VITAMIN K & HEALTHY AGEING

María Lucía Camacho Barcia

UNIVERSITAT ROVIRA i VIRGILI

VITAMIN K & HEALTHY AGEING

LUCÍA CAMACHO BARCIA

DOCTORAL THESIS

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DOCTORAL THESIS

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UNIVERSITAT ROVIRA i VIRGILI

Human Nutrition Unit

Department of Biochemistry and Biotechnology

Rovira i Virgili University

Reus, Tarragona

2020

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Mònica Bulló, Associate Professor of Nutrition and Food Science at the Department of Biochemistry and Biotechnology of Rovira i Virgili University,

I STATE:

That the present study, entitled "VITAMIN K & HEALTHY AGEING", presented by Ms. Lucía Camacho Barcia 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 fulfils the requirements for an international mention.

Reus, 9th March 2020

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Jordi Salas Salvadó, Professor of Nutrition and Food Science at the Department of Biochemistry and Biotechnology of Rovira i Virgili University,

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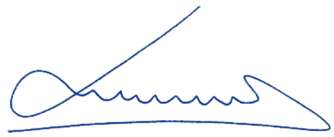

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"All we have to decide is what to do with the time that is given us"

J.R.R. Tolkien, 1954.

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ABSTRACT

ENGLISH

The demographic transition headed for an older society is becoming a challenge for the global social economy, especially for the public health care systems. Evidence, supporting different promoters of health on age-related conditions is necessary in order to improve the quality of life towards a heathier ageing process. Vitamin K has been suggested to play a modulatory role in ageing and age-related mechanisms such as oxidative stress, inflammation and insulin resistance. However, the current evidence regarding vitamin K's association with the risk of age-related diseases is still scarce.

The main objective of this work was to evaluate, the association between the dietary vitamin K1 intake and the risk of cataracts, diabetic nephropathy and retinopathy, cognitive function decline and dementia. The present work was conducted in the framework of the PREDIMED and the PREDIMED-plus studies, both large multicentre, parallel, randomized clinical trials carried out on elderly Mediterranean populations at high cardiovascular risk.

Results derived from the present work showed significant positive associations between dietary vitamin K1 intake and the risk of cataracts, diabetic nephropathy and dementia. Likewise, an increment in the consumption of vitamin K1 was associated with better performance in cognitive functioning scores.

The main conclusion derived from this Doctoral Thesis is that compared to a low intake, a high dietary vitamin K1 intake is associated with healthy ageing through decreasing the risk of different age-related diseases among an elderly Mediterranean population at high cardiovascular risk.

RESUMEN

CASTELLANO

La transición demográfica hacia una sociedad más envejecida se ha convertido en un desafío para la economía social global, especialmente para los sistemas públicos de salud. Evidencia que soporte diferentes promotores de salud en condiciones relacionadas con la edad son necesarias para mejorar la calidad de vida hacia un proceso de envejecimiento más saludable. Se ha sugerido previamente que la vitamina K podría desempeñar un papel modulador en el proceso de envejecimiento y los mecanismos relacionados con la edad, como el estrés oxidativo, la inflamación y la resistencia a la insulina. Sin embargo, la evidencia actual sobre la asociación del consumo de vitamina K y el riesgo de enfermedades asociadas al envejecimiento es aún escasa.

El objetivo principal de este trabajo fue evaluar la asociación entre la ingesta dietética de vitamina K1 y el riesgo de cataratas, nefropatía y retinopatía diabética, el deterioro de la función cognitiva y la demencia. El presente trabajo se realizó en el marco de los estudios PREDIMED y PREDIMED-plus, ambos grandes ensayos clínicos aleatorizados, multicéntricos, paralelos realizados en poblaciones Mediterráneas de edad avanzada con alto riesgo cardiovascular.

Los resultados derivados del presente trabajo mostraron asociaciones positivas significativas entre el consumo dietético de vitamina K1 y el riesgo de cataratas, nefropatía diabética y demencia. Asimismo, un incremento en el consumo de vitamina K1 se asoció con un mejor desempeño en los scores de función cognitiva.

La conclusión principal derivada de esta Tesis Doctoral es que, en comparación con una ingesta baja, un alto consumo dietético de vitamina K1 se asocia con un envejecimiento saludable al disminuir el riesgo de diferentes patologías asociadas al envejecimiento en una población Mediterránea de edad avanzada con alto riesgo cardiovascular.

RESUM

CATALÁ

La transició demogràfica cap a una societat més envellida ha esdevingut un desafiament per l'economia social global, especialment pels sistemes de salut públics. Per tal de millorar la qualitat de vida fins un procés d'envelliment més saludable, son necessàries evidències que suportin diferents promotors de salut en condicions relacionades amb l'edat. Prèviament, s'ha suggerit que la vitamina K podria desenvolupar un paper modulador en el procés de l'envelliment i els mecanismes relacionats amb l'edat, com l'estrès oxidatiu, la inflamació i la resistència a la insulina. No obstant, l'evidència actual sobre l'associació de la ingesta de vitamina K i el risc de malalties associades a l'envelliment és escassa.

L'objectiu principal d'aquest treball va ser avaluar l'associació entre la ingesta dietètica de vitamina K1 i el risc de cataractes, nefropatia i retinopatia diabètica, el deteriorament de la funció cognitiva i la demència. La present tesi doctoral s'ha realitzat en el marc dels estudis PREDIMED i PREDIMED-plus, ambdós grans assajos clínics aleatoritzats, multicèntrics i paral•lels, realitzats en poblacions mediterrànies d'edat avançada amb elevat risc cardiovascular.

Els resultats derivats del present treball van mostrar associacions positives significatives entre el consum dietètic de vitamina K1 i el risc de cataractes, nefropatia diabètica i demència. Tanmateix, un increment en la ingesta de la vitamina K1 es va associar amb una millor puntuació en índexs de funció cognitiva.

La conclusió principal obtinguda d'aquesta tesi doctoral és que, en comparació amb una ingesta baixa, un consum dietètic elevat de vitamina K1 s'associa amb un envelliment saludable al disminuir el risc de diferents patologies associades a l'envelliment d'una població mediterrània d'edat avançada amb elevat risc cardiovascular.

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ABBREVIATIONS

ACR	Albumin-to-creatinine ratio
AD	Alzheimer's disease
AGEs	Advanced glycation end products
AI	Adequate intake
ANOVA	Analysis of variance
BMD	Bone mineral density
BMI	Body mass index
BUN	Blood urea nitrogen
CDT	Clock Drawing Test
CHD	Coronary heart disease
CI	Confidence Intervals
CKD	Chronic kidney disease
CRP	C-reactive protein
CVD	Cardiovascular diseases
cVKDP	Carboxylated vitamin K dependent proteins
DC	Diabetes complications
DLBC	Dementia with Lewy Bodies Consortium
DN	Diabetic nephropathy
DNA	Deoxyribonucleic acid
DR	Diabetic retinopathy
eGFR	Estimated glomerular filtration rate
EVOO	Extra -virgin olive oil
FAO	Food and Agriculture Organization
FFQ	Food Frequency Questionnaire
GGCX	γ-glutamyl carboxylase enzyme
Gla	γ-carboxylglutamate
Glu	Glutamate residues
HR	Hazard Ratios
IKK	Nuclear factor kB kinase
IL-6	Interleukin-6
iNPH	Idiopathic normal-pressure hydrocephalus
IR	Insulin resistance
MeDiet	Mediterranean diet

MGP	Matrix Gla protein
MMSE	Mini-Mental State Examination
NF-kB	Nuclear factor kB
NHS	Nurses' Health Study
NINCDS-ADRDA	National Institute of Neurologic, Communicative Disorders and Stroke – Alzheimer's disease and Related Disorders Association
NINDS-AIREN	National Institute of Neurological Disorders and Stroke
OC	Osteocalcin
OR	Odds Ratio
PREDIMED	PREvención con DIeta MEDiterránea
QoL	Quality of Life
RDA	Recommended Dietary Allowance
ROS	Reactive oxygen species
RTC	Randomized clinical trials
SASP	Senescence-associated secretory phenotype
SD	Standard deviations
T2D	Type 2 diabetes
TMT	Trail Making Test
TNF-α	Tumour necrosis factor-α
ucVKDP	Uncarboxylated vitamin K dependent proteins
ULs	Upper Limits
UN	United Nations
USDA	United States Department of Agriculture
VF	Verbal Fluency Test
VKDP	Vitamin K Dependent Proteins
WAIS III - DS	Wechsler Adult Intelligence Scale III - Digit Span
WHO	World Health Organization

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A. INTRODUCTION

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1. HEALTHY AGEING

From a biological point of view, ageing is the consequence of an accumulation of molecular and cellular damages that occur over time. This process naturally results in a progressive decline of the physical and mental capacities that gradually increases the risk of several diseases and latter death[1]. The World Health Organization (WHO) defined healthy ageing as "the process of developing and maintaining the functional ability that enables wellbeing in older age". In the last decades, advances in science and technology have increase our life expectancy making the rate of elderly population dramatically higher. Changes in the public health action are necessary to confront this demographic transformation, including the way we conceive it. The new concept "healthy ageing" was built in terms of health and wellbeing but also enabling the elderly active participation in society. Public health strategies are now trying to focus on improving the individuals capacities of remain active and independent, enhancing the physical and mental capacities, rather than focusing only in the absence of disease[2].

1.1. Demographic and epidemiological changes

Worldwide population is rapidly ageing. For the first time in history, the number of people aged 60 years and older outnumber the children younger than 5 years old. Predictions are that, by the year 2050, the number of people over 60 years will increase from a 12% to a 22% of the world's proportion population, reaching up to a total number of two billion people[3]. In Europe, the projections shows the same tendency, an increase from 17.4% to a nearly 30% by 2060 of the population aged 65 and older[4].

As showed in figure 1, this demographic transition will change the shape of the world population pyramid. The United Nations (UN) projects that it will shift from a stage with high birth rates and high death rates, to a one with low birth rates, low death rates[5].

VITAMIN K & HEALTHY AGEING

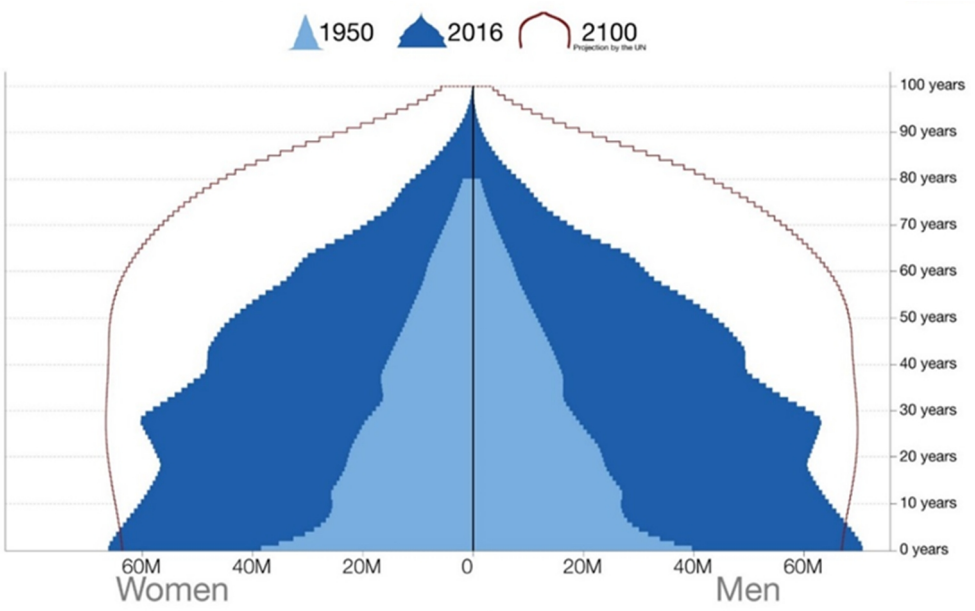

Figure 1. World population pyramid in 1950, 2016 and 2100 projection. Age distribution of the world population by sex on the years 1950, 2016 and UN Population Division´s projections until 2100[5].

This phenomenon is determined by two major causes, improvements in longevity and a decline in the global fertility rates. The increase in the life expectancy has established that in many countries, especially in developed ones, the oldest old (people aged 85 or older) are now the fastest growing part of the total population. This age group is projected to increase, on a global level, 351% by the year 2050[6].

As this demographic transition is a recent global event, most countries have not yet generated an effective health response to the new disease patterns associated to ageing populations. It is projected that the global burden of disease in older people will increase in line with the older population growth. Since population ageing is the most important driver of the chronic disease epidemic, the grater increase will particularly occur in disorders strong associated with age like heart diseases, cancer, dementia, diabetes and chronic obstructive pulmonary disease[7]. The patterns of healthcare economic costs will be influenced by these changes.

Nowadays the global burden of disease associated to older people ascends to a 23 %, numbers expected to grow in the next year. From this percentage, mental disorders are reported as the leading cause of disability and ill health[7].

