MEDiterránea) trial (PREDIMED website: http://www.predimed.es) [24]. The PREDIMED study (registered at http://www.controlled-trials.com as ISRCTN35739639) was a randomized, multi center, parallel-group clinical trial conducted in Spain between October 2003 and December 2010. The main aim of the trial was to evaluate the effectiveness of the MedDiet on the primary prevention of CVD; the principal results have been published elsewhere [25]. Briefly, the study included 7447 men (aged 55–80 years) and women (aged 60–80 years) without CVD at enrolment but who were at high cardiovascular risk. They were eligible if they had either type 2 diabetes or at least three of the following cardiovascular risk factors: hypertension (systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$90 mmHg or taking antihypertensive drugs), hypercholesterolemia (high LDL cholesterol $\geq$160 mg/dL or taking hypolipidemic medication), low high-density lipoprotein ($\leq$50 mg/dL in women or $\leq$40 mg/dL in men), overweight/obesity (BMI $\geq$ 25 kg/m^2), current smoking or family history of premature coronary heart disease. Exclusion criteria included alcohol or drug abuse, severe chronic illness, presence of BMI $\geq$40 kg/m^2 and allergy or intolerance to olive oil or nuts. For the current analysis, we excluded participants with type 2 diabetes at baseline (n = 3614), and those who lacked measures of blood glucose control (n = 292), who had implausible daily energy intake (<500 or >3500 kcal/d for women and <800 or >4000 kcal/d for men [26]) or who had not completed the baseline Food Frequency Questionnaire (FFQ) (n = 98). We also excluded participants without follow-up (n = 94). The final analysis included 3349 non-diabetic individuals. The protocol was approved by the institutional review boards of the respective recruiting centers, and written informed consent was provided by all participants included in the study.

Dietary assessment

Trained dietitians quantified dietary intake using a validated semi-quantitative FFQ at baseline and yearly during the follow-up [27]. The Pearson correlation coefficient and the intraclass correlation coefficient (ICC) were used to explore the reproducibility of the FFQ for food groups and energy and nutrient intake. The reproducibility and validity of the FFQ for legumes were 0.47 (ICC 0.63), and 0.29 (ICC 0.40), respectively [27]. Legumes consumption

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was assessed using four items from the FFQ (lentils, chickpeas, dry beans and fresh peas). The consumption frequency was measured in nine categories (ranging from never or almost never to >6 servings/day) for each food item. The responses to each item were transformed to daily frequency and then multiplied by the portion size (in grams) in order to obtain grams per day consumed during the follow-up. The consumption of energy, nutrients and food groups was calculated using Spanish food composition tables [28,29].

Assessment of other covariates

At baseline and yearly during the follow-up, participants completed a 47-item questionnaire about lifestyle, medical history and medication use and a validated Spanish version of the Minnesota Leisure Time Physical Activity Questionnaire [30]. To assess adherence to the MedDiet, a 14-item validated questionnaire was filled in for each participant [31]. One question was about legume consumption, two questions about meat and one about fish [31]. In order to control for the overall dietary pattern, we used this MedDiet questionnaire score but removed the variable related to legume consumption for the main analysis. Therefore, a 13-point score was used as a covariate in the models. For the substitution analysis of meat and fish, we used an 11-point score after additionally removing the variables related to meat intake and a 12-point score after removing the variable related to fish intake as covariates in the models.

Fasting blood samples, and anthropometric and blood pressure measurements were collected from all participants by trained personnel. Blood pressure was measured in triplicate (recording the mean of the three values), with an interval of 5 min between each measurement, using a validated oscillometer (Omron HEM705CP, Hoofddorp, The Netherlands), Weight and height were measured with participants in lightweight clothing and no shoes using calibrated scales and a wall-mounted stadiometer.

Ascertainment of type 2 diabetes mellitus

Type 2 diabetes was a prespecified secondary outcome in the PREDIMED trial. New-onset type 2 diabetes was identified following the American Diabetes Association criteria [32]; namely, fasting plasma glucose levels of ≥7.0 mmol/L (≥126.1 mg/dL) or 2-h plasma glucose levels of ≥11.1 mmol/L (≥200.0 mg/dL) after an oral dose of 75 g of glucose. Yearly, physicians-investigators of each center, who were blinded to the intervention, completed a review of all the participants' medical records. When new cases of type 2 diabetes were identified based on a diagnosis reported in the medical charts or on a fasting blood glucose values during routine biochemical analyses (done at least once per year), these reports were sent to the PREDIMED Clinical Events Committee, whose members were also blinded to treatment allocation. Only when the new onset type 2 diabetes case was verified within the next 3 months, using the same criteria, the adjudication committee definitively confirmed the end point [10]. Only confirmed diabetes events that occurred between 1 October 2003 and 1 December 2010 were included in the analyses.

Statistical analyses

To take advantage of the yearly dietary assessment and to better represent the long term diet [33], we used the cumulative average from baseline to the last FFQ before the new-onset of type 2 diabetes or to the last available FFQ (in those individuals without type 2 diabetes incidence). Participants were categorized into quartiles of consumption of total legumes, lentils, chickpeas, dry beans and fresh peas adjusted for energy intake using the residuals method [26].

The baseline characteristics of the study population were presented as means ± SD for quantitative variables, and percentages and numbers for categorical variables. One way ANOVA and Chi-square tests were used to assess differences in baseline characteristics according to quartiles of energy-adjusted cumulative average consumption of total legumes and the different subtypes. Cox regression models were fitted to assess the hazard ratios (HRs) and 95% confidence intervals (CIs) of type 2 diabetes according to quartiles of consumption of total legumes, lentils, chickpeas, dry beans and fresh peas. To appraise the linear trend, the median consumption within each quartile was included in the Cox regression models as a continuous variable. Model 1 was adjusted for sex, age (continuous), intervention group, baseline leisure time physical activity (METs-min/day), smoking status (never, current or former), educational level (primary education, secondary education or academic/graduate), fasting plasma glucose (<100 mg/dL or ≥100 mg/dL), prevalence of hypertension (yes/no), prevalence of hypercholesterolemia (yes/no), use of antihypertensive medication (yes/no), use of hypolipidaemic medication (yes/no) and cumulative average alcohol consumption in grams per day (continuous and adding a quadratic term). Model 2 was additionally adjusted for cumulative average of the 13-point screener (excluding legumes) of MedDiet adherence as a continuous variable. Model 3 was further adjusted for BMI (kg/m^2). All models were stratified by recruitment center. The first quartile was used as a reference category in all models. For each participant, we calculated the time variable as the interval between the randomization and the date of type 2 diabetes diagnosis, death from any cause or the last visit, whichever came first. To test the statistical interaction between quartiles of total legumes, lentils, chickpeas, dry beans, fresh peas and potential confounding variables such as sex, intervention group and BMI, the product terms were included in the multivariable model. Because no significant interactions were observed, the product terms were removed. We conducted subsequent multivariate analyses to examine the HRs of substituting half a serving/day of legumes (30 g in raw) for half a serving/day of another protein-rich food, such as meat (75 g), fish (75 g) and eggs (30 g), and another carbohydrate-rich food, such as bread (38 g), rice (30 g raw), baked potato (100 g) and pasta (30 g raw). These dietary variables were included as continuous variables in the same model, adjusted for the covariates listed above. The differences in their β-coefficients, variance and covariance were used to calculate the β-coefficient ± SE for the substitution effect, and the HRs and 95% CI were calculated from these parameters.

To test the robustness of our results, we conducted two sensitivity analyses: a) adjusting for updated BMI instead of baseline BMI to evaluate the impact of changes in body weight; and b) censoring participants at the time of diagnosis of cancer or CVD (myocardial infarction, stroke) because these diseases may lead to changes in diet [34].