1.2. Health decline in ageing

As age increases, also does many health disorders that can have a significant impact on the person's everyday life. Despite of the presence of one or more diseases, many older adults remain healthy and maintain good functional ability and well-being. Even though there is a marked diversity on the changes experienced at individual level, there is a general trend when the whole population is considered[1].

The underlying changes of this stage of life are characterised by a lifelong gradual accumulation of molecular and cellular damage. All these changes result in a generalized impairment of many organic functions that increases the risk of disease and death. The decline in the immune system enhance the susceptibility to infectious diseases and several chronic conditions, like diabetes or atherosclerosis characterized by a pro-inflammatory state[8]. As people age, the presence of multiple chronic conditions at the same time, multimorbidity, is more likely to occur. This has a significant impact on functionality and quality of life. Multimorbidity not only increases the risk of mortality, but also the individual risk of declines in capacities and the economic rates of health-care[9].

Changes in the movement functions are very characteristic during the ageing process. As the muscle mass trends to decline, so does the strength and the musculoskeletal function[10]. With age, bone density tends to drop, especially among premenopausal women, increasing significantly the risk of osteoporosis. The higher risk of fractures have serious implications in the quality of life, increasing disability and mortality[11].
Ageing is also frequently associated with declines in sensory functions. Hearing and vision loss have important implications for the older adult everyday lives.

These sensory impairments affect communication; contribute to social isolation and loss of autonomy. Consequently, the presence of visual and hearing impairment have been independently associated with the decision to retire from employment[12].

Deterioration of some cognitive functions, such as memory and speed of information processing, is also quite common in older people. However, this decrease in the cognitive capacities vary greatly among persons and is influenced by many factors, including the presence of chronic diseases conditions and the use of medication, but also by the years of education, lifestyle, socioeconomic status and health care access[13].

2. MECHANISMS OF AGEING: OXIDATIVE STRESS, INFLAMMATION AND INSULIN RESISTANCE

Ageing is not a linear multifaceted phenomenon, but a sum of distinct aspects that occur at different levels (Figure 2) such as molecular, cellular, physiologic and functional levels, as well as by the disease state[14].

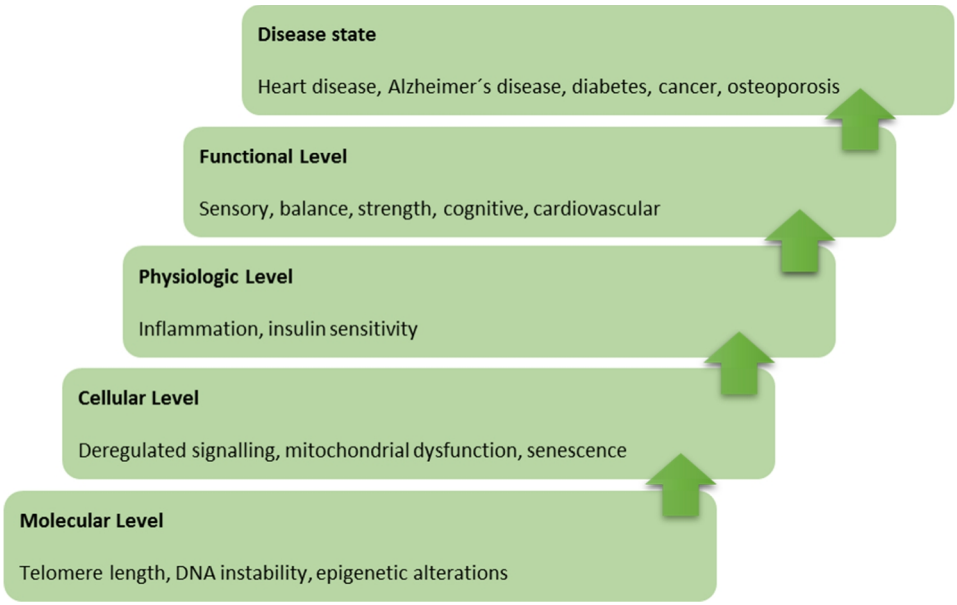

Figure 2. Multifaceted aspects of ageing. Modified from Marsman, D et al.[14]

At molecular level, telomere attrition, genomic instability and epigenetic alterations are believed to determine cellular lifespan. It has been proposed that the accumulation of unrepaired molecular damage leads to cellular and tissue dysfunction, resulting in an increased frailty and age-related diseases. The exposure of sources of damage over a lifespan vary among individuals. Some are intrinsic, like reactive oxygen species (ROS), and others extrinsic such as irradiation or exposure to pollutants. Other contributing factors that can influence this individual variety can be genetics, epigenetics, diet and physical activity[15]. ROS are constantly generated in the organism; yet, oxidative stress occurs when an abnormally high concentration is reached. Damaging effects produced by

oxidative stress play a key role in the ageing mechanisms[16]. It has been suggested that lipoxidative damage of the cells membrane phospholipids[17], proteins and DNA damage contributes to this age-related changes[18].

However, oxidation related damage is not the only mechanism associated; ageing is also characterized by a chronic low-grade pro-inflammatory state defined as "inflamm-ageing". This process, mediated by the age-related increases in inflammatory mediators such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α), appear to contribute to the inception and progression of age-related chronic diseases like cardiovascular diseases (CVD), dementia and osteoarthritis among others[18].

Likewise, ageing increments in the TNF-α and IL-6 concentrations can also influence insulin action, and may be one of the reasons associated to the development of insulin resistance (IR) observed in elderly humans[19]. Moreover, increased insulin signaling on energy metabolism could increment the generation of ROS, leading to cellular damage. IR leads to chronic hyperglycaemia, which also promotes ageing through accumulation of advanced glycation end products (AGEs)[20].

More recently, another concept was presented: "garb-ageing". It refers to the accumulation of "molecular garbage", consisting on altered macromolecules that result from the damage or dead cells or organelles. The aggregation of these molecules happens because of an age-impaired decline in cell autophagy. The accumulation of this "garbage molecules" lead to a continuous activation of inflammasome in macrophages[21].

All these stressors can trigger cell senescence, a quiescent cell state with a pro-inflammatory phenotype. This altered phenotype is known as senescence-associated secretory phenotype (SASP) and is related with the production of elevated levels of pro-inflammatory cytokines and other pro-inflammatory molecules. With ageing, SASP accumulates in tissues promoting local and systemic inflammation[22].

3. ROLE OF NUTRITION IN HEALTHY AGEING

Nutritional requirements change through the course of life. For the elderly in particular, nutrition can play a determinant role as one of the fundamental modifiable factors that can affect have the potential to promote and maintain a healthy ageing process[23]. Diet and lifestyle, added to a healthy body weight, are crucial for healthy aging and for reducing the risk of developing disease, maintaining functional independence and therefore promoting continued independent living[24].

Diet quality, defined as proper nutrient variety and adequate caloric intake, have been associated with better functional status and with higher physical and emotional Quality of Life (QoL) scores in older adults[25]. Otherwise, poor nutritional status in elder people increases the susceptibility to disease and the severity of illness[26]. A healthy lifestyle that incorporates a well-balance diet and physical activity is associated with a healthful, active and independent old age[27]. However, ageing increases the risk of malnutrition due to several physiological and social changes inherent of this period of life, and achieving an optimal nutritional status becomes a difficult task. Evidence suggest that, in an elder population, a poor nutritional status increases the susceptibility to disease[28]. For this reason, several health organizations recommend specific dietary guidelines for healthy elderly population[29,30].

Numerous dietary factors, including total energy, specific nutrients and other bioactive compounds may influence individually and collectively the cell damage, and therefore, the ageing process. Nonetheless, the strongest evidence nowadays is focused on those 'key' nutrients or food groups in the context of the whole diet[31,32]. There is robust evidence that adhering to Mediterranean-style dietary patterns, with high consumption of nutrient-dense food, unsaturated fatty acids and anti-oxidants contributes to a healthy ageing process. The PREDIMED study intervention showed clear evidence that a Mediterranean dietary pattern improved several cardiovascular related factors, such as blood pressure, LDL-cholesterol

and fasting glucose in a high cardiovascular risk elderly population[33] suggesting that is never too late to migrate to a healthy dietary pattern. Additionally, a meta-analysis of prospective studies and a recent review showed that adherence to a Mediterranean dietary pattern was associated with significantly reduced incidence of CVD and cancer and reduce mortality for both diseases, as well as a reduced total mortality[34,35].

Beyond the evidence of positive effects of healthy dietary patterns on ageing, and focusing on the hypothesis that damage to cellular mechanisms and tissues result from oxidative stress and inflammatory process, interest rises in whether certain antioxidant dietary compounds, mainly vitamins and minerals, might limit oxidative stress and inflammatory damage and lower the risk of some age-related diseases[36]. There is emerging evidence that omega-3 fatty acids, B vitamins, vitamin D and calcium could play a role in healthy ageing[37]. Increased intakes of omega-3 fatty acids have been related with improvements in memory faculties[38,39] and in levels of leucocyte telomere oxidative stress[40], as well as higher muscle protein synthesis[41]. Vitamin B_{12} and folic acid intake were linked to better cognitive function[42], and vitamin D, especially in combination with calcium, with fracture prevention[43]. Vitamin K has emerged as an important ageing nutrient with pleotropic effects on metabolism being object of the present thesis.

4. VITAMIN K

4.1. Definition, structure and dietary sources

The term 'vitamin K' refers to a family of fat-soluble compounds that share a naphthoquinone ring as a common chemical structure, but differ in the poly-isoprenyl lipophilic side chain in length and saturation[44]. Vitamin K exists naturally in two forms: Phylloquinone (also known as vitamin K1), present in all photosynthetic plant organisms, green algae and certain cyanobacteria; and Menaquinones (usually refer as vitamins K2), primarily from bacterial origin. Phylloquinone molecular form has a partially unsaturated side chain formed of one isopentenyl followed by three isopentyl units, while menaquinones have a fully unsaturated side chain composed of between 2 and 13 isopentenyl units[45] (Figure 3).

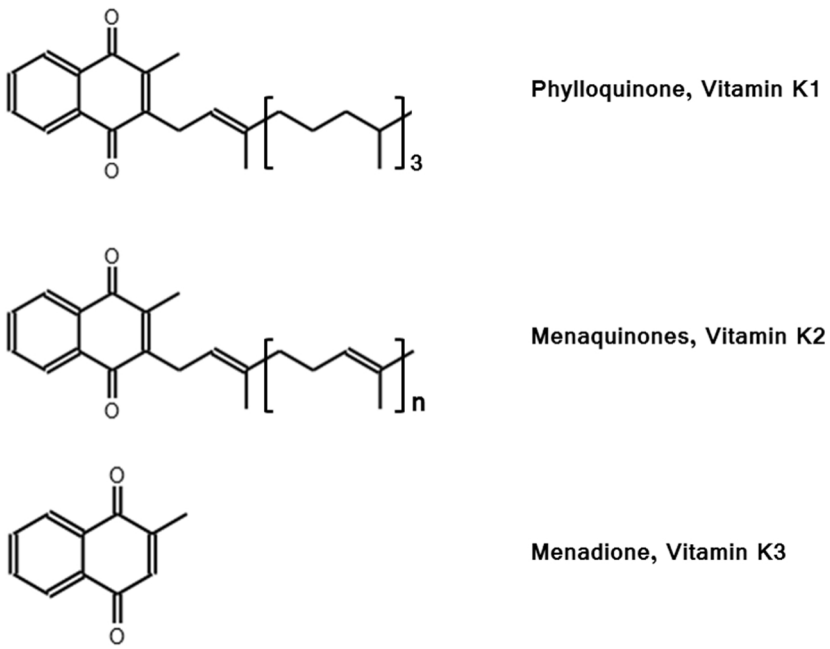

Figure 3. Molecular forms of vitamin K.

The majority of Menaquinones contain between 4 and 10 repeated isoprenoid units. Their names are designated based on the length of their side chain, usually call MK-n, being "n" the number of isoprenoid units. Human microbiota produces almost every type of Menaquinone, in particular the long-chain ones. Menaquinone-4 is the only type that does not come from bacterial origin but from tissue-specific conversion from phylloquinone. MK4 can also be produced by a re-alkylation step from Menadione, present in the animal feeds or consumed by humans as pharmaceutical drug. Menadione is a synthetic form, also known as vitamin K3. Its molecular structure is constitute uniquely by a naphthoquinone ring, without the isoprenoid side chain[44,45].

Vitamin K1 is the primary dietary source for humans. The highest content is found in green leafy vegetables and certain vegetable oils. On the other hand, dietary intake of Menaquinones forms of vitamin K comes from fermented products like fermented types of dairy and fermented vegetables (Table 1).

MK-4 is found in other animal-based foods, like some types of meats and eggs. Even though Phylloquinone food content is higher, its quantification also results easier because food composition data for Menaquinones is very limited. MK-4, MK-7 and MK-9 are the menaquinones most widely studied, and in consequence, the only ones that can be found in food composition data[46,47].