Data were analysed using a commercially available software program Stata 14 (StataCorp) and statistical significance was set at a 2-tailed *P* value <0.05.

Results

During a median follow-up of 4.3 years, 266 incident cases of type 2 diabetes were documented. Baseline characteristics of the study population according to energy-adjusted quartiles of total legume intake are presented in Table 1. Participants in the highest quartile of total legume consumption were less likely to have higher education and had higher BMI and fasting plasma glucose levels than those in the bottom quartile. They also had a lower

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Table 1
Baseline characteristics of the study population according to cumulative average quartiles of energy-adjusted total legume consumption.[a]

	Quartile of total legume consumption				*P*-value[b]
	Q1 (lowest) n = 838	Q2 n = 837	Q3 n = 837	Q4 (highest) n = 837	
Total legume consumption, (g/day)	9.64 ± 3.55	16.09 ± 1.41	21.57 ± 1.89	34.60 ± 17.24	
Lentils, g/day	3.58 ± 2.31	5.26 ± 2.13	7.28 ± 2.34	9.97 ± 6.06	
Chickpeas, g/day	2.71 ± 2.02	4.31 ± 1.70	6.06 ± 2.051	9.17 ± 5.53	
Fresh peas, g/day	1.14 ± 1.92	2.57 ± 2.15	2.90 ± 2.84	6.24 ± 14.70	
Dry beans, g/day	2.20 ± 1.97	3.95 ± 1.85	5.32 ± 2.67	9.22 ± 6.22	
Age, years	66 ± 6	67 ± 6	67 ± 6	67 ± 6	0.31
Women, % (n)	59.90 (502)	62.37 (522)	63.80 (534)	62.60 (524)	0.42
Smoking habit, % (n)					0.31
Never	60.74 (509)	60.81 (509)	64.40 (539)	63.92 (535)	
Former	21.72 (182)	22.82 (191)	22.10 (185)	20.79 (174)	
Current	17.54 (147)	16.37 (137)	13.50 (113)	15.29 (128)	
Education, % (n)					0.01
Primary	72.43 (607)	75.87 (635)	76.58 (641)	78.49 (657)	
Secondary	16.59 (139)	16.97 (142)	16.01 (134)	15.05 (126)	
University/graduate	10.98 (92)	7.17 (60)	7.41 (62)	6.45 (54)	
Intervention group, n (%)					0.13
MedDiet + EVOO	32.22 (270)	33.09 (277)	34.65 (290)	33.09 (277)	
MedDiet + Nuts	35.20 (295)	35.72 (299)	36.56 (306)	31.66 (265)	
Control group	32.58 (273)	31.18 (261)	28.79 (241)	35.24 (295)	
BMI, kg/m^2	29.95 ± 3.65	29.68 ± 3.48	30.16 ± 3.54	30.17 ± 3.65	0.02
Leisure time physical activity, METs.min/day	234.21 ± 236.78	228.65 ± 214.85	226.27 ± 215.75	237.12 ± 220.54	0.74
Hypertension, % (n)	90.57 (759)	92.11 (771)	91.52 (766)	92.95 (778)	0.34
Hypercholesterolemia, % (n)	84.25 (706)	83.27 (697)	85.19 (713)	85.90 (719)	0.47
Current medication use, % (n)					
Use of antihypertensive agents	75.78 (635)	76.46 (640)	77.18 (646)	78.61 (658)	0.55
Use hypolipidemic agents	47.37 (397)	49.58 (415)	50.78 (425)	51.25 (429)	0.39
Fasting plasma glucose, mg/dL	97.57 ± 15.22	97.26 ± 12.93	98.81 ± 16.51	99.32 ± 14.79	0.01
Nutrient intake[c]					
Total energy, kcal/day	2381 ± 564	2196 ± 469	2239 ± 533	2230 ± 504	<0.01
Total fat, % of total energy	38.68 ± 6.53	39.13 ± 6.11	38.02 ± 6.15	37.00 ± 6.56	<0.01
Carbohydrate, % of total energy	42.37 ± 7.23	42.09 ± 6.57	42.89 ± 6.43	44.11 ± 7.19	<0.01
Protein, % of total energy	15.75 ± 2.70	16.19 ± 2.64	16.59 ± 2.641	16.69 ± 2.81	<0.01
Alcohol, g/day	10.52 ± 16.71	9.10 ± 12.11	9.09 ± 14.10	7.74 ± 12.33	<0.01
Dietary fibre, g/day	22.43 ± 7.02	24.02 ± 6.27	25.76 ± 6.93	28.98 ± 8.59	<0.01

Abbreviations: Q, quartile; MedDiet, Mediterranean diet; EVOO, extra virgin olive oil; METs, metabolic equivalent.
[a] Data are expressed as means ± SD for continuous variables and percentage and number (n) for categorical variables.
[b] P value for differences between quartiles were calculated by chi-square or ANOVA tests for categorical and continuous variables, respectively.
[c] All dietary variables were adjusted for total energy intake.

intake of total energy, dietary fat and alcohol but a higher intake of carbohydrates, protein and dietary fibre. Baseline characteristics according to quartiles of different type of legume consumption are described in Supplemental Table 1.

During follow-up, the median cumulative average intake was 19.76 g/d for total legumes, 6.58 g/d for lentils, 4.98 g/d for chickpeas, 4.69 g/d for dry beans and 2.76 g/d for fresh peas (Table 2).

In multivariable analyses (Table 3), participants in the highest quartile of consumption of total legumes had a lower risk of developing type 2 diabetes even after adjusting for the overall dietary pattern score and BMI (HRs: 0.65; 95% CI: 0.43, 0.96; *P* trend = 0.04) than those in the lowest quartile. Likewise, those in the highest quartile of lentil intake had a 33% lower risk of type 2 diabetes incidence (HRs 0.67; 95% CI: 0.46–0.98; *P* trend = 0.05) than those in the bottom quartile. Comparing the 4th *vs* the 1st quartile of chickpeas consumption a borderline significant inverse association with type 2 diabetes development was observed (HRs 0.68; 95% CI: 0.46–1.00; *P* trend = 0.06). No significant associations were observed between fresh peas and dry beans, and the risk of type 2 diabetes. The results were similar when the consumption of total legumes, lentils, chickpeas, dry beans and fresh peas was modelled as a continuous variable per 30 g/day increase (Supplemental Table 2). We observed an inverse association between total legumes (HR: 0.55; 95% CI: 0.32, 0.93; *P*-value = 0.03) and lentil consumption (HR: 0.18; 95% CI: 0.05, 0.65; *P*-value = 0.01) with incident type 2 diabetes, while consumption of chickpeas, dry beans and fresh peas was unrelated.

Table 2
Energy-adjusted cumulative average legume consumption during follow-up in the study population.[a]

	Means ± SD	Median	Interquartile range
Legumes	20.84 ± 9.60	19.76	15.42–24.75
Lentils	6.73 ± 3.51	6.58	4.37–8.46
Chickpeas	5.67 ± 3.25	4.98	3.90–7.37
Dry beans	5.33 ± 3.59	4.69	3.48–8.70
Fresh peas	3.12 ± 5.15	2.76	0.92–4.18

[a] Data are expressed in raw grams per day.

Results were similar when model 3 was adjusted by quintiles of cumulative consumption of nuts, olive oil, fish and fruits and vegetables instead of using the MedDiet score (data not shown).

Figure 1 shows the potential impact on type 2 diabetes development of theoretically substituting half a serving/day of total legumes for half a serving/day of food rich in protein or carbohydrates. For foods rich in protein, the risk of type 2 diabetes was 50% lower when half a serving/day of legumes was substituted for half a serving/day of eggs. However, although there was a trend toward lower risk of type 2 diabetes, the association was non-significant when fish or meat were replaced with legumes [(HR: 0.58; 95% CI: 0.32, 1.05; *P*-value = 0.07) and (HR: 0.59; 95% CI 0.34,

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Table 3
HRs (95% CIs) of type 2 diabetes incidence according to energy-adjusted quartiles of cumulative average consumption of legumes and its specific subtypes.

Quartiles of legumes consumption					
	1 (lowest)	2	3	4 (highest)	*P*-trend
Legumes					
Cases/person-years	85/3479	72/3465	62/3456	47/3397	
Median (P25, P75), g/day	12.73 (10.38, 14.29)	17.63 (16.59, 18.70)	21.97 (20.85, 23.23)	28.75 (26.45, 32.66)	
Crude model	1 (ref.)	0.84 (0.61–1.15)	0.74 (0.54–1.03)	0.55 (0.37–0.81)	<0.01
Multivariable model 1	1 (ref.)	0.84 (0.61–1.15)	0.81 (0.58–1.12)	0.59 (0.40–0.87)	0.01
Multivariable model 2	1 (ref.)	0.85 (0.62–1.17)	0.84 (0.60–1.17)	0.62 (0.42–0.93)	0.03
Multivariable model 3	1 (ref.)	0.87 (0.63–1.19)	0.86 (0.61–1.20)	0.65 (0.43–0.96)	0.04
Lentils					
Cases/person-years	99/3465	60/3492	55/3465	52/3376	
Median (P25, P75), g/day	3.77 (2.89, 4.17)	5.62 (4.71, 6.15)	7.66 (7.12, 8.23)	8.88 (8.66, 9.49)	
Crude model	1 (ref.)	0.62 (0.45–0.86)	0.60 (0.43–0.85)	0.60 (0.42–0.87)	0.01
Multivariable model 1	1 (ref.)	0.67 (0.48–0.92)	0.61 (0.43–0.88)	0.63 (0.44–0.91)	0.02
Multivariable model 2	1 (ref.)	0.69 (0.50–0.96)	0.64 (0.45–0.92)	0.66 (0.45–0.96)	0.04
Multivariable model 3	1 (ref.)	0.69 (0.50–0.96)	0.66 (0.46–0.94)	0.67 (0.46–0.98)	0.05
Chickpeas					
Cases/person-years	79/3470	80/3477	60/3461	47/3390	
Median (P25, P75), g/day	3.05 (1.77, 3.57)	4.35 (4.14, 4.73)	6.15 (5.58, 6.71)	8.59 (7.99, 9.13)	
Crude model	1 (ref.)	1.08 (0.79–1.47)	0.80 (0.57–1.13)	0.60 (0.42–0.88)	0.01
Multivariable model 1	1 (ref.)	1.10 (0.80–1.50)	0.89 (0.62–1.27)	0.65 (0.45–0.96)	0.03
Multivariable model 2	1 (ref.)	1.10 (0.80–1.51)	0.92 (0.64–1.31)	0.67 (0.46–0.99)	0.05
Multivariable model 3	1 (ref.)	1.08 (0.79–1.49)	0.91 (0.64–1.30)	0.68 (0.46–1.00)	0.06
Dry beans					
Cases/person-years	77/3453	75/3480	56/3455	58/3410	
Median (P25, P75), g/day	2.16 (0.68, 2.99)	4.16 (3.84, 4.46)	5.67 (5.08, 6.30)	8.45 (7.66, 9.22)	
Crude model	1 (ref.)	1.02 (0.74–1.40)	0.77 (0.55–1.08)	0.83 (0.57–1.19)	0.31
Multivariable model 1	1 (ref.)	1.03 (0.75–1.41)	0.80 (0.56–1.12)	0.87 (0.61–1.26)	0.50
Multivariable model 2	1 (ref.)	1.05 (0.76–1.44)	0.83 (0.59–1.17)	0.91 (0.62–1.31)	0.65
Multivariable model 3	1 (ref.)	1.05 (0.77, 1.45)	0.84 (0.59, 1.18)	0.93 (0.65, 1.35)	0.75
Fresh peas					
Cases/person-years	61/3418	76/3441	65/3487	64/3458	
Median (P25, P75), g/day	0.22 (0, 0.61)	1.85 (1.33, 2.32)	3.53 (3.15, 3.87)	5.06 (4.52, 7.06)	
Crude model	1 (ref.)	1.18 (0.84–1.66)	0.89 (0.62–1.28)	0.89 (0.61–1.29)	0.22
Multivariable model 1	1 (ref.)	1.26 (0.89–1.79)	1.03 (0.71–1.49)	0.97 (0.66–1.42)	0.51
Multivariable model 2	1 (ref.)	1.26 (0.89–1.79)	1.04 (0.72–1.50)	0.97 (0.66–1.41)	0.50
Multivariable model 3	1 (ref.)	1.27 (0.89–1.81)	1.04 (0.72–1.51)	0.97 (0.66–1.42)	0.49

Cox regression models were used to assess the risk of diabetes incidence by quartiles of cumulative average of intake of legumes and legume subtypes. Multivariable model 1 was adjusted for age (y), sex, intervention group, cumulative average consumption of alcohol (continuous and adding a quadratic term), smoking status (never, former, or current smoker), educational level (primary education, secondary education, or academic/graduate), leisure-time physical activity (metabolic equivalent task minutes/day), baseline hypertension (yes/no), hypercholesterolemia (yes/no), use of antihypertensive medication (yes/no), use of lipid-lowering drugs (yes/no) and fasting plasma glucose at baseline (<100 mg/dL or ≥100 mg/dL). Model 2 was further adjusted for cumulative average of the 13-point screener (excluding legumes) of MedDiet adherence (continuous). Model 3 was additionally adjusted for BMI (kg/m^2). Extremes of total energy intake (>4000 or <800 kcal/day in men and >3500 or <500 kcal/day in women) were excluded.

1.03) (*P*-value = 0.07), respectively]. For carbohydrate-rich food, a 44%, 47%, 52% and 51% lower risk of type 2 diabetes development was observed when wholemeal bread, white bread, rice and baked potato, respectively, were replaced with legumes.

Our results were significant in two different sensitivity analyses. When we examined the impact of changes in BMI on the association of legume consumption and type 2 diabetes risk adjusting for updated BMI instead of baseline BMI, total legumes lentils and chickpeas consumption was associated with a lower risk of type 2 diabetes [HR: 0.65; 95% CI: 0.43, 0.96; *P*-trend = 0.03 (fourth quartile *vs* first quartile of legumes consumption), HR: 0.68; 95% CI: 0.47, 0.98; *P*-trend = 0.05 (fourth quartile *vs* first quartile of lentils consumption)] and HR: 0.67; 95% CI: 0.46, 0.99; *P*-value = 0.05 (fourth quartile *vs* first quartile of chickpeas consumption). The associations were non-significant for the intake of dry beans and fresh peas. When study participants with cancer or CVD incidence during follow-up were censored after they were diagnosed —on the assumption that their diet could have changed after the diagnosis— the results were similar. Individuals in the highest quartile of total legumes and lentils consumption had a lower risk of type 2 diabetes development than those in the lowest quartile [(HR: 61; 95% CI: 0.40, 0.93; *P*-trend = 0.03) and (HR: 60; 95% CI: 0.41, 0.90; *P*-trend = 0.04), respectively]. Otherwise, chickpeas, dry-beans and fresh-peas consumption was not associated with type 2 diabetes incidence.

Discussion

To the best of our knowledge, this is the first prospective study conducted in senior Mediterranean individuals at high cardiovascular risk evaluating the association between consumption of total legumes and its different varieties, and type 2 diabetes. The present study revealed that a higher consumption of total legumes, especially lentils, was associated with a lower risk of type 2 diabetes development. The consumption of chickpeas was borderline significantly associated with a lower risk of type 2 diabetes incidence. Nonetheless, the intake of dry beans and fresh peas was not associated with type 2 diabetes risk. It should be underlined that the theoretical effect of substituting half a serving/day of legumes for half a serving/day of other foods rich in protein or carbohydrates, including eggs, wholemeal and white bread, rice or baked potato, was associated with a significant lower risk of type 2 diabetes incidence. These findings provide new insights into the role of legume consumption in preventing type 2 diabetes.