Table 1. Vitamin K content of common foods.

Food	Major form of vitamin K	Total concentration (µg/100 g)
	Vegetables	
Chard	Phylloquinone	830
Spinach	Phylloquinone	483
Collard	Phylloquinone	440
Beet greens	Phylloquinone	400
Broccoli	Phylloquinone	180
Cabbage	Phylloquinone	145
Green peas	Phylloquinone	36
Iceberg Lettuce	Phylloquinone	35
	Oils	
Soybean oil	Phylloquinone	184
Canola oil	Phylloquinone	127
Cottonseed oil	Phylloquinone	60
Olive oil	Phylloquinone	60
	Other food	
Blue cheese	Menaquinone-9	189
	Menaquinone-7	
Margarine, 80% fat	Phylloquinone	92
Hard cheeses	Menaquinone-9	51
Soft cheeses	Menaquinone-9	40
Sauerkraut	Phylloquinone	30
	Menaquinone-6	
Curd cheese	Menaquinone-9	19
Chicken liver	Menaquinone-4	14.1
Beef (High Fat)	Menaquinone-4	8.1

Data from Booth, SL (2012)[46] and Schurgers, L et al. (2000)[47].

4.2. Metabolism

After its ingestion, all the dietary forms of vitamin K are incorporated into mixed micelles through the action of bile and pancreatic enzymes, like other forms of dietary fats. Later, they are absorbed by the enterocytes of the proximal small intestine. After the absorption, are incorporated into chylomicrons and transported to the liver, where are repackaged into very low-density lipoproteins, form in which are carried in the circulation. Very few amounts of vitamin K circulates in blood, since is rapidly metabolized and excreted. Vitamin K1 is the major circulating form, though MK-7 can also be present in plasma but at lower

concentrations. Excretion via is primarily by faeces through bile, but a minor proportion is excreted in the urine. There is very little information about the absorption and transport of the vitamin K produced by the gut microbiota, and it is still unknown if the circulation menaquinones are derived from the intestinal flora, the diet or the combination of both[45,48].

Until recently, the liver was the only known site of vitamin K storage. However, due to the discovery of several vitamin K-dependent processes in extra-hepatic tissues, it is thought that there may be stores in other tissues like adipose and bone tissue[45].

4.3. Bioavailability, recommendations, deficiency and toxicity

There are very limited data on the bioavailability of the different forms of vitamin K from food on the literature. Phylloquinone free form has an absorption rate of approximately 80%. However, in plant foods is strongly bound to chloroplasts, making its absorption rate significantly decrease. The vitamin K1 present in oils has higher rates of absorption due to its fat-soluble structure and its free form[46]. Likewise, the consumption of vegetables at the same time as fats improves its phylloquinone absorption. There is also insufficient evidence about the absorption of dietary menaquinones, though it has been suggested that long-chain ones could have higher absorption rates than vegetables phylloquinone[45].

Regarding the gut microbiota produced menaquinones; research has shown that considerable quantities of long-chain MKs are present in the large bowel. It is still unclear the amount of MKs that is obtained by this mechanism, as well as its absorption[45,49].

The recommended intakes of vitamin K are expressed in adequate intakes (AIs), which are the intake levels necessary to ensure the nutritional adequacy. The AIs are based on vitamin K intakes in healthy population groups and are established because there is insufficient evidence to develop a Recommended

Dietary Allowance (RDA). In their recommended nutrient intake, WHO/FAO included the Phylloquinone amount of 1µ per kilogram of body weight per day[50]. The United States of Americas' Institute of Medicine on the other hand, set out the AI for vitamin K as 120µg/day for men and 90µg/day for women[51].

Vitamin K deficiency in adults is very rare; however, it can occur more frequently in newborn babies during the first weeks of infancy. This can happen for several reasons including a low placenta transfer of phylloquinone, and a low content of vitamin K in the breast milk. In adults, the deficiency is usually restricted to people with malabsorption problems or associated to some medication consumption like anticoagulants, bile acid sequestrants and lipase inhibitors. When this happens, there is a decrease in the prothrombin activity of blood and in consequence, the prothrombin time increases significantly. This promote haemorrhage and bleeding, the classic signs of this vitamin deficiency[44,51].

There are no Upper Limits (ULs) established for vitamin K because no adverse effects associated to its dietary consumption have been reported. However, amounts higher than 5mg/day of synthetic menadione in infants have shown to produce haemolytic anaemia and hyperbilirubinemia and consequently is no longer use as therapeutic agent[51].

4.4. Biochemical roles and mechanisms of action

Vitamin K was discovered in 1929, as part of experiments on sterol metabolism. Immediately was associated with blood coagulation. Major understanding of the mechanism of action happened with the discovery of γ-carboxylglutamate (Gla) in the 70s. Through this breakthrough, we acknowledge today the vitamin K major role as a co-factor for γ-glutamyl carboxylase enzyme (GGCX), essential for the γ-carboxylation of specific glutamate residues (Glu) to Gla. Among these Gla containing proteins, there are blood coagulation factors (prothrombin and factors VII, IX and X), but also proteins associated with bone metabolism

VITAMIN K & HEALTHY AGEING

(osteocalcin and Matrix Gla Protein) and cell signalling (Gas6) involved in the stimulation of cell proliferation[44,52].

The γ-carboxylation through the action of vitamin K is what confers function to these proteins, converting Glu into Gla. This proteins were latter known as vitamin K Dependent Proteins (VKDP)[53] (Figure 4).

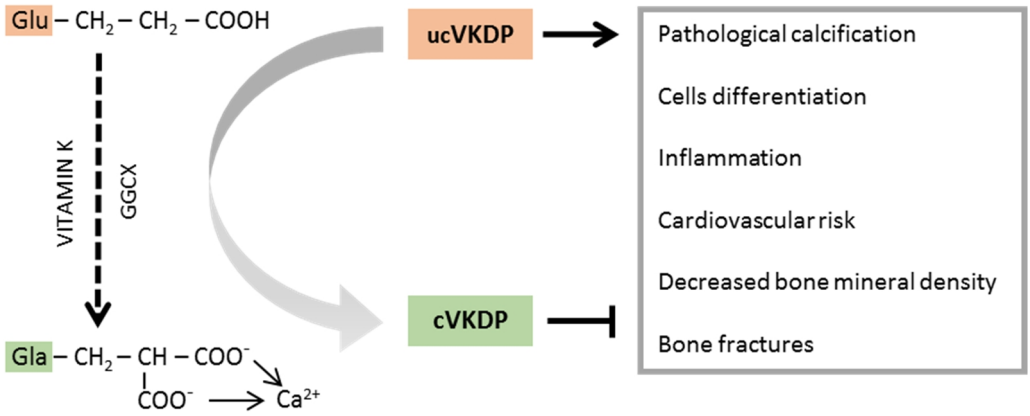

Figure 4. Vitamin K roles as co-factor of γ-glutamyl carboxylase enzyme (GGCK). Uncarboxylated vitamin K dependent proteins (ucVKDP) have been implicated in several pathological processes associated to age-related diseases, while the carboxylated form of these proteins (cVKDP) inhibit these processes giving a health protective role. Modified from Simes et al. (2019)[54].

The indisputable role of this micronutrient in health is the maintenance of normal coagulation. There are seven vitamin K–dependent coagulation proteins: prothrombin (factor II), factors VII, IX and X, protein C, protein S and protein Z. All of them are synthesized in the liver. They contain between 10 and 12 Gla residues, which enable the binding of the proteins to the phospholipid surfaces of the blood platelets and endothelial cells at the site of injury. Prothrombin and factors VII, IX and X action in the clotting cascade results in the formation of the fibrin clot[55].

Vitamin K also participate in vascular calcification, converting Matrix Gla protein (MGP), a VKDP present in vascular tissue, into MGP carboxylated form that

inhibits calcification in arterial and other soft tissues[56]. In the bone metabolism, vitamin K works as an enzyme cofactor for three VKDPs present in bone tissue: osteocalcin (OC), MGP and Protein S[52]. OC is the primary non-collagenous protein present in bone and is produce during bone formation by osteoblast. The presence of three Gla residues on its structure, allows it to bind to hydroxyapatite crystals in bone[57].

The γ-carboxylation of OC is thought to be the primary mechanism underlying the protective effect of vitamin K on the bone. MGP contains five Gla residues, and unlike OC, is expressed in several tissues and cell types including muscle cells, though it only accumulates in calcified tissues. It functions as an inhibitor of calcification. Protein S on the other hand, is only present in bone tissue, but its role in the bone metabolism is still undetermined[52]. Although its exact mechanism is still unclear, vitamin K is thought to function also as a regulator of bone mineral maturation, and to intervene in bone remodelling and calcification process[57].

4.4.1. Vitamin K emerging functions: oxidative stress, inflammation and insulin sensitivity

Recently, new roles (Figure 5) have been associated to vitamin K independent from its activity as co-factor for the GGCK.

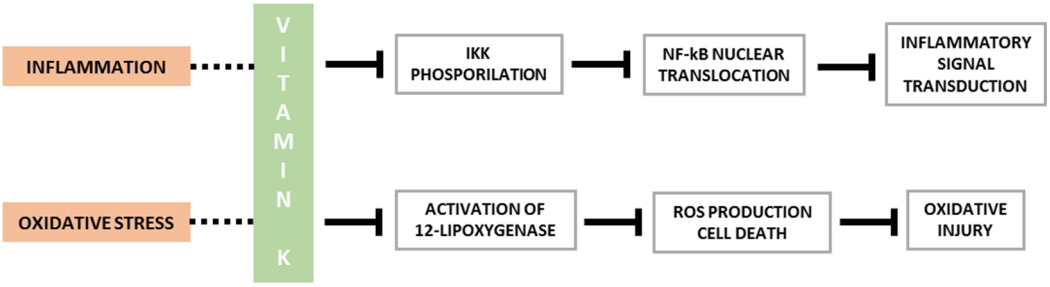

Figure 5. Independent GGCK vitamin K roles. Vitamin K inhibits pathways associated to both inflammation and oxidative stress. IKK, nuclear factor kB kinase; NF-kB, nuclear factor kB. Modified from Simes et al. (2019)[54].

Both phylloquinone and MK-4 have shown anti-inflammatory effects, by either decreasing cytokines and other inflammatory markers or inhibiting its formation[58–61]. These two vitamin K forms are thought to play this role by suppressing nuclear factor kB (NF-kB) signal transduction. Activated NF-kB induces the secretion of several pro-inflammatory molecules like TNF-α, IL6 and MCP1 among others[58].

In addition, it has been suggested a vitamin K protective effect against oxidative stress through blocking ROS generation. Studies have shown a protective effect of both vitamin K1 and MK-4 against oxidative damages. Even though the exact mechanisms remains undefined, in nervous tissue, it has been observed an inhibiting the activation of 12-Lipoxygenase, an enzyme with essential role in oxidative cellular death[62,63].

Beneficial effects of vitamin K on insulin sensitivity and glucose homeostasis have been reported by several studies. The underlying mechanisms have not yet been recognised, though some hypothesis were raised. One, the vitamin K anti-inflammatory effect decreasing cytokines and other inflammatory markers, which is implicated in the pathology of insulin sensitivity[58,60,61]. Other, is the effect of VKDP OC action as a mediator in the endocrine pathway. The carboxylated form of OC could influence insulin sensitivity acting on pancreatic β-cells, increasing their proliferation and the insulin secretion[64–66].

5. AGEING AND VITAMIN K

Ageing is a major risk factor for chronic diseases and metabolic problems. Vitamin K has been suggested to play a protective role against age-related diseases, such as CVD, type 2 diabetes (T2D), osteoporosis, sarcopenia and several neurological disorders.

The effect of vitamin K on osteoporosis and bone health is the most studied. Most observational studies have associated high phylloquinone intakes with lower risk of hip fracture, but the association with bone mineral density (BMD) is less consistent. A meta-analysis including 19 randomized clinical trials (RTC) concluded that vitamin K2 treatment was able to improve vertebral bone mineral density and reduce fracture risk in osteoporotic postmenopausal women. No effect was observed in the postmenopausal women without osteoporosis group[67]. In addition, the effect of vitamin K supplementation on BMD and fractures in adults was assessed in a systematic review and a meta-analysis with 36 RCT. Their results show that vitamin K supplementation appears to have no effect on vertebral fracture outcomes and little effect on BMD, for postmenopausal and osteoporotic patients[68].

Prospective observational studies and clinical trials have also shown a T2D risk reduction, improvements in insulin sensitivity and glucose metabolism with higher vitamin K consumption. In a large sample of the Framingham Offspring Cohort, higher intake of phylloquinone was associated with better insulin sensitivity and glycaemic status[69]. Previous analysis from our group, performed in the PREDIMED study cohort, associated higher vitamin K dietary intakes with a reduced risk of T2D[70]. In the same line, we searched for an association between dietary phylloquinone intake and peripheral adipokines and other T2D and IR metabolic risk markers. Our results showed improvements in these metabolic markers on those subjects allocated in the upper tertile of one-year changes in dietary phylloquinone intake compared to those in the lowest tertile of change[61].