To date there has been little evidence of the effect of legume consumption on the risk of type 2 diabetes. In agreement with our results, in the India's Third National Family Health Survey, total legume consumption was associated with a reduced prevalence of type 2 diabetes in adult women, but not in men [17]. In a similar manner, in the prospective Shanghai Women's Health Study, total legume consumption and the intake of three mutually exclusive

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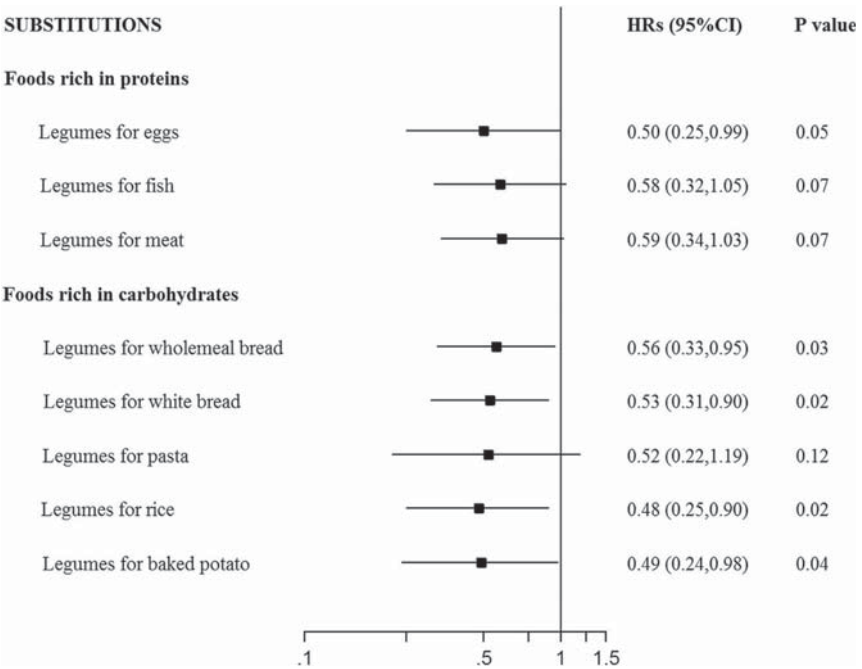

Fig. 1. The impact of substituting half a serving/day of legumes for half a serving/day of foods rich in proteins or carbohydrates on risk of type 2 diabetes. All HRs were adjusted for age (y), sex, intervention group, cumulative average of alcohol intake (continuous, adding a quadratic term), total energy intake (kcal/d), smoking status (never, former or current smoker), educational level (primary education, secondary education or academic/graduate), leisure-time physical activity (metabolic equivalent task minutes/d), baseline hypertension (yes/no), hypercholesterolemia (yes/no), use of lipid-lowering drugs (yes/no), use of antihypertensive drugs (yes/no), fasting plasma glucose at baseline (<100 mg/dL or ≥ 100 mg/dL), MedDiet adherence (13-point score) except for meat (11-point score) and fish (12-point score) and BMI (kg/m^2). Stratified by recruitment center. Extremes of total energy intake (>4000 or <800 kcal/d in men and >3500 or <500 kcal/d in women) were excluded. Half a serving/day corresponds to 30 g of raw legumes, eggs, pasta and rice; 38 g of bread; 75 g of fish and meat; 100 g of baked potato.

legume groups (soybeans, peanuts and other legumes) were associated with a protection against type 2 diabetes development [22].

Other epidemiological studies, however, have not supported this protective role of total legume consumption on type 2 diabetes risk [18–21,23]. For example, in the Indian Migration Study, no cross-sectional association was observed between legume consumption and type 2 diabetes prevalence [18]. Likewise, Meyer and co-workers [19] found no association between legume consumption and type 2 diabetes incidence in older women during six year of follow-up. Similarly, no significant association was observed in the Malmö Diet and Cancer study [21] and in the Women's Health Study [20] after evaluating a large sample consisting of 27,140 men and women, and 38,018 female health professionals, respectively. Contrary to our results, in the Nurse's health Study [23], a higher risk of type 2 diabetes was observed in those individuals in the 5th quintile *vs* those in the 1st quintile of total legume consumption. However, when they analysed the consumption as an increase of one serving/day, non-significant association was observed.

The discrepancies between our results and those of the aforementioned studies, could be explained by differences in the study design. For instance, in the present analysis we used cumulative average consumption as exposure, while all the other prospective studies, except the Nurse's Health study and the Shanghai Women's Health Study [22,23], used a single measurement at baseline. Discrepancies could also be due to the different characteristics of the study population studied. Participants from the present study were European Mediterranean individuals at high risk of CVD. However, the other studies have been conducted in Asian [17,18,22], American [19,20,23] and Northern European populations [21]. Another possible explanation is the way as the outcome was defined. In the present study, diabetes was defined following the definition of the American Diabetes Association, but in most of the studies [17,19,20,22,23] diabetes was self-reported. Finally, subtypes of legumes included in the analysis as well as the amount consumed also could explain the heterogeneity in the results. In the present study, we considered legumes as the sum of lentils, chickpeas, dry beans and fresh peas, and the main contributors to total legumes consumption were lentils and chickpeas (both subtypes inversely associated with type 2 diabetes risk in the current analysis). Nonetheless, of the 5 previous prospective published studies, only two of them distinguished between the different subtypes of legumes included [19,23], and contrary to us, they did not include lentils and chickpeas in the analysis.

Various mechanisms could explain the protective role of legume consumption against type 2 diabetes. Legumes are a low-energy but nutrient-dense food group [6] and their consumption, like that of other seeds, could improve cardiometabolic health due to their unique composition in bioactive nutrients and phytochemicals and the complex interplay among them [35]. Recently, the intake of vegetable protein, of which legumes are a good source, has been recently associated with a lower risk of type 2 diabetes in two large US prospective cohorts [36]. Legumes also contain significant amounts of calcium, potassium and magnesium, minerals which intake has been inversely related to type 2 diabetes risk [37–39].

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Furthermore, legumes also contain high amounts of polyphenols [40], predominantly phenolic acids and flavonoids with antioxidant and anti-inflammatory properties [6], which may also protect against type 2 diabetes [41]. In addition, legumes are rich in fibre [6], which is associated with higher satiety [42] and improvements in the control of body weight [43], glucose metabolism [44] as well as lower risk of type 2 diabetes incidence [45]. Another explanation for the beneficial effect on type 2 diabetes risk may be the low glycemic index of legumes, which might blunt glycemic excursions and, therefore, pancreatic insulin secretion, both of which are mechanisms involved in the development of type 2 diabetes. In this context, a pooled analysis of randomized clinical trials demonstrated that the consumption of pulses alone or combined with a low glycemic index diet rich in fibre improves markers of long term glycemic control in individuals with or without type 2 diabetes [46]. In addition, a higher risk of type 2 diabetes has been associated with high glycemic index diets, which highlights the biological plausibility of these associations [47,48].

To date, no studies have evaluated the associations between the consumption of lentils, dry beans, chickpeas, or fresh peas and type 2 diabetes incidence. In our study, lentil consumption was significantly associated with a lower risk of type 2 diabetes. The higher flavonoid content in cooked lentils compared to other cooked pulses may explain this finding [49]. However, further studies are needed to better understand the effect of lentil consumption on the incidence of type 2 diabetes and elucidate the underlying biological mechanisms.

We also examined the effect of substituting legumes for other carbohydrate-or protein-rich foods on the risk of type 2 diabetes. Our novel results suggest that replacing eggs, bread, rice or baked potato with legumes has a beneficial effect on type 2 diabetes. These findings support our previous suggestions of legumes as a good substitute for energy dense animal protein sources [6] and other more rapidly digestible carbohydrates [50].

Our study has limitations. First, because type 2 diabetes was a secondary outcome of the PREDIMED study, these analyses conducted in a subgroup of participants without type 2 diabetes should be considered exploratory in nature. Second, our sample population was comprised of older elderly Caucasian individuals at high cardiovascular risk, which limits the extrapolation of our results to other populations. Third, although we used a validated FFQ to assess diet, measurement errors are inevitable.

Our study also has strengths, such as the use of repeated dietary measurements, which allows as to reduce the random measurement error produced by within-person variation and dietary changes during follow-up; the control for many potential confounding variables; the inclusion of sensitivity analyses; and the accurate and blind assessment of incident cases of type 2 diabetes.

Conclusions

In summary, the current data suggests that a frequent consumption of legumes and particularly lentils could provide benefits on type 2 diabetes development in senior adults at high cardiovascular risk. The substitution of legumes for other protein- or carbohydrate-rich foods is also associated with a lower risk of type 2 diabetes. The present study supports an increased consumption of legumes for type 2 diabetes prevention. However, given the mixed results from previous researches, further studies are needed to confirm our findings and elucidate which mechanisms are involved.

Authors' responsibilities

ER, DC, RE, MF, LS-M, FA, RML-R, M Fiol, JL and JS-S *designed the research*. NB-T, AD-L, NR-E, ER, PB-C, DC, RE, MF, LS-M, FA, RML-R, M Fiol, JMS-L, JD-E, OP and JS-S *conducted the research*. NB-T and JS-S *analysed the data*. NB-T, AD-Land JS-S *wrote the paper*. NB-T and JS-S *had primary responsibility for final content*. All authors read and approved the final manuscript.

Conflict of interest statement and Funding sources

The authors disclose no conflict of interest related with the article.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.clnu.2017.03.015.

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Supplemental Table 1. Baseline characteristics of study population according to cumulative average quartiles of energy-adjusted subtypes of legumes consumption[1]

	Lentils		Chickpeas		Dry beans		Fresh peas	
	Q1	Q4	Q1	Q4	Q1	Q4	Q1	Q4
Lentils, g/d[2]	2.6 ± 1.6	10.9 ± 5.6‡	5.2 ± 4.6	8.5 ± 4.5‡	5.5 ± 4.6	8.6 ± 4.7‡	6.5 ± 4.4	7.1 ± 4.7‡
Chickpeas, g/d	4.3 ± 4.3	7.0 ± 4.5‡	1.9 ± 1.6	10.3 ± 4.8‡	4.3 ± 4.3	7.8 ± 4.9‡	5.4 ± 4.4	6.2 ± 4.1‡
Fresh peas, g/d	3.2 ± 12.1	3.4 ± 4.4	2.4 ± 4.3	4.1 ± 10.0‡	3.0 ± 11.2	4.1 ± 10.0‡	0 ± 0.6	8.2 ± 14.3‡
Dry beans, g/d	3.8 ± 4.4	6.7 ± 5.2‡	3.5 ± 4.1	7.3 ± 5.7‡	1.0 ± 1.3	10.6 ± 5.3‡	5.0 ± 4.9	5.8 ± 4.8‡
Age, years	66 ± 6	67 ± 6‡	66 ± 6	67 ± 6‡	67 ± 6	67 ± 6‡	66 ± 6	67 ± 6‡
Women, % (n)	53.9 (452)	73 (611)‡	53.3 (447)	68.6 (574)‡	64.0 (536)	60.9 (510)‡	55.5 (465)	70.5 (590)‡
Smoking habit, % (n)								
Never	58.6 (491)	71.6 (599)‡	56.8 (476)	67.0 (561)‡	62.2 (521)	62.8 (526)	56.9 (477)	67.9 (568)‡
Former	22.6 (189)	17.1 (143)‡	24.0 (201)	19.6 (164)‡	21.4 (179)	22.0 (184)	24.2 (203)	18.8 (157)‡
Current	18.9 (158)	11.4 (95)‡	19.21 (161)	13.4 (112)‡	16.5 (138)	15.2 (127)	18.9 (158)	13.4 (11)‡
Education, % (n)								
Primary	71.4 (598)	82.1 (687)‡	71.0 (595)	79.8 (668)‡	72.8 (610)	78.6 (658)	77.6 (650)	74.8 (626)
Secondary	17.3 (145)	12.5 (105)‡	18.5 (155)	13.7 (115)‡	17.5 (147)	14.3 (120)	14.9 (125)	17.6 (147)
University/graduate	11.3 (95)	5.4 (45)‡	10.5 (88)	6.5 (54)‡	9.7 (81)	7.1 (59)‡	7.5 (63)	7.7 (64)
Intervention group, n (%)								
MedDiet + EVOO	35.2 (295)	31.3 (262)‡	35.0 (293)	33.6 (281)	31.0 (260)	34.4 (288)‡	36.5 (306)	31.7 (265)‡
MedDiet + nuts	35.2 (295)	35.8 (300)‡	35.4 (297)	34.7 (290)	37.0 (310)	34.1 (285)‡	35.7 (299)	34.7 (290)‡
Control group	29.6 (248)	32.9 (275)‡	29.6 (248)	31.8 (266)	32.0 (268)	31.5 (264)‡	27.8 (233)	33.7 (282)‡
BMI, kg/m^2	29.8 ± 3.5	30.2 ± 3.6‡	29.8 ± 3.6	30.2 ± 3.7‡	30.0 ± 3.7	30.1 ± 3.7‡	29.9 ± 3.6	30.2 ± 3.6‡
Leisure time physical activity, METs.min/d	248 ± 237	207 ± 180‡	247 ± 239	218 ± 203‡	224 ± 221	237 ± 223	236 ± 240	216 ± 195‡
Hypertension, % (n)	91.7 (768)	92.2 (772)	89.5 (750)	92.4 (773)‡	91.8 (769)	92.2 (772)	90.8 (761)	93.1 (779)
Hypercholesterolemia, % (n)	82.0 (687)	87.3 (731)‡	81.3 (681)	87.8 (735)‡	84.7 (710)	84.4 (706)	84.1 (705)	87.0 (728)‡
Current medication use, % (n)								
Use of antihypertensive agents	76.6 (642)	78.1 (654)	74.2 (622)	78.7 (659)	76.5 (641)	78.6 (658)	76.0 (637)	79.6 (666)
Use hypolipidemic agents	45.2 (379)	54.0 (452)	46.4 (389)	53.6 (449)‡	48.1 (403)	52.8 (442)‡	48.3 (405)	54.0 (452)‡
Fasting plasma glucose, mg/dl	97.3 ± 14.7	98.8 ± 14.8‡	98.1 ± 16.6	99.2 ± 14.6	97.5 ± 14.3	99.2 ± 16.7	98.9 ± 15.3	98.1 ± 13.5
Nutrient intake								
Total energy, kcal/day	2545 ± 485	1930 ± 368‡	2561 ± 586	2091 ± 371‡	2396 ± 642	2204 ± 418‡	2492 ± 380.4	2047 ± 486‡
Total fat, % of total energy	38.9 ± 6.4	37.5 ± 6.7‡	38.1 ± 6.5	37.8 ± 6.7‡	38.1 ± 6.6	37.1 ± 6.5‡	37.4 ± 6.5	38.6 ± 6.5‡