The association between vitamin K and CVD has been also wildly studied. It is hypothesised that vitamin K dietary consumption decreases the risk of CVD. However, evidence is inconsistent. Most cross-sectional studies find an inverse association between vitamin K2 intake and incidence of coronary heart disease (CHD) and coronary calcification[71,72]. The Rotherham Study assessed the association of both K1 and K2 intake with coronary calcification and CHD, finding only a protective effect of K2 on total CHD[72]. A prospective study with a total population of 16.057 elderly women showed that dietary total vitamin K2 was significantly associated with lower incidence of CHD across an eight years follow-up[73].

5.1. Cataracts and vitamin K

A cataract is a clouding of the crystalline lens causing a decrease in vision. Untreated cataract is the major cause of blindness in developed countries. It represents more than 50% of all cases of loss of vision worldwide that constitutes about 20 million people[74]. In the last years, the progressive ageing of the population has been linked to the increase of incidence and prevalence of cataract. In Europe, the prevalence increases from 30% for population between 60 and 69 years to 64% in those over 70 years of age[75]. Cataract blindness decreases the quality of life on the elderly community, being associated with depression and higher mortality rates[76,77]. Additionally it affects the productivity of the blind, but also their caretakers[77].

Ageing is considered the strongest predictor of cataract development, but other risk factors such as obesity, diabetes, family history of cataracts, diet, smoking, alcohol and lower socioeconomic status are also recognized as risk factors[78].

Surgical removal of the cataract lens and replacement with a synthetic one is the only definitive treatment[78]. Even though it is a relatively simple surgery, it is not free of risk and several barriers still limits it access. Although the cost of the surgery in developed countries is relatively low, in lower socioeconomic

levels represents a significant expenditure. In addition, the number and distribution of ophthalmologist worldwide is a major limiting factor[75].

The exact mechanisms underlying cataract formation are still unclear but growing evidence suggests a potential role of inflammatory and oxidative processes[79,80]. As diet can modulate inflammation and oxidation, several studies have prospectively assessed the relationship between some food or specific dietary components and the process of cataractogenesis. In this regard, three different meta-analyses of cohort studies have shown that higher intakes of vitamin E[81], vitamin C[82] and vitamin A[83] are associated with a reduced risk of cataract. Similarly, the dietary intake of lutein and zeaxanthin has been shown to be associated with a reduced risk of age-related cataract in a dose-response manner[84]. High intakes of fruit and vegetables have also been associated with a lower prevalence of cataract or cataract surgery in an elderly population[85].

There is evidence on a study conducted in diabetic rats that treatment with vitamin K1 may protect against cataract by affecting the homeostasis of blood glucose and minimizing subsequent oxidative and osmotic stress. The inhibition of diabetic-cataract could be produced by modulating lens Ca2+ homeostasis and vitamin K1 hypoglycaemic effect through its direct action on the pancreas[86].

5.2. Diabetes Complications and vitamin K

Diabetes is a complex chronic metabolic disorder that occurs when either the pancreas is not being able to produce insulin or the organism is not capable of use it effectively. This leads to hyperglycaemias that associates with several complications as well as altered lipid metabolism, subclinical inflammation and increased oxidative stress[87].

It has become a major global health problem with approximately 425 million people affected by the year 2017, and a projected figure of 629 million by 2045. Type 2 diabetes is the most common expression of the pathology, present in the 90% of the total cases[88].

Constant high blood glucose levels increase the risk of developing numerous health problems. Diabetes complications (DC) are most commonly separated into two sub-divisions: microvascular, due to damage to small blood vessels; and macrovascular, due to damage to larger blood vessels. Microvascular complications include retinopathy, nephropathy and neuropathy. Macrovascular complications, on the other hand, include CVD diseases and peripheral artery disease[89].

Diabetic nephropathy (DN) represents the leading cause of end-stage kidney disease in developed countries. It affects approximately 25% of patients with T2D[90,91]. DN is clinically characterized by increased albuminuria levels and low rates of glomerular filtration, though its physiopathology is defined by the chronic hyperglycaemia that leads to a production of reactive oxygen species and an increment in inflammation markers that concludes in the declined renal function[90]. Additionally to the high blood glucose levels, its development and progression is promoted by a variety of risk factors including obesity, dyslipidaemia, and elevated blood pressure among others[91].

Diabetic retinopathy (DR) is the leading cause of blindness among working-age adults[92]. It is characterised by progressive vascular alterations, caused majorly by chronically high blood sugar, which produces proliferative lesions and clinically significant macular oedema[93]. Inflammation may be one of the leading causes, mediating deleterious effects in the neuronal and vascular components of the retina. Chronic retinal inflammation has been found from early phases to advanced stages. However, the development of DR is also mediated through metabolic changes, oxidative stress, and AGEs accumulation[94].

In previous analysis from our group, we observed that vitamin K inhibit the production of inflammatory cytokines associated with insulin response[61], and that its dietary intake was associated with a reduced risk of T2D[70]. However, there is still uncertainty whether vitamin K has any effect in the development of the different complications associated to T2D. In the best of our knowledge, there

is only one study conducted in animals demonstrating that the subcutaneous administration of vitamin K for three months decreased blood glucose and prevented microalbuminuria. The treatment with vitamin K also reduced oxidative stress and protected renal physiology by modulating Ca2+ and Na+/K+-ATPases[95]. No study has assessed the longitudinal association between dietary vitamin K and diabetic nephropathy or retinopathy in humans.

5.3. Cognitive Impairment and vitamin K

Cognitive impairment is a chronic condition characterized by the loss of memory, concentration and the depletion of the ability to learn new things. It affects the decisions making and in consequence the persons everyday life. Up to one third of cases of cognitive impairment ends in dementia. Although the impairment is less severe than in dementia, it can also result in the inability to live independently[30]. It is a costly condition for the health system; reports show that people with cognitive impairment have three times more hospital stays than other individuals hospitalized for other conditions[96].

Diverse factors determine the progress of the impairment. Cardiovascular disease, alterations in inflammatory markers, hypertension, high cholesterol, high body mass index (BMI) amongst several other medical risk factors are highly related[97]. Presence of diabetes has also been associated with cognitive impairment[98,99]. Changes in the brain structure and function may be related to the duration of the diabetes, as well as with the glycaemic control, although the exact mechanisms are not clearly understood[99]. Specifically in the elderly population with T2D, cognitive dysfunction has been associated with poor diabetes self-management[100]. Other possible related contributors could be insulin resistance, oxidative stress and vascular disease[101]. Either way, modifiable risk factors, like lifestyle, play also a crucial role[102].

In the last years, evidence that vitamin K could have a role in the cognitive response has increased. Among other actions in brain, is known for its

involvement in sphingolipid metabolism. Sphingolipids, major components of cell membranes, are present in particularly high concentrations in cells of the central nervous system. These complex membrane lipids were initially valued for its structural role. Nowadays research showed them as important players in cellular processes such as proliferation, differentiation, senescence, cell–cell interaction, and transformation[103]. Alterations in sphingolipid metabolism have been associated to neurodegenerative disorders such as Alzheimer and Parkinson disease and other neurodegenerative ageing processes[104].

A study examining vitamin K and cognitive function has found that higher dietary vitamin K1 intake was associated with better cognition performance and behaviour among older adults[105]. In the same line, a cross-sectional analysis linked serum vitamin K1 concentration with better verbal episodic memory performances[106].

5.4. Dementia and vitamin K

Dementia is a progressive deterioration in cognition, function and behavior. Is a syndrome, usually of a chronic or progressive nature, in which exists a deterioration in cognition function beyond of what might be expected from normal ageing. It affects several dimensions of the cognitive function including the memory and language function and other behavioral abilities[107]. Nowadays, the prevalence reaches up to 50 million cases around the world, being considered one of the major causes of disability and dependency among older people. This number will double in the next twenty years, with bigger increases in developing countries. The most common cause of dementia is the Alzheimer's disease (AD), approximately 70% of the cases[107,108].

Even though the mechanisms underlying the neuropathological changes are widely studied, it remains unclear. The brain atrophy caused by this neuropathological deterioration is associated with vascular lesions produced by a compromised metabolism with elevated oxidative stress and inflammation[109]. Obesity, diabetes and metabolic syndrome have been also highly related[110,111]. There are several

non-modifiable risk factors that are also highly associated, most important ageing and genetic predisposition. Most authors conclude that the probable causes are genetics sum up to environmental factors[112]. Recently, insulin resistance has also been considered as a risk factor per se, potentially inducing a bioenergetic shift in peripheral and central nervous system energy metabolism[113].

Diet seems to be one of the modifiable factors associated with this neuropathology. Evidence supported by different observational studies suggest that antioxidant-rich foods, like fruits and vegetables, and those food with a demonstrated insulin down lowering effects, may have a protective effect from cognitive decline and AD. Consequently, healthy dietary patterns, such as the Mediterranean diet, high in antioxidants and unsaturated fatty acids, were associated with a reduced incidence of AD. In contrast, diets rich in saturated and trans fats, and deficient in some micronutrients such as vitamin B or D are considered deleterious nutritional factors[114–116].

Emerging evidence supports the potential role of vitamin K in the nervous system. It is well known that vitamin K modulates the activities of key enzymes of the sphingolipid biosynthetic pathway and their metabolism. Moreover, the discovery of Gas6, a vitamin K–dependent protein, and the characterization of its signaling actions in neurons and in various glial cell types have straighten out other mechanisms through which vitamin K can influence the nervous system[117].

Due to the recent link found between insulin resistance and AD, our groups' observations associating vitamin K inhibition of the production of inflammatory cytokines with the insulin response, may be other related mechanism[61]. In an observational study patients with early stages of AD had significant lower vitamin K dietary intake compared to age and sex matched heathy participants[118]. In the same line, a positive association between serum phylloquinone concentrations and performances in verbal episodic memory was reported, suggesting a specific role of this vitamin in memory consolidation[119].

B. JUSTIFICATION

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The world is facing a demographic transition towards an older population structure. For the first time in history, people over 60 years old represents the fastest growing age group. Even though life expectancy has extended, the years gained are often lived with disability and disease. Therefore, it is extremely important to understand the etiopathogenic factors related to the ageing process.

At a physiological level, ageing is characterised by a generalized impairment in many body functions that increases vulnerability to environmental challenges and rises the risk of disease and death. Within prevalent diseases such as Type 2 Diabetes represents a major health challenge in our aging society that increases the risk of morbidity, disability and frailty in the elderly. The poor management of this disease can cause long-term damages that can result in associated complications. Changes in sensory functions associated with vision causes severe visual impairment with implications for the everyday lives of older people. Cataracts, being the leading cause of blindness, and diabetic retinopathy can restrain mobility, increase the risk of falls and accidents and affect the interpersonal interactions. The same applies to the cognitive impairment affected population. The impact of severe levels of impairment can lead to the incapacity to perform everyday tasks, resulting in the inability to live independently. People with cognitive impairment report three times more hospital stays than individuals hospitalized for other conditions. Alzheimer's disease and other dementias are one of the most expensive diseases to treat. Payment for these persons Medicaid are nine times higher than for other patients.

For this reason, lifestyle is recognized to play an important role in the aetiology of several chronic diseases modulating different ageing processes. In particular, diet is one of these lifestyle modifiable risk factors that has been largely studied in last years in the context of ageing. To determine possible associations between diet and the incidence of diseases or conditions related to ageing is crucial to develop and implement primary health care approaches promoting healthy ageing, and maintaining a functional ability and improving the individual quality of life in this aged population.

Therefore, there is an urgent need to understand the role that an adequate nutrition and/or specific food or food components intake could play helping to achieve healthy ageing. We know that nutrition and other lifestyle interventions have the potential to promote healthy ageing preventing, slowing or reversing age-related diseases that affect the capacity and functional ability. Evidence shows that a Mediterranean dietary pattern promotes health, increases longevity and reduces the risk of non-communicable diseases. Still, undernutrition and micronutrient deficiency are a common problem among older population, and partially responsible for the unhealthy status prevalent in this age. Even though evidence shows beneficial effect of several nutrients and other food components, there are still a lot of research gaps that keep us from identifying the most efficient strategies to develop better guidance on nutrition for the healthy elderly.

Vitamin K has been suggested to play a modulatory role in ageing and age-related mechanisms such as oxidative stress, inflammation and insulin resistance. However, the current evidence regarding vitamin K's association with the risk of disease is still scarce. Predimed and Predimed-Plus studies, due to their longitudinal designs, provide optimal frameworks with large elderly populations that can help us elucidate the potential role of vitamin K improving healthy ageing by lowering the risk of age-related conditions.

Because of the limited amount of evidence and the relevancy of the theme, the research questions for this thesis is:

- Does vitamin K1 dietary intake associate with a decreased risk of cataracts, diabetes complications, cognitive impairment and dementia in an elderly population?

C. HYPOTHESIS AND OBJECTIVES

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1. HYPOTHESIS

High dietary vitamin K1 intake is associated with healthy ageing through a decrease risk of different age-related health conditions.

2. OBJECTIVES

The main objective of this thesis was to evaluate the effect of dietary vitamin K1 intake in different age-related health conditions.

Specific objectives:

To examine, among an elderly Mediterranean population at high cardiovascular risk, the association between the dietary vitamin K1 intake and:

- The risk of incident cataract.
- The risk of diabetic nephropathy and diabetic retinopathy.
- The risk of cognitive functioning decline.
- The risk of dementia.

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D. MATERIAL AND METHODS

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1. The PREDIMED (PREvención con DIeta MEDiterránea) study

1.1. Study design and main objective

The PREDIMED study was a large multi-centre, parallel group, randomized controlled clinical trial conducted by eleven different recruitment centres in Spain. The main objective of the study was to assess the effects of Mediterranean diet (MeDiet) on the primary prevention of cardiovascular disease, being the primary outcomes a composite of myocardial infarction, stroke and cardiovascular death by these causes.

Secondary aims were to assess the effects of dietary changes on the risk of clinical events of secondary outcomes: death of any cause, myocardial infarction, diabetes, dementia and different types of cancer; and intermediate end-points such as changes in blood pressure, fasting blood glucose, lipid profile and markers of inflammation and cardiovascular risk.

1.2. Study population

The study population were community-dwelling high CV risk persons, without CVD at the time of enrolment, with ages between 55 and 80 years old for men and from 60 to 80 years for woman.

For meeting the inclusion criteria, participants needed either to present a diagnosis of type 2 diabetes, or to have three or more of the following metabolic risk factors:

- Being a current smoker (>1 cigarette/day during the last month)
- Present hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mmHg or under antihypertensive medication)
- Present levels of LDL-c ≥ 160 mg/dl or lipid-lowering therapy
- Present levels of HDL-c ≤ 40 mg/dl in men or ≤ 50 mg/dl in women
- Have a BMI ≥25 kg/m^2

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- Have a family history of premature CHD (definite myocardial infarction or sudden death before 55 years in father or male 1st-degree relative, or before 65 years in mother or female 1st-degree relative)

The major exclusion criteria were:

- Having a documented history of previous CVD, including CHD, stroke or clinical peripheral artery disease
- Have a medical condition that could interfere with the participation in a nutrition intervention study (digestive disease, nut or olive oil allergy or major neurological, psychiatric or endocrine disease)
- The impossibility to follow the intervention (religious reasons or presence of chewing or swallowing disorders)
- Present any other medical condition that would limit survival to less than one year
- Having a drug or alcohol abuse problem.
- Present a BMI > 40 kg/m^2

1.3. Randomization and Intervention

Participants were assigned in a 1:1:1 ratio to the corresponding intervention group: two intensive behavioural counselling and nutrition education interventions with MeDiet, one supplemented with extra-virgin olive oil (EVOO), (MeDiet+EVOO), and other supplemented with nuts (MeDiet+Nuts); and a third group advise to follow a low-fat diet.

PREDIMED dietitians were responsible for the dietary intervention. The first and second intervention groups received education on MeDiet, based on a 14-point score questionnaire measuring the adherence to MeDiet. Both had indications of ad libitum fat intake, as long as it was derived from fatty fish, olive oil and nuts. Participants in the MeDiet+EVOO group were provided with 1 litre of oil per week and the MeDiet+Nuts group with 30g/day of a mix of walnuts,

hazelnuts and almonds. The low-fat control group did not receive any MeDiet education, but they were advised to follow a low-fat diet.
Each intervention group received separately group sessions where the intervention was reinforced. Participants were provided of written material including weekly meal plans and cooking recipes.

1.4. Specific measurements

At baseline and in each annual follow up visit several different variables were measured (Table 2).

Table 2. Measurement schedule in the PREDIMED trial.

	Baseline	1Y	2Y	3Y	4Y	5Y	6Y
Eligibility Questionnaire	•						
General Questionnaire	•						
14-item MeDiet Questionnaire	•	•	•	•	•	•	•
Food Frequency Questionnaire	•	•	•	•	•	•	•
Follow-up Questionnaire*		•	•	•	•	•	•
Tolerance Questionnaire		•	•	•	•	•	•
Physical Activity Questionnaire	•	•	•	•	•	•	•
Abandonment Questionnaire**		•	•	•	•	•	•
ECG	•	•	•	•	•	•	•
Blood Sample	•	•		•		•	•
Urine Sample	•	•		•		•	•

*Includes measurements of weight, height, waist circumference, BP and ankle-brachial blood pressure index.
**Only if applicable. ECG, electrocardiograms; Y, year.

Different questionnaires were filled during the follow-up with the objective of collecting all the necessary measurements in the study. The eligibility questionnaire gathers the socio-demographic information and the smoking habits, as well as verify the inclusion and exclusion criteria. The general questionnaire included medical conditions, use of medication, anthropometry and blood pressure. The tolerance questionnaire assessed the potential adverse effects of the intervention.

The 14-item MeDiet questionnaire was a semi-quantitative score intervention tool, used to measure the adherence to Mediterranean Diet[120].
In order to assess physical activity, we use the validated Spanish version of the Minnesota Leisure-Time Physical Activity questionnaire[121].

1.4.1. Dietary assessment

Dietary intake Information were provided by a yearly administrated 137-items semi-quantitative Food Frequency Questionnaire (FFQ). To ensure a better quality of the measurements, the FFQ was validated for the Spanish population[122]. Trained dietitians completed it at baseline and yearly thereafter, in a face-to-face interview with the participant.
Energy, macro and micro nutrient intake were estimated using Spanish food composition tables[123,124].

Information on vitamin K1 intake was assessed using the United States Department of Agriculture (USDA) nutrient database as this information is not available in the Spanish food composition tables[125]. The reproducibility and relative validity of the 137-items FFQ used in the study was validated for dietary vitamin K1 consumption using 3-day dietary records as reference. The reproducibility for Vitamin K1 dietary intake estimated by the Pearson correlation coefficient (r) was 0.755 with an interclass correlation coefficient of 0.860 ($P < 0.001$)[61].

1.4.2. Anthropometric and blood pressure measurements

Anthropometric measures were determined by trained personnel with calibrated equipment at baseline and at each annual visit during the follow-up. Weight was measured using a calibrated balance beam scale with the subject barefoot and wearing light clothes. The height measurements were taken using a wall-mounted calibrated stadiometer. Waist circumference was measured using an

anthropometric measuring tape, at a horizontal plane midway between the lowest rib and the iliac crest.

Blood pressure was assessed in triplicate using a validated semiautomatic oscillometer (Omron HEM-705CP; Omron Healthcare). The mean of the second and third measurement was recorded.

1.4.3. Biological samples and biochemical determinations

At baseline and in years 1, 3, 5 and 6 (or final visit of follow-up) we collected and processed fasting blood and spot urine samples for subsequent biochemical analyses. Biochemical measurements included fasting blood glucose, uric acid, creatinine and blood urea nitrogen (BUN); total HDL-c and LDL-c; triglycerides, total protein and albumin.

Additionally, a complete blood count and routine biochemical measurements were performed yearly in the Primary Care Centre together with a routine urine exam.

1.5. Ascertainment of clinical outcomes

An External Adjudication Committee, whose members were blinded to the dietary intervention and to the participants' dietary behaviours, confirmed all clinical outcomes. Only events that were definitively confirmed by this committee and that occurred between October 1, 2003, and June 30, 2012 (date of the last update in the extended follow-up of the PREDIMED cohort, after the end of the trial), were included in the analyses.

1.5.1. Cataracts events

Cataract outcome was a pre-specified secondary outcome of the PREDIMED Trial, and was defined as the occurrence of cataract surgery at any time during the study.

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For the assessment of incident cataract surgery, participants were specifically asked during their yearly visit if they had undergone cataract surgery. The occurrence of cataract surgery was also confirmed by annually reviewing the computer-based records of general practitioners. The cataract outcome was defined by the ophthalmologist and externally confirmed by the independent adjudication committee. Cases of traumatic cataracts and those that emerged after intraocular surgery, such as vitrectomy or glaucoma surgery were excluded. In cases of bilateral surgery in the same patient, only the first event was considered in our time-to-event analyses.

1.5.2. Diabetic nephropathy and retinopathy events

Even though diabetic nephropathy and diabetic retinopathy were not pre-specified secondary outcome of the PREDIMED trial, given that nearly half of participants had T2D, these two complications were included as relevant outcomes in all interim analyses supervised by the Data Safety Monitoring Board.

New-onset diabetic nephropathy events assessment were based on the information recorded in the clinical records. We also included incident cases of DN defined by the chronic kidney disease (CKD) progression from moderate to severe (stage 3 or greater) or albuminuria progressing during follow-up. The kidney disease progression was defined as a sustained estimated glomerular filtration rate (eGFR) value <60 mL/min/1.73m^2 based on serum creatinine, whereas the albuminuria progression was defined as the transition from normo- to micro- or macro-albuminuria (Albumin-to-creatinine ratio [ACR] ≥30 mg/g). Serum creatinine and ACR were measured at least once a year in the 67% and 43% of participants, respectively. Both progresses were confirmed by at least two consecutive measurements during follow-up. In the present study, serum creatinine-based eGFR was calculated with the CKD-Epi equation[126].

The end-point for diabetic nephropathy was determine as the time to first occurrence. An ophthalmologist diagnosed all new-onset diabetic retinopathies and

after were ascertained by the adjudication events committee. Participants were considered to have sight-threatening DR if they had undergone laser photocoagulation, intra-vitreal anti-vascular endothelial growth factor injections, and/or vitreoretinal surgery. Since Spain's public health systems advise yearly fundus examination by an ophthalmologist to detect early diabetic retinopathy, in the present report we presume that all participants were free of diabetic retinopathy at baseline.

1.5.3. Dementia Events

As one of the previously specified secondary outcomes of the PREDIMED study, an independent adjudication committee externally confirmed all dementia events, including Alzheimer's disease and other dementias (frontotemporal lobar degeneration, Lewy body dementia, normal pressure hydrocephalus and vascular dementia).

Alzheimer's disease was defined by the National Institute of Neurologic, Communicative Disorders and Stroke – Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA) criteria, published on 1984 and rectified in 2011[127].

For other dementias the diagnosis criteria used were: the Dementia with Lewy Bodies Consortium (DLBC) for dementia with Lewy bodies[128], the National Institute of Neurological Disorders and Stroke (NINDS-AIREN) criteria for vascular dementia[129], consensus criteria for frontotemporal lobar degeneration[130] and idiopathic normal-pressure hydrocephalus (iNPH) guidelines for normal pressure hydrocephalus[131].

2. The PREDIMED-Plus study

2.1. Study design and main objectives

The PREDIMED-Plus study is an ongoing multicentre randomized, parallel-group primary prevention trial conducted in 23 different centres in Spain.

Its main objective is to evaluate the effect of an intensive weight-loss lifestyle intervention program with an energy reduced Mediterranean diet, physical activity promotion and behavioural support on the incidence of cardiovascular events compared to an unrestricted caloric Mediterranean control diet.

2.2. Study population

Participants are community-dwelling adults, men aged 55–75 years and women aged 60–75 years, with overweight or obesity (BMI 27–40 kg/m^2), who at baseline met at least three components of the metabolic syndrome from the joint statement of the International Diabetes Federation, National Heart, Lung and Blood Institute and the American Heart Association criteria[132]:

- Hypertriglyceridemia [≥150 mg/dL (≥1.7 mmol/L)] or drug treatment for elevated triglycerides.
- Low concentrations of HDL cholesterol [<50 mg/dL (<1.3 mmol/L) and <40 mg/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 hyperglycaemia.
- Elevated waist circumference for European individuals (≥88 cm in women and ≥102 cm in men).

Major exclusion criteria included:

- Inability to provide written informed consent or communicate with study staff.
- Documented history of previous CVD.
- Being permanent institutionalized or long-stay resident in a nursing home)
- Have an active malignant cancer or history of malignancy within the last 5 years.
- Inability to follow the recommended intervention diet or to perform physical activity.
- Food allergy to any component of the Mediterranean diet.
- Immunodeficiency or HIV-positive status.
- Cirrhosis or liver failure.
- Serious psychiatric disorders
- Alcohol abuse or addiction (or total daily alcohol intake >50g) or drug abuse within the past 6-months.

2.3. Randomization and Intervention

Each recruiting centre randomly assigned eligible candidates to one of the two intervention groups using a centrally controlled, computer-generated random-number system. Participants were randomly assigned with stratification by centre, sex, and age group (<65, 65-70, >70 years). Married or unmarried couples were randomized together.