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Carbohydrate, % of total energy	42.3 ± 7.1	43.4 ± 7.1‡	43.0 ± 7.3	43.4 ± 7.1‡	43.1 ± 7.3	43.9 ± 7.0‡	43.9 ± 7.0	42.4 ± 7.0‡
Protein, % of total energy	15.3 ± 2.5	17.4 ± 2.8‡	15.6 ± 2.7	16.8 ± 2.7 ‡	15.1 ± 2.8	16.6 ± 2.7‡	15.6 ± 2.3	17.1 ± 2.9‡
Alcohol, g/day	10.1 ± 17.4	8.0 ± 9.0‡	10.2 ± 17.8	7.9 ± 10.4‡	9.2 ± 16.4	8.7 ±13.1	9.3 ± 16.1	8.0 ± 10.5‡
Dietary fiber , g/day	25.0 ± 8.3	21.5 ± 6.2‡	25.7 ± 9.3	26.5 ± 8.3‡	24.6 ± 9.2	27.5 ± 8.8‡	26.7 ± 8.5	25.0 ± 9.6‡

[1]Data are expressed as mean ± SD for continuous variables and percentage and number (n) for categorical variables.
[2]All dietary variables were adjusted for total energy intake.
‡P value <0.05. Chi-square or ANOVA test were used for categorical variables and for continuous variables, respectively.
Abbreviations: Q, quartile; MedDiet, Mediterranean Diet; EVOO, extra virgin olive oil; METs, metabolic equivalent.

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Supplemental Table 2. HRs and 95% CIs of type 2 diabetes associated with 30g/day increase consumption of energy-adjusted legumes and its different subtypes.

	HR	95% CI	P-value
Legumes			
Crude Model	0.42	(0.25-0.73)	<0.01
Model 1	0.48	(0.29-0.81)	0.01
Model 2	0.52	(0.31-0.88)	0.02
Model 3	0.55	(0.32, 0.93)	0.03
Lentils			
Crude Model	0.12	(0.04-0.42)	<0.01
Model 1	0.14	(0.04-0.50)	<0.01
Model 2	0.17	(0.05-0.60)	0.01
Model 3	0.18	(0.05, 0.65)	0.01
Chickpeas			
Crude Model	0.32	(0.08-1.23)	0.10
Model 1	0.42	(0.11-1.61)	0.21
Model 2	0.48	(0.12-1.85)	0.29
Model 3	0.50	(0.13, 1.93)	0.32
Fresh peas			
Crude Model	0.38	(0.10-1.48)	0.16
Model 1	0.47	(0.14-1.57)	0.22
Model 2	0.47	(0.14-1.57)	0.22
Model 3	0.48	(0.14, 1.64)	0.24
Dry beans			
Crude Model	0.43	(0.13-1.41)	0.16
Model 1	0.52	(0.17-1.57)	0.24
Model 2	0.60	(0.20-1.78)	0.35
Model 3	0.67	(0.22-2.02)	0.48

Multivariable model 1 was adjusted for age (y), sex, intervention group, cumulative average of alcohol intake (continuous, adding a quadratic term), smoking status (never, former, or current smoker), educational level (primary education, secondary education, or academic/graduate), leisure-time physical activity (metabolic equivalent task minutes/d), baseline hypertension (yes/no), hypercholesterolemia (yes/no), use of antihypertensive medication (yes/no), use of lipid-lowering drugs (yes/no) and fasting plasma glucose at baseline (<100mg/dL or ≥100mg/dL). Model 2 was further adjusted for cumulative average of MedDiet adherence (13-point score). Model 3 was additionally adjusted for baseline BMI (kg/m^2). All models were stratified by recruitment center. Extremes of total energy intake (>4000 or < 800 kcal/d in men and >3500 or <500 kcal/d in women) were excluded.

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UNIVERSITAT ROVIRA I VIRGILI
DIETARY FACTORS ASSOCIATED WITH METABOLIC SYNDROME AND TYPE 2 DIABETES RISK
Nerea Becerra Tomás

VI. Discussion

UNIVERSITAT ROVIRA I VIRGILI
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The present doctoral thesis has sought to evaluate in deeper manner the association between the consumption of meat, dairy products and legumes in relation to MetS and T2D risk in Mediterranean elderly individuals at high CVD risk.

The results derived from this thesis provide new insights to better understand the role that the abovementioned food groups could play in the observed differences in relation with T2D and MetS risk among dietary patterns, particularly between Western and Mediterranean diets.

Each of the three articles presented in this dissertation included a discussion section. Nevertheless, other important aspects and additional considerations that were not commented in the articles are more extensively discussed below.

1. General discussion

The present prospective study conducted in elderly participants at high CVD risk showed: a) a beneficial association between the consumption of low-fat dairy products (milk and yogurt), low-fat milk and yogurt (total, low-fat and high-fat) and the risk of MetS development; and a positive association between cheese consumption and the incidence of MetS; b) a positive association between the consumption of total, red and processed red meat and the risk of MetS development, and an inverse association with poultry; c) a lower risk of T2D incidence with high total legume, and particularly lentils, consumption.

As a consequence of the traditional belief that fat consumption exerted detrimental effects on health, the consumption of dairy products has been reduced. However, novel evidences have emerged suggesting that dairy products may protect against different chronic diseases[348]. Some studies have indicated that not all types of fat have the same health properties, suggesting that those from dairy products may have beneficial effects on T2D and MetS risk[349]. Despite this, most of the studies conducted so far did not distinguish between high-fat and low-fat options, probably because of limitations in methods used to collect dietary data. The FFQ used in the PREDIMED study provides with the opportunity to differentiate across different varieties of commonly-consumed dairy products. In our study, whole-fat dairy products consumption was not associated with the risk of MetS, whereas low-fat dairy options

showed an inverse association. Some controversies exist in this field. Some studies have observed a lower risk of MetS associated with high consumptions of whole-fat and cheese[317], whereas others have observed a higher risk or lower risk of MetS incidence in individuals who consumed more whole-fat or low-fat dairy products, respectively[318]. These divergent results, apart from inherent differences in study populations, could be probably due to the nutrient composition of dairy products, which may vary across countries (i.e. inherent differences between breeds of dairy animals), and the use of different serving sizes. Moreover, technological innovations afforded the production of a wide variety of new dairy products, which nutritional composition has been altered by removing or adding several components, mainly minerals and vitamins. All of these new varieties have not been considered in any of the studies published up to date (including the present study), and could also contribute to the disparities in the observed results.

Findings from the present study also showed an inverse association between total, low-fat and whole-fat yogurt and the risk of MetS incidence. Although previous cross-sectional studies support a beneficial effect of yogurt on MetS risk[350,351], prospective results from the CARDIA study did not find any significant association[317]. Yogurt results from the fermentation process driven by microorganisms, in which different metabolites with potential health properties may be also produced. Besides, these bacteria could interact with the host in a positive manner. Therefore, although the nutritional profile of yogurt is comparable with that of milk, the fermentation process of yogurt could enhance its nutritional value. Moreover, it has been recently reported that yogurt has a low-glycemic index[352], and findings from prospective studies have shown a positive association between low-glycemic index diets and the risk of T2D and MetS[180,192]. One concern regarding yogurt is whether all types of yogurts have the same health effects. Whereas plain and artificially-sweetened yogurts have a low-glycemic index (mean of 27), sugar-sweetened yogurts have higher glycemic index (mean of 41)[352]. Unfortunately, in the present study we could not evaluate the possible different effects of these yogurt varieties because our FFQ was limited to total, whole-fat and low-fat yogurt. Results derived from this dissertation also demonstrated that the MetS promoting effect of meats