Participants allocated to the intervention group were prescribed an energy-restricted MedDiet, complemented by physical activity promotion and behavioural support. The purpose of the intervention is to accomplish specific weight-loss objectives: an average reduction of ≥8% of the initial body weight and an average reduction of ≥5% of initial waist circumference in the first 6-months, and maintaining these reductions throughout the duration of the study. Dietary advice focused on typical and seasonal MedDiet foods: extra-virgin olive oil, raw

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nuts, fruits and vegetables, whole grains, legumes, lean meat and fish, and low-fat dairy products; as well as a reduced consumption of animal fats, sugar-sweetened beverages, processed foods and refined grains.

The physical activity program included aerobic activities or any equivalent activity of moderate intensity. The recommendations were adapted to the participants' preferences and they were encouraged to engage in resistance, balance, and flexibility training twice or more a week.
The behavioural support included strategies and practical tools to facilitate their long-term adherence to the dietary and physical activity recommendations.

Participants in the control group received non intensive educational sessions with the same content used in the PREDIMED study, an energy-unrestricted traditional MedDiet with emphasis on improving dietary quality but without calorie control. No specific advice regarding physical activity or weight losing was provided to participants in the control group.

2.4. Specific measurements

At the screening visit, baseline, 6-month and in each annual follow-up visits, several variables were measured (Table 3).

Different questionnaires were used to collect information during the trial. The eligibility questionnaire gathers the socio-demographic information and the smoking habits, as well as verify the inclusion and exclusion criteria. The general questionnaire included medical history, family history of disease and use of medication.

Physical activity questionnaires included a short version of the Minnesota leisure physical activity questionnaire[121] and the Nurses' Health Study (NHS) sedentary lifestyle questionnaire[133].

Two MeDiet questionnaires are used as compliance assessment tools. The intervention group uses a 17-item energy restricted MeDiet adherence questionnaire, while the control group uses the 14-item that was utilized in the PREDIMED study[120].

Table 3. Measurement schedule in the PREDIMED-Plus trial.

	S	B	6m	1Y	2Y	3Y	4Y	5Y	6Y	7Y	8Y
Eligibility Q	•										
General Q		•									
Anthropometric measurements	•	•	•	•	•	•	•	•	•	•	•
FFQ (143 items)	•		•	•	•	•	•	•	•	•	•
MeDiet Q (17/14 items)		•	•	•	•	•	•	•	•	•	•
Physical Activity Q	•	•	•	•	•	•	•	•	•	•	•
Chair test	•	•	•	•	•	•	•	•	•	•	•
Accelerometry	•	•	•	•	•	•	•	•	•	•	•
Follow-up Q			•	•	•	•	•	•	•	•	•
ECG	•		•	•	•	•	•	•	•	•	•
Blood pressure measurement	•	•	•	•	•	•	•	•	•	•	•
Blood sample		•	•	•		•		•		•	•
Morning spot urine sample		•	•	•		•		•		•	•
Cognitive-neuropsychological tests	•				•		•		•		•
Psychopathological Q	•			•	•	•	•	•	•	•	•

ECG, electrocardiograms; m, month; Q, questionnaire; S, screening; Y, year.

2.4.1. Anthropometric and blood pressure measurements

At each visit, trained personnel with calibrated equipment determined and recorded different anthropometric measures: body weight, waist and hip circumference. All are determined in duplicate, with participants in light clothing and without shoes or accessories. Height was measured with a stadiometer at baseline. Systolic and diastolic blood pressure are yearly evaluated, using a validated semiautomatic oscillometer (Omron HEM-705CP, Netherlands) after 5 minutes of rest in-between measurements and determine in triplicate.

2.4.2. Biological samples and biochemical determinations

Fasting blood samples are collected in order to perform biochemical analyses. These include fasting plasma glucose and lipid profile (total cholesterol, HDL-c, LDL-c and triglycerides) among other measurements.

2.4.3. Dietary assessment

The validated 143-item FFQ used in the PREDIMED study is administrated to evaluate the total food intake at each annual follow-up visit [122]. For the estimation of the total energy, macro and micronutrients we used Spanish food composition tables[123,124].

The dietary intake of vitamin K1 was estimated using the USDA nutrient database, as this information is not available in the Spanish food composition tables[125].

2.4.4. Cognitive functioning assessment

The wholly PREDIMED-Plus cohort completed a battery of cognitive-neuropsychological tests at baseline and every two years thereafter. The objective of this assessment is to evaluate changes in the cognitive function through time. The test included in this analysis were the Mini-mental State Examination, the Clock Drawing Test, the WAIS-III Digit Span tasks, the Verbal Fluency tests and the Trail Making Test. All of them have been standardized for the Spanish population in the age range of the study.

Cognitive decline was settled for the Mini-Mental State Examination with a 24 score cut-off and for the Clock Drawing Test when the score was less or equal than 4. For the other tests, cognitive decline were defined based on the study population mean score and a -1.5 SD cut-off, except for the TMT that the cut-off was +1.5 SD[134].

Mini-Mental State Examination (MMSE)

The MMSE is a scored form of cognitive mental functioning. It is a rapid tool to assess cognitive loss with a quick administration of normally 5-10 minutes. It consists of a variety of questions grouped into seven categories each representing a different cognitive domain or function, with a maximum score of 30 points. It assesses only the cognitive aspects of mental functions, excluding queries about mood, the form of thinking and abnormal mental experiences[135]. It has high levels of sensitivity for identifying individuals with cognitive impairment. Due to this, its availability in several languages and its practical structure, it has become a widely used tool in clinical practice, epidemiological studies and community surveys. Even though it should not be used as a diagnostic tool to identify dementia by itself, it is one of the test recommended by the NINCDS-ADRDA to document the clinical diagnosis of Alzheimer's disease[136].

Clock Drawing Test (CDT)

The CDT is a hand sketch based neurological test widely used in neuropsychology for the assessment of neurological and cognitive impairments, such as Alzheimer's diseases and other dementias. In this test, the participant is asked to draw a clock in a piece of paper with the numbers and the hands showing a specific time. The sketch is later evaluated by a clinician that searches for abnormalities in the drawings, such as poor number position, omission of the numbers, incorrect sequencing or missing clock hands and even the presence of insignificant unrelated writing[137].

Wechsler Adult Intelligence Scale III - Digit Span (WAIS III - DS)

The WAIS III is a comprehensive intelligence test that provides a variety of scores regarding the subject intellectual abilities. Its original structure contains fourteen subsets, including the Digit Span tasks. WAIS has been employed to assess several aspects of cognitive functioning, and specifically DS have been used in both clinical practice and research to assess attention and working memory[138].

DS has two different subsections: DS-Forward and DS-Backward. The task requires that the participants repeat sequences of digits of increasing length forward and then in reverse order. DS-Forward recalls for attention and short-term memory capacity and DS-Backward recall for working memory capacity[138,139].

Verbal Fluency Test (VF)

Verbal fluency tasks are a verbal functioning often used as neuropsychological assessment. In research, they have been included as tools for the evaluation of cognitive impairment in persons with neurodegenerative diseases[140,141]. The VF tests assesses both verbal ability and executive control using phonemic and semantic fluency tasks[140].

The phonemic fluency task consists in verbalize, in a minute time, as many words as possible starting with a given letter. In the semantic fluency task, participants have to name, also in 60 seconds, as many unique words within a semantic category (e.g. animals) as possible, without repeating. The final number of unique correct words the participant accomplishes corresponds to the total participant's raw score[142].

Trail Making Test (TMT)

The TMT is a useful assessment tool to investigate executive function and speed of processing. It is a good measure of overall cognitive functioning, although older age usually affects the performance, even in absence of any cognitive impairment[143].

It consists of two parts, A and B, which have to be performed as rapidly and precisely as possible. TMT-A consist on 25 circles randomly distributed in a piece of paper, with a series of numbers from 1 to 25. Participants are requested to connect consecutive numbers in the correct order drawing a line from circle to circle in the minimum time possible. In the TMT-B, participants are also asked to connect consecutive circles but instead of only numbers, half circles contain letters from A to L and the other half numbers from 1 to 12. The task consists in alternating numeric and alphabetic sequence. The score on each different part is represented by the seconds required to complete the task[144].

3. STUDY POPULATION FOR THE ANALYSES

This doctoral thesis included data from two different trials, the PREDIMED and the PREDIMED-Plus studies. Each result chapter analyses a different outcome variable and therefore have a different effective sample size.

Chapter 1: Cataracts outcome from PREDIMED study

A total of 5680 subjects were included in the present analysis. From the entire PREDIMED sample of 7447 participants, we excluded for this analysis subjects with bilateral cataract at baseline and those who had developed it during the first year (n=1536). In addition, we excluded those participants with incomplete dietary intake data at baseline (n=78) and those with extremes of total energy intake (>4000 or <800 kcal/d in men and >3500 or <500 kcal/d in women; n=153).

Chapter 2: Diabetic complications outcome from PREDIMED study

For the current analysis we excluded those subjects without T2D at baseline (n=3833), those subjects who were outside of the predefined values for total energy intake (>4000 or <800 kcal/d in men and >3500 or <500 kcal/d in women; n=64), and those without baseline food frequency questionnaire (FFQ) information (n=41). We therefore analysed a subset of 3509 participants. This was the final sample for the retinopathy cases. However, for the analysis of nephropathy, we additionally excluded subjects who lacked measurements at baseline or who did not have at least two consecutive urinary albumin/creatinine ratio or serum creatinine measurements that enable to determine the onset of disease during the follow-up (n=933). Participants were also excluded (n=491) if they had any of the following conditions at baseline at two consecutive visits: albuminuria (urinary ACR ≥ 30 mg/g) or impaired renal function (eGFR <60 mL/min/1.73m^2), two widely used measures for assessing kidney dysfunction. The final sample size for the assessment of diabetic nephropathy incidence was 2085 participants.

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Chapter 3: Cognitive impairment outcome from PREDIMED-Plus study

From the entire population of the PREDIMED-Plus study (n=6874), we excluded those participants lacking or without the complete dietary information at the two-year visit (n=2957) at the time of the analysis. Additionally, were also excluded those subjects who were outside the predefined values for total energy intake (>4000 or <800 kcal/d in men and >3500 or <500 kcal/d in women; n=180). The final sample included in this analysis ascend to 3737 participants.

Chapter 4: Dementia outcome from PREDIMED study

The entire sample included in this analysis was 7216 subjects. From the total of 7447 PREDIMED participants those with extremes of total energy intake (>4000 or <800 kcal/d in men and >3500 or <500 kcal/d in women; n=153), as well as the ones that had incomplete dietary data at baseline (n=78) were excluded.

4. STATISTICAL ANALYSES

Detailed information about the statistical approaches of each result chapter is fully described in each publication. Either way, a brief overview of the general statistical methods is presenter hereunder.

The baseline participants' descriptive data and the differences among groups are presented as means ± standard deviations (SDs) for continuous variables and as numbers and percentages (%) for categorical variables. Differences between groups were evaluated using either one-way ANOVA or the χ^2 test.

Multivariable time-dependent Cox proportional hazard models with robust variance and considering intra-cluster correlations were fitted to assess the associations between vitamin K1 consumption and the risk of cataracts, dementia and diabetic complications. Results are presented as Hazard Ratios (HRs) and 95% Confidence Intervals (CIs). Follow-up time was calculated as the time between recruitment and the date of the appearance of the event, death, and loss of follow-up or end of the study, whichever came first. To assess death outcome the strategies included a yearly review of medical records and consultation of the National Death Index. Loss of follow-up was defined when was impossible to contact with the participant or follow them through the National Health System.

To evaluate the association between 2-year changes in vitamin K1 dietary intake and all cognitive function tests scores, multivariate logistic regression models were applied. Results are presented as Odds Ratios (ORs) and 95 % CI. Participants who shared their household were randomized in the same intervention group and were included as cluster in all models to consider intra-cluster correlations.

All the statistical tests were 2-tailed and the level of significance was $P < 0.05$. Statistical analyses were carried out with SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA), R software v3.5.1 (www.r-project.org, R Development Core Team, 2012) and STATA 14 (StataCorp, College Station, TX).

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E. RESULTS

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CHAPTER 1

Association of Dietary Vitamin K1 intake with the incidence of cataract surgery in an adult Mediterranean population: A secondary analysis of a Randomized Clinical Trial

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JAMA Ophthalmology | Brief Report

Association of Dietary Vitamin K_1 Intake With the Incidence of Cataract Surgery in an Adult Mediterranean Population
A Secondary Analysis of a Randomized Clinical Trial

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IMPORTANCE Cataract, one of the most frequent causes of blindness in developed countries, is strongly associated with aging. The exact mechanisms underlying cataract formation are still unclear, but growing evidence suggests a potential role of inflammatory and oxidative processes. Therefore, antioxidant and anti-inflammatory factors of the diet, such as vitamin K_1, could play a protective role.

OBJECTIVE To examine the association between dietary vitamin K_1 intake and the risk of incident cataracts in an elderly Mediterranean population.

DESIGN, SETTING, AND PARTICIPANTS A prospective analysis was conducted in 5860 participants from the Prevención con Dieta Mediterránea Study, a randomized clinical trial executed between 2003 and 2011. Participants were community-dwelling men (44.2%) and women (55.8%), and the mean (SD) age was 66.3 (6.1) years.

MAIN OUTCOMES AND MEASURES Dietary vitamin K_1 intake was evaluated using a validated food frequency questionnaire. The time to the cataract event was calculated as the time between recruitment and the date of the occurrence to cataract surgery, the time to the last visit of the follow-up, date of death, or the end of the study. Hazard ratios and 95% CIs for cataract incidence were estimated with a multivariable Cox proportional hazards model.

RESULTS Participants were community-dwelling men (44.2%; n = 868) and women (55.8%; n = 1086), and the mean (SD) age was 66.3 (6.1) years. After a median of 5.6 years follow-up, we documented a total of 768 new cataracts. Participants in the highest tertile of dietary vitamin K_1 intake had a lower risk of cataracts than those in the lowest tertile (hazard ratio, 0.71; 95% CI, 0.58-0.88; $P = .002$), after adjusting for potential confounders.

CONCLUSIONS AND RELEVANCE High intake of dietary vitamin K_1 was associated with a reduced risk of cataracts in an elderly Mediterranean population even after adjusting by other potential confounders.

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Supplemental content

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A cataract is a clouding of the crystalline lens that causes a decrease in the vision. Aging is its major predictor, but it is also associated with obesity, type 2 diabetes, diet, smoking, and alcohol, among others.[1,2] Inflammation and oxidation could underlie the cataract formation. Because diet modulates these processes, the relationship between food or specific dietary components and cataractogenesis has been prospectively assessed. Meta-analyses of cohort studies showed that higher intakes of some vitamins[3-5] were associated with a reduced cataract risk. Similarly, high intakes of fruit and vegetables are also associated with a lower prevalence of cataract or cataract surgery.[6]

Because vitamin K has anti-inflammatory and antioxidant properties and is related to glucose and insulin metabolism, we hypothesize that dietary vitamin K_1 intake would be associated with a decreased risk of cataracts among an elderly Mediterranean population.

Key Points

Question Is dietary vitamin K associated with a decreased risk of cataract among an elderly Mediterranean population?

Findings In this secondary analysis of 5860 participants in a randomized clinical trial, participants in the highest tertile of dietary vitamin K_1 intake had a lower risk of cataract than those in the lowest tertile.

Meaning These findings suggest that a high intake of dietary vitamin K_1 is associated with a reduced risk of cataract in an elderly Mediterranean population.

Methods

The analysis was conducted in the Prevención con Dieta Mediterránea (PREDIMED) study, a parallel-group, randomized clinical cardiovascular prevention trial in participants at high cardiovascular risk (http://www.predimed.es). The detailed protocol was published elsewhere.[7] All participants included in the study provided written informed consent according to a protocol approved by the institutional review boards of all the recruiting centers. At baseline and yearly thereafter, a food frequency questionnaire was administered, and data on energy and nutrient intake were obtained using Spanish food composition tables. Vitamin K_1 intake was estimated using the US Department of Agriculture nutrient database. Cataract was a prespecified secondary outcome defined as the occurrence of cataract surgery during the study. The outcome was externally confirmed by an independent adjudication committee. Traumatic cataracts and those that emerged after intraocular surgery were excluded. In cases of bilateral surgery in the same patient, only the first event was considered (eMethods in the Supplement).

Baseline descriptive data were presented as mean (SD) and percentages using analysis of variance and χ^2 test. Dietary variables were adjusted for total energy intake using the residuals method. We averaged food consumption from the baseline to the end of the follow-up or to the last follow-up food frequency questionnaire before the occurrence of cataract surgery. Multivariable Cox proportional hazard models were fitted to assess the association between dietary vitamin K_1 intake and the risk of cataract surgery. Follow-up time was calculated as the time between recruitment and the date of the event, death, or end of study, whichever came first. Hazard ratios (HRs) and 95% confidence intervals were calculated. A sensitivity analysis was conducted excluding all cataract surgeries that occurred during the first year of the trial. The level of significance was $P < .05$, and all P values were 2-sided. Statistical analyses were conducted using SPSS, version 19.0 (SPSS Inc) and Stata, version 14 (StataCorp).

Results

We included 5860 participants in the analysis after excluding those with extremes of energy intake, incomplete dietary data at baseline, bilateral cataract, or for whom bilateral cataract could not be discarded at baseline.

During a median of 5.6 years of follow-up, 768 participants underwent cataract surgery. Baseline characteristics of study participants are shown in **Table 1**. Individuals in the highest tertile of mean energy-adjusted dietary vitamin K_1 intake had a lower waist circumference, were more physically active, and were less inclined to smoke. Baseline dietary intake is presented in the eTable in the Supplement. Participants in the highest tertile of the mean energy-adjusted dietary vitamin K_1 intake had a lower risk of cataract surgery than those in the lowest tertile (HR, 0.71; 95% CI, 0.58-0.88; $P = .002$) (**Table 2**). The survival curves and the number of participants at risk in each tertile are shown in **Figure**. The results of the sensitivity analysis did not differ from the general one, showing a lower risk of cataract surgery in the highest tertile of energy-adjusted dietary vitamin K_1 intake (HR in the 3rd tertile, 0.75; 95% CI, 0.60-0.92; $P = .02$).

Discussion

These data suggest that higher intake of dietary vitamin K_1 is associated with a reduced risk of cataract. A lens is a complex tissue with a high protein content, minimum turnover, and a powerful antioxidant system. However, during the aging process, lens proteins are subject to posttranslational modifications, and the antioxidant system is compromised. Other chronic conditions, such as hyperglycemia and inflammation, also contribute to the cataract formation.[2,8]

Several epidemiologic studies have shown an inverse association between diets rich in antioxidant and anti-inflammatory vitamins and the risk of cataract, although the results of clinical trials failed to establish any causal association.[9] In this study, we found an inverse association between higher intake of dietary vitamin K_1 and the risk of cataract surgery. Our results could be partly explained by the antioxidant and anti-inflammatory properties attributed to this vitamin.[10] Moreover, dietary vitamin K_1 intake has been also associated with lower circulating glucose levels and a delay in the insulin response to

Table 1. Baseline Characteristics of 5860 Prevención con Dieta Mediterránea Participants by Tertiles of Baseline Dietary Vitamin K_1 Intake

Variable	Energy-Adjusted Baseline Vitamin K_1 Intake, µg/d, No. (%)			*P* Value[a]
	T1 (n = 1953)	T2 (n = 1954)	T3 (n = 1953)	
Women	1001 (51.3)	1086 (55.6)	1182 (60.5)	<.001
Age, mean (SD), y	66.3 (6.3)	66.4 (6.0)	66.1 (5.9)	.41
BMI, mean (SD)	30.1 (3.9)	29.8 (3.8)	29.9 (4.1)	.07
Waist circumference, mean (SD), cm	101.3 (10.1)	100.0 (10.5)	100.1 (10.7)	<.001
Leisure-time physical activity, mean (SD), MET min/d	213.0 (224.7)	235.4 (239.6)	245.2 (248.4)	<.001
Glucose, mean (SD), mg/dL	119.3 (38.3)	120.5 (40.3)	123.1 (42.6)	.011
Total cholesterol, mean (SD), mg/dL	211.9 (37.8)	217.1 (44.4)	218.1 (40.3)	.007
Triglycerides, mean (SD), mg/dL	151.6 (103.0)	140.5 (75.8)	145.9 (85.1)	.09
HDL cholesterol, mean (SD), mg/dL	51.6 (13.1)	53.8 (14.8)	54.0 (13.3)	<.001
Diabetes	881 (45.1)	903 (46.2)	991 (50.7)	.001
Hypertension	1607 (82.3)	1655 (84.7)	1593 (81.6)	.03
Hypercholesterolemia	1414 (72.4)	1370 (70.1)	1453 (74.4)	.01
Smoking status				
Never	1115 (57.1)	1190 (60.9)	1217 (62.3)	<.001
Current	342 (17.5)	299 (15.3)	240 (15.3)	
Former	496 (25.4)	465 (23.8)	496 (25.4)	
Education				
Primary education	1481 (75.8)	1490 (76.2)	1509 (77.3)	.57
Secondary education	333 (17.1)	314 (16.1)	296 (15.1)	
Higher education	87 (7.1)	150 (7.7)	148 (7.6)	
Intervention group				
Mediterranean diet with EVOO	658 (33.7)	684 (35.0)	670 (34.3)	.006
Mediterranean diet with nuts	598 (30.6)	647 (33.1)	684 (35.0)	
Control diet	697 (35.7)	623 (31.9)	599 (30.7)	
Cataract				
Prevalence	77 (3.9)	80 (4.1)	72 (3.7)	.80
Incidence	263 (13.5)	260 (13.3)	245 (12.5)	.66

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); EVOO, extra virgin olive oil; HDL, high-density lipoprotein; MET, metabolic equivalent of task; PREDIMED, Prevención con Dieta Mediterránea; T, tertile.

SI conversion factor: To convert HDL cholesterol to millimoles per liter, multiply by 0.0259; total cholesterol to millimoles per liter, multiply by 0.0259; glucose to millimoles per liter, multiply by 0.0555; triglycerides to millimoles per liter, multiply by 0.0113.

[a] *P* values are based on the difference between tertiles of mean energy-adjusted dietary vitamin K_1 intake (analysis of variance for the continuous variables and χ^2 test for categorical variables).

Table 2. Adjusted HRs of Cataracts According to Tertiles of Vitamin K_1 Intake[a]

Variable	Energy-Adjusted Average Dietary Phylloquinone Intake, µg/d			*P* Value for Trend
	T1 (n = 1953)	T2 (n = 1954)	T3 (n = 1953)	
Vitamin K_1 intake, median (IQR), µg/d	249.4 (206.7-281.7)	353.6 (329.6-378.0)	496.7 (444.4-597.2)	NA
Cases/person-year, No.	10 420	11 203	11 204	NA
Crude model, HR (95% CI)	1 (Reference)	0.93 (0.79-1.10)	0.71 (0.60-0.85)	<.001
Model 1, HR (95% CI)[b]	1 (Reference)	0.96 (0.80-1.15)	0.71 (0.58-0.88)	.002

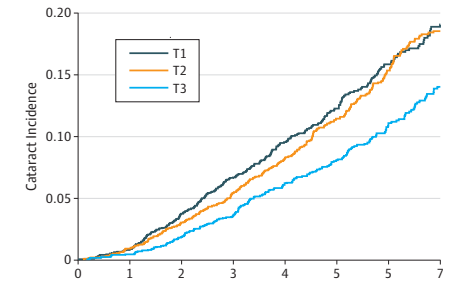
Abbreviatons: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HR, hazard ratio; IQR, interquartile range; MET, metabolic equivalent of task; NA, not applicable.

[a] Cox regression was used to evaluate the risk of cataracts according to tertiles of baseline dietary vitamin K intake and mean dietary energy-adjusted vitamin K intake.

[b] Model 1 was adjusted for sex, age, BMI, recruiting center, intervention group, smoking (never, current, past), leisure time activity (MET/d), education (primary education, secondary education, higher education), cataract event at baseline, diabetes at baseline, hypertension, hypercholesterolemia, and use of anticoagulant and for baseline dietary variables in energy-adjusted tertiles (vegetables, fruits, legumes, cereals, dairy, meat, fish, olive oil, and nuts), Vitamin C, alcohol, and alcohol squared in grams per day.

glucose infusion.[11] In this regard, dietary vitamin K_1 intake was associated with a reduced risk of new-onset type 2 diabetes in the PREDIMED trial after 5.5 years of follow-up.[12] A 51% lower risk of diabetes was reported in those participants who increased their vitamin K_1 intake during the follow-up. Similarly, a 2014 study[13] conducted in diabetic rats demonstrated that the treatment with vitamin K_1 decreased blood and lens glucose concentrations and reduced the levels of lens sorbitol that could help prevent cataract formation.[13] Therefore, the protective effect of vitamin K_1 intake on cataract surgery risk observed in our study helps to reinforce these findings in animals and extend them to the human population.