differs by meat subtype. The consumption of total meat, and particularly red meat and processed meat, was associated with a higher risk of MetS incidence. These findings are in line with other previous prospective studies reporting a positive association between these subtypes of meat consumption and the risk of MetS development[308,309]. White meat consumption has not been extensively investigated in prospective studies. Previously, only one study specifically evaluated its consumption with the risk of MetS incidence, showing no significant associations[310]. In our study, poultry intake *per se,* and the replacement of red meat and processed red meat with poultry has been inversely associated with the risk of MetS development. It is well known that red meat and processed red meat have higher amounts of heme iron and SFAs than poultry. Moreover, processed red meat contains other additives, such as nitrites, nitrates and salt, which have been associated with a higher risk of T2D and endothelial dysfunction[353,354]. The replacement of red meat and processed meat with poultry could displace the intake of these constituents, and therefore, reduce the risk of MetS. It is important to point out that studies published to date did not take into account the type of cooking methods used, which may represent another important aspect to consider when evaluating the effects of meat consumption on human health. A recent study conducted in the framework of the NHS demonstrated that high-temperature and/or open-flame cooking methods for red meat could be positively associated with the risk of T2D beyond red meat intake[355]. Therefore, it would be of great interest evaluating the effect of different cooking methods not only for red meat, but also for processed red meat and poultry. Another important aspect to take into consideration in observational epidemiological studies, is the difficulty in accurately estimating meat consumptions[356] in view of the fact that usually meat is consumed as a part of composite meals, including other food components such as pasta, legumes or vegetables.

Importantly, findings from the present doctoral thesis showed, on one hand, a beneficial association between total legumes consumption and the risk of T2D development. These observations reinforce the results derived from the theoretical replacement analyses of red meat and processed red meat, which showed a lower

risk of MetS incidence when its consumption was replaced with legumes. This food group has a unique nutritional composition value characterized by high amount of dietary fiber, minerals (calcium, potassium and magnesium), vitamins, bioactive components (mainly polyphenols) and plant proteins. Moreover, it is also considered a low-glycemic index food[269]. The synergy between all of these healthy components could be the responsible for the beneficial effects on T2D prevention associated to its consumption.

On the other hand, findings from the substitution analyses suggest that legumes could be a good alternative for other protein- and carbohydrate rich foods such as, eggs, bread, rice and baked potato, highlighting the importance of choosing this food rather than others in the context of a healthy dietary pattern.

Finally, we also reported, for the first time, the association between different non-soy legumes subtypes and the risk of T2D incidence. Only lentils consumption was associated with a lower risk of T2D development. It is difficult to elucidate the possible mechanisms responsible for this protection. We speculate that it could be due to the flavonoid content of lentils because its amount is higher compared to the other type of legumes and the consumption of this polyphenol has been associated to a lower risk of T2D in a meta-analysis of prospective cohort studies[357]. Large well-conducted randomized clinical trials are needed in order to clarify its effects on T2D risk and elucidate the possible mechanisms implicated in this association.

The epidemiological studies derived from the present thesis suggest that a dietary pattern with high consumption of low-fat dairy products and yogurt (regardless the fat content), together with the preference for poultry rather than red meat or processed red meat, and high frequency consumption of legumes, would be beneficial for the prevention of MetS and T2D among individuals at high CVD risk.

Balance and variety is essential to healthy eating. Therefore, dietary patterns, as the MedDiet, which also include high frequency consumption of whole grains, vegetables, fruits, nuts and vegetable oils, along with the aforementioned characteristics, should be encouraged for the reduction of MetS and T2D incidence in elderly Mediterranean individuals at high cardiovascular risk.

2. Strengths and limitations

Several strengths and limitations derived from the present work deserve to be tackled. The first limitation is about sampling. Our study population included Mediterranean elderly individuals at high cardiovascular risk. Therefore, we cannot extrapolate our results to others and, consequently, further studies conducted in other populations are needed in order to confirm our findings.

Second, we should be careful when drawing inference about causation, given the observational nature of the study design, which only allows us to claim associations.

Third, also due to the observational design, results might be difficult to interpret because people often adopt other healthy or unhealthy behaviors (for instance increase or decrease physical exercise, reducing or increasing smoking, eating other healthy or unhealthy foods, etc.) associated to the consumption of the analyzed foods, which clearly affect their health status. Although we took into account these lifestyle factors adjusting all statistical models for it, residual confounding factors there may still be present.

Fourth, we used a FFQ to estimate habitual dietary intake, which is susceptible for measurement error because participants are unable to exactly report their consumption of foods during the preceded year. However, FFQ is the method of dietary data collection that has been most widely used in large epidemiological studies. Moreover, the FFQ of the PREDIMED study was validated against a 3-day food record, which is an independent superior reference method.

Fifth, MetS and T2D were secondary end-points in the PREDIMED study, making the results exploratory in nature.

The present work also has some strengths that need to be highlighted. The relatively long follow-up; the control for a large number of potential confounders; the use of yearly updated FFQ that allows us to better represent the long-term diet and to reduce the random measurement error due to within person variation; the analyses of different subtypes of dairy products, meat and legumes consumption; the diagnosis of T2D was based on the criteria proposed by the ADA, and was confirmed

with a second analytics conducted within the following 3 month; the End-point Adjudication Committee was blinded to the treatment allocation.

VII. Conclusions

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The conclusions derived from this prospective cohort study of Mediterranean elderly individuals at high cardiovascular risk are presented as a response of each hypothesis specified at the beginning of the present doctoral thesis:

Hypothesis 1: A greater intake of dairy products is associated with a reduced risk of MetS or any of its components incidence.

- Total dairy consumption was not associated with the risk of MetS incidence. Higher consumption of low-fat dairy products, yogurt (total, low-fat, and whole-fat yogurt) and low-fat milk was associated with a reduced risk of MetS incidence and some if its components. Contrary, higher consumption of cheese was related to a higher risk of MetS incidence.

Hypothesis 2: Higher consumption of meat, and particularly red meat and processed red meat, is associated with an increased risk of MetS or any of its features development.

- Total meat consumption was associated with a higher risk of MetS development and some of its components. The risk differs according to the subtype of meat consume. Whereas red meat and processed red meat consumption was associated with a higher risk, poultry consumption was associated with a lower risk.
- The theoretically replacement effect of red meat and processed red meat for other protein-rich foods such as eggs, fish, poultry and legumes is associated with a lower risk of MetS incidence.

Hypothesis 3: Higher consumption of legumes is inversely associated with the risk of T2D incidence.

- Total legume consumption, particularly lentils, was associated with a lower risk of T2D incidence. The consumption of chickpeas, dry beans and fresh peas was not associated with T2D risk.

The substitution effect of legumes for eggs, rice, bread and baked potato is associated with a lower risk of T2D incidence.

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VIII. Global and future insights

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MetS and T2D are metabolic disturbances that can be prevented with changes in lifestyle. Results from the present research add further scientific evidence to understand the role that dairy products, meat and legumes consumption plays on the risk of MetS and T2D development. Importantly, it could help to form the basis for dietary guidelines recommendations for preventing both chronic diseases.

Based on our findings, we could consider several important aspects regarding future research in the field of the aforementioned foods and the prevention of MetS and T2D:

- It is important to conduct prospective studies in different populations from diverse geographic areas in order to confirm our results and could extrapolate it to the general population.

- Dietary assessment tools used in epidemiological studies should be improved, in order to better represent the usual consumption:
    - Considering all new varieties of dairy products (i.e. plain, artificially-sweetened or sugar-sweetened yogurts)
    - Taking into consideration the type of cooking methods
    - Standardizing serving sizes

  This would help to compare findings from different studies and to translate the results to the general population in a clear, unambiguous and easily understandable manner.
- Long-term randomized clinical trials with large number of participants, evaluating the effect of different varieties of dairy products, meat and legumes on the risk of MetS and T2D development are required in the future to stablish solid based-evidence recommendations.

- Due to the fact that mechanisms implicated in the associations between dairy products, meat and legumes consumption and the risk of MetS and T2D are not fully understood, it is important to focus future investigations in this sense.The risk of T2D and MetS could vary according to the genetic background. Therefore, studies based on gene-diet interactions are also warranted in order to better understand the relation between specific

genetic loci, the consumption of the dairy products, meat and legumes and the risk of MetS and T2D incidence.

- In the last decade special attention has been focused on microbiota. It has been reported that changes in its composition in response to both internal and external stimulus are associated with different diseases such as obesity, T2D and MetS. As consequence, evaluating the effect of diet-microbiota interactions and the risk of MetS and T2D would be of great interest to describe other new possible mechanisms regarding the observed associations in the present doctoral thesis. Moreover, a better understanding of the role of the diet-microbiota interaction in human health will allow the development of new preventive and therapeutic measures.

IX. References

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X. Appendices

UNIVERSITAT ROVIRA I VIRGILI
DIETARY FACTORS ASSOCIATED WITH METABOLIC SYNDROME AND TYPE 2 DIABETES RISK
Nerea Becerra Tomás

1. Scientific contributions

Publications derived from the present work:

Babio N, **Becerra-Tomás N**, Martínez-González MÁ, Corella D, Estruch R, Ros E, Sayón-Orea C, Fitó M, Serra-Majem L, Arós F, Lamuela-Raventós RM, Lapetra J, Gómez-Gracia E, Fiol M, Díaz-López A, Sorlí JV, Martínez JA, Salas-Salvadó J; PREDIMED Investigators. Consumption of Yogurt, Low-Fat Milk, and Other Low-Fat Dairy Products Is Associated with Lower Risk of Metabolic Syndrome Incidence in an Elderly Mediterranean Population. J Nutr. 2015;145(10):2308-16. PubMed PMID: 26290009.

Becerra-Tomás N, Babio N, Martínez-González MÁ, Corella D, Estruch R, Ros E, Fitó M, Serra-Majem L, Salaverria I, Lamuela-Raventós RM, Lapetra J, Gómez-Gracia E, Fiol M, Toledo E, Sorlí JV, Pedret-Llaberia MR, Salas-Salvadó J. Replacing red meat and processed red meat for white meat, fish, legumes or eggs is associated with lower risk of incidence of metabolic syndrome. Clin Nutr. 2016;35(6):1442-1449. PubMed PMID: 27087650.

Becerra-Tomás N, Díaz-López A, Rosique-Esteban N, Ros E, Buil-Cosiales P, Corella D, Estruch R, Fitó M, Serra-Majem L, Arós F, Lamuela-Raventós RM, Fiol M, Santos-Lozano JM, Díez-Espino J, Portoles O, Salas-Salvadó J; PREDIMED Study Investigators. Legume consumption is inversely associated with type 2 diabetes incidence in adults: A prospective assessment from the PREDIMED study. Clin Nutr. 2017 Mar 24. pii: S0261-5614(17)30106-1. doi: 10.1016/j.clnu.2017.03.015. [Epub ahead of print] PubMed PMID: 28392166.

Other publications:

Becerra-Tomás N, Estruch R, Bulló M, Casas R, Díaz-López A, Basora J, Fitó M, Serra-Majem L, Salas-Salvadó J. Increased serum calcium levels and risk of type 2 diabetes in individuals at high cardiovascular risk. Diabetes Care. 2014;37(11):3084-91. PubMed PMID: 25139884.Babio N, Martínez-González MA, Estruch R, Wärnberg J,

Recondo J, Ortega-Calvo M, Serra-Majem L, Corella D, Fitó M, Ros E, **Becerra-Tomás N**, Basora J, Salas-Salvadó J. Associations between serum uric acid concentrations and metabolic syndrome and its components in the PREDIMED study. Nutr Metab Cardiovasc Dis. 2015;25(2):173-80. PubMed PMID: 25511785.

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2. Participation in national and international conferences

Conference: III World Congress of Public Health Nutriton. Las Palmas de Gran Canaria, Spain. 9-12 November 2014**.**
Authors: Becerra-Tomás N, Estruch R, Bulló M, Casas R, Díaz-López A, Basora J, Fitó M, Serra-Majem L, Salas-Salvadó J**.**
Title: Increased serum calcium levels and risk of type 2 diabetes in individuals and high cardiovascular risk.
Format: Poster.
Publication: Int J Comm Nutr 2014; 0S: 162.

Conference: VI Symposium Ciber Fisiopatologia de la Obesidad y Nutrición – Obesity and Nutrition in the 21st Century. Madrid, Spain. 20-22 November 2014**.**
Authors: Becerra-Tomás N, Estruch R, Bulló M, Casas R, Díaz-López A, Basora J, Fitó M, Serra-Majem L, Salas-Salvadó J**.**
Title: Increased serum calcium levels and risk of type 2 diabetes in individuals and high cardiovascular risk.
Format: Poster.
Publication: Abstract book; p.40

Conference: III Congreso de la Federación Española de Sociedades de Nutrición y Dietética (FESNAD). Sevilla, Spain. 5-7 November 2014**.**

Authors: Babio Sánchez N, **Becerra-Tomás N**, Martínez-González MA, Corella Piquer D, Estruch Ribas R, Ros Rahola E, Sayón-Orea C, Fitó Colomer M, Serra-Majem L, Arós Borau F, Lamuela-Raventós RM, Lapetra Peralta J, Gómez-Gracia E, Fiol Sala M, Pintó Sala X, Díaz-López A, Martínez Hernández JA, Salas-Salvadó J**.**

Title: Ingesta de leche, yogur y otros productos lácteos y riesgo de desarrollar síndrome metabólico en una población con alto riesgo cardiovascular.

Format: Poster.

Publication: Nutr Clin Med 2015; 1: 33

Conference: 34th International symposium on diabetes and nutrition. Prague, Czech Republic. 29 June-1 July 2016**.**

Authors: Becerra-Tomás N, Babio N, Martínez-González M.A, Corella D, Estruch R, Ros E, Fitó M, Serra-Majem Ll, Salaverria I, Lamuela-Raventós R.M, Lapetra J, Gómez-Gracia E, Fiol M, Toledo E, Sorlí J.V, Pedret-Llaberia M.R, Salas-Salvadó J.

Title: Replacing red meat and processed red meat for White meat, fish, legumes or eggs is associated with lower risk of incidence of metabolic syndrome.

Format: Poster.

Conference: 35th International symposium on diabetes and nutrition. Skagen, Denmark. 19-22 June 2017**.**

Authors: Becerra-Tomás N, Díaz-López A, Rosique-Esteban N, Ros E, Buil-Cosiales P, Corella D, Estruch R, Fitó M, Serra-Majem L, Arós F, Lamuela-Raventós RM, Fiol M, Santos-Lozano JM, Díez-Espino J, Portoles O, Salas-Salvadó J; PREDIMED Study Investigators.

Title: Legume consumption is inversely associated with type 2 diabetes incidence in adults: A prospective assessment from the PREDIMED study.

Format: Oral communication. Young Investigator Award

3. Mobility

Length: 3 months (March - May 2017)

Institution: Department of Nutritional Sciences, Faculty of Medicine, University of Toronto and Toronto 3D Knowledge Synthesis and Clinical Trials Unit, Clinical Nutrition and Risk Factor Modification Centre, St. Michael's Hospital, Toronto, ON, Canada.

Supervisor: John L Sievenpiper.

Objective: Participate in the confection of a European Association for the Study of Diabetes (EASD) commissioned systematic review and meta-analysis with GRADE assessment of the Mediterranean diet and cardiovascular disease in diabetes (PROSPERO 2017:CRD42017057885).

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DIETARY FACTORS ASSOCIATED WITH METABOLIC SYNDROME AND TYPE 2 DIABETES RISK
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