Figure. Nelson-Aalen Estimates of Incidence of Cataracts by Tertiles of Vitamin K_1 Intake



No. at risk

T1	1953	1923	1847	1713	1433	1091	722	481
T2	1954	1927	1870	1779	1528	1216	975	689
T3	1953	1939	1906	1818	1552	1228	960	653

Limitations

Some limitations of this study should be mentioned. First, because food frequency questionnaires were used for the dietary assessment and vitamin K_1 contents were extracted from the US Department of Agriculture food composition database because no Spanish composition tables include the vitamin K contents of food, we cannot discard errors in the estimation of vitamin K_1 intake. However, although the food frequency questionnaire used was not specifically validated for vitamin K_1 intake, an intraclass correlation coefficient of 0.81 was found.[14] Second, because we have no data on vitamin K_1 plasma levels, we cannot discard physiological conversions of dietary vitamin K_1 that do not account for the circulating active form of vitamin K. However, a high association between dietary vitamin K_1 intake and plasma vitamin K_1 levels has been demonstrated previously.[15] Third, because the study was conducted in elderly participants at high cardiovascular risk living in a Mediterranean country, our findings may not be extrapolated to other populations. Finally, although we cannot completely discard a potential different access to healthier dietary habits according to the socioeconomic status, we have adjusted the Cox model by food groups to avoid the residual confounding. Likewise, in Spain, cataract surgery is included in the National Health System, thus removing any bias regarding access to the surgery procedures according to socioeconomic status. The strengths of this study include its longitudinal design, the use of repeated dietary measurements during follow-up, and the accurate assessment of incident cataract surgery.

Conclusions

The results of this study suggest a protective role of high vitamin K_1 dietary intake on cataract incidence in a senior Mediterranean population even after adjusting by other potential confounders. Further studies and trials are required to confirm these results.

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VITAMIN K & HEALTHY AGEING
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CHAPTER 2

Dietary vitamin K intake is associated with a lower risk of diabetes nephropathy but not diabetes retinopathy

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VITAMIN K & HEALTHY AGEING
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Clinical Nutrition

Dietary vitamin K1 intake is associated with a lower risk of diabetic nephropathy but not diabetic retinopathy

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Abstract:	Background: Vitamin K dietary intake has been associated with an improvement of the insulin response and sensitivity, along with a reduced risk of Type 2 Diabetes (T2D). However, whether vitamin K1 has any effect in the development of the different complications associated to T2D has not been yet explored. Objective: The present analysis aimed to evaluate, for the first time, the association between dietary intake of vitamin K1 and the risk of diabetic nephropathy (DN) and diabetic retinopathy (DR) in an elderly Mediterranean population at high cardiovascular risk. Methods: A post-hoc analysis was conducted in patients with T2D participating in the PREDIMED study who were free of DN and DR at enrolment and had available data on dietary intake (n=3509). At baseline and yearly thereafter, vitamin K1 dietary intake was repeatedly evaluated using a validated food-frequency questionnaire. Multivariable-adjusted Cox regression models were fitted to estimate the Hazard Ratios (HR) for the new onset of diabetic nephropathy and retinopathy. Results: Through a mean follow-up of 5.9 years, we identified a total of 165 new cases

	of diabetic nephropathy and 70 diabetic retinopathy. After adjusting for potential confounders, those participants allocated in the upper tertile of the yearly updated dietary vitamin K1 intake had a significant lower risk of diabetic nephropathy compared to those in the lowest tertile (HR [95%CI]; 0.59 [0.39, 0.90] P for trend= 0.009). However, no significant association was found for the diabetic retinopathy risk. Conclusion: A high dietary intake of vitamin K1 could have a protective effect against diabetic nephropathy but not on retinopathy in an elderly Mediterranean population.
Opposed Reviewers:	

Dietary vitamin K1 intake is associated with a lower risk of diabetic nephropathy but not diabetic retinopathy

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Short running head: Dietary vitamin K1 intake and diabetic complications.

ABSTRACT

Background: Vitamin K dietary intake has been associated with an improvement of the insulin response and sensitivity, along with a reduced risk of Type 2 Diabetes (T2D). However, whether vitamin K1 has any effect in the development of the different complications associated to T2D has not been yet explored.

Objective: The present analysis aimed to evaluate, for the first time, the association between dietary intake of vitamin K1 and the risk of diabetic nephropathy (DN) and diabetic retinopathy (DR) in an elderly Mediterranean population at high cardiovascular risk.

Methods: A post-hoc analysis was conducted in patients with T2D participating in the PREDIMED study who were free of DN and DR at enrolment and had available data on dietary intake (n=3509). At baseline and yearly thereafter, vitamin K1 dietary intake was repeatedly evaluated using a validated food-frequency questionnaire. Multivariable-adjusted Cox regression models were fitted to estimate the Hazard Ratios (HR) for the new onset of diabetic nephropathy and retinopathy.

Results: Through a mean follow-up of 5.9 years, we identified a total of 165 new cases of diabetic nephropathy and 70 diabetic retinopathy. After adjusting for potential confounders, those participants allocated in the upper tertile of the yearly updated dietary vitamin K1 intake had a significant lower risk of diabetic nephropathy compared to those in the lowest tertile (HR [95%CI]; 0.59 [0.39, 0.90] P for trend= 0.009). However, no significant association was found for the diabetic retinopathy risk.

Conclusion: A high dietary intake of vitamin K1 could have a protective effect against diabetic nephropathy but not on retinopathy in an elderly Mediterranean population.

Introduction

Type 2 Diabetes (T2D) is one of the leading causes of cardiovascular disease worldwide, and affects almost 425 million people by the year 2017 with a projected figure of 629 million by 2045 [1]. People with T2D are at increased risk of many other complications, including nephropathy and retinopathy, that are related to interconnected mechanisms including insulin resistance, hyperglycaemia, low-grade inflammation and oxidative stress [2,3].

Diabetic nephropathy (DR) represents the leading cause of kidney disease in developed countries, affecting approximately to 25% of subjects with T2D [4,5]. DN is characterized by increased albuminuria levels and inflammation markers that progressively decline the glomerular filtration rate (GFR) and promotes the elevation of the blood pressure [4]. Diabetic retinopathy (DR) is the leading cause of blindness among working-age adults [6] and is characterised by progressive vascular alterations which produce proliferative lesions and clinically significant macular oedema [7]. Inflammation may be one of the leading causes of DR, mediating deleterious effects in the neuronal and vascular components of the retina and chronic retinal inflammation has been found from early phases to advanced stages. However, the development of DR is also mediated through metabolic alterations like hypertension, hyperglycaemia and dyslipidaemias; as well as through oxidative stress, and advanced glycation end products (AGEs) accumulation [8]. Therefore, dietary strategies focused on improving insulin resistance, inflammation and oxidation are of potential interest for diabetes complications and public health.

Vitamin K is a fat-soluble vitamin with numerous effects among health. Its role in the blood-clotting cascade, calcium metabolism and bone health have been extensively studied [9,10]. But the role of vitamin K as an anti-inflammatory [10–12] and anti-oxidant factor [13,14], as well as its relation with insulin response and sensitivity [15,16] has renewed its interest in nutrition and human health.

In previous analysis conducted by our group, we observed that higher intake of vitamin K1 was associated with an improvement of circulating inflammatory and other metabolic markers associated with insulin metabolism and type 2 diabetes [17]. Consistently, dietary

vitamin K has been associated with a reduced risk of T2D [16,18] However, it is still uncertainty whether vitamin K has any effect in the development of the different diabetic complications. In the best of our knowledge, there is only one study demonstrating that the subcutaneous administration of vitamin K for three months in rats decreased blood glucose and prevented microalbuminuria. Vitamin K also reduced oxidative stress and protected renal physiology by modulating Ca2+ and Na+/K+-ATPases [19]. No study has assessed the longitudinal association of dietary vitamin K on diabetic nephropathy and retinopathy in humans. The present research explores, for the first time, the association between dietary vitamin K1 intake and the risk of DN and DR in an elderly diabetic Mediterranean population at high cardiovascular risk.

Methods

Study design and population

The present analysis was performed in the framework of the PREDIMED study, a large multicentre and parallel group controlled clinical trial conducted in Spain between October 2003 and December 2010. The main aim of this trial was to evaluate the effectiveness of the Mediterranean Diet (MedDiet) on the primary prevention of cardiovascular disease [20]. A total of 7447 high cardiovascular risk individuals were randomized to one of the three intervention groups: MedDiet supplemented with extra-virgin olive oil (EVOO), MedDiet supplemented with mixed nuts, or advice on a low-fat diet (control diet). Participants who shared a household (n=425) were assigned to the same intervention group; and participants of one of the recruiting centres (n=467) were allocated by clusters instead of using individual randomization. Subjects included in the study were men aged 55-80 years and women aged 60-80 years, free of cardiovascular disease at enrolment, but with either T2D or three or more of the following cardiovascular risk factors: hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg, or antihypertensive drugs), hypercholesterolemia (LDL cholesterol ≥160 mg/dL or medication), low HDL cholesterol (≤50 mg/dL in women or ≤40 mg/dL in men), 25≤BMI≥40 kg/m^2, current smoking, or family history of premature coronary heart disease. Exclusion criteria have been reported previously [20]. All participants included in the study provided written informed consent according to a protocol approved by the institutional review boards of all the recruiting centres. For the current analysis we excluded those subjects without T2D at baseline (n=3833), those subjects who were outside of the predefined values for total energy intake (>4000 or <800 kcal/d in men and >3500 or <500 kcal/d in women) (n=64), and those without baseline food frequency questionnaire (FFQ) information (n=41). We therefore analysed a subset of 3509 participants. For the analysis of nephropathy, we additionally excluded subjects who lacked measurements at baseline or who did not have at least two consecutive urinary albumin/creatinine ratio or serum creatinine

measurements that enable to determine the onset of disease during the follow-up (n=933). Participants were also excluded (n = 491) if they had any of the following conditions at baseline at two consecutive visits: albuminuria (urinary ACR≥30 mg/g) or impaired renal function (eGFR <60 mL/min/1.73 m^2), two widely used measures for assessing kidney dysfunction. The final sample size for the assessment of diabetic nephropathy incidence was 2085 participants.

Dietary assessment

Total energy, macro and micronutrient intake were assessed by a 137-item semi-quantitative validated food frequency questionnaire [21]. The information was gathered annually in face-to-face interviews with the participants performed by trained dietitians. Energy and nutrient intake were estimated using Spanish food composition tables [22,23]. Vitamin K1 intake was estimated using the United States Department of Agriculture (USDA) nutrient database as this information is not available in the Spanish food composition tables [24]. Reproducibility and relative validity of the FFQ used in the study was validated for dietary vitamin K1 intake via the intra-class correlation coefficient. The FFQ was administered twice to explore reproducibility at one year, and four 3-day dietary records were used as reference to explore validity. Vitamin K1 intake reproducibility was estimated by Pearson correlation (r=0.755) with an interclass correlation coefficient of 0.860 (p<0.001) [17]. A validated 14-item Screener, that includes 12 questions on food intake frequency and 2 questions on food consumption habits, was administrated to assess the adherence to the Mediterranean Diet [25].

Ascertainment of incident diabetic nephropathy and retinopathy

Clinical outcomes were confirmed by an External Adjudication Committee. New-onset of diabetic nephropathy was based on assessments recorded in the clinical records. Despite DN and DR were not prespecified secondary outcome of the PREDIMED trial, given that nearly half of participants had T2D, these two complications were included as a relevant outcomes in all interim analyses supervised by the Data Safety Monitoring Board. We also

included incident cases of DN defined by the chronic kidney disease (CKD) progression from moderate to severe (stage 3 or greater) or albuminuria progressing during follow-up. The kidney disease progression was defined as a sustained estimated glomerular filtration rate (eGFR) value <60 mL/min/1.73 m^2 based on serum creatinine, whereas the albuminuria progression was defined as the transition from normo- to micro- or macro-albuminuria (Albumin-to-creatinine ratio [ACR] ≥30 mg/g). Serum creatinine and ACR were measured at least once a year in the 67% and 43% of participants, respectively. Both progresses were confirmed by at least two consecutive measurements during follow-up. The end-point for diabetic nephropathy was determine as the time to first occurrence. New-onset diabetic retinopathy was considered after the diagnoses by an ophthalmologist and ascertained by the adjudication events committee. Participants were considered to have sight-threatening DR if they had undergone laser photocoagulation, intra-vitreal anti-vascular endothelial growth factor injections, and/or vitreoretinal surgery. Since Spain's public health systems advise yearly fundus examination by an ophthalmologist to detect early diabetic retinopathy, in the present report we presume that all participants were free of diabetic retinopathy at baseline.

Only events that were definitively confirmed by the adjudication committee and that occurred between October 1, 2003, and June 30, 2012 (date of the last update in the extended follow-up of the PREDIMED cohort, 2 years after the end of the trial), were included in the analyses.

Anthropometrical and Biochemical measurements

Additional information was collected from subjects' medical record, including the use of medication, at baseline and yearly during the follow-up. Trained personnel with calibrated equipment determined anthropometric measures such as weight, height and waist circumference, as well as blood pressure in triplicate with a validated semiautomatic oscillometer (Omron HEM-705CP, Hoofddorp, the Netherlands). The validated Spanish

version of the Minnesota Leisure-Time Physical Activity questionnaire was administered at each visit in order to assess physical activity [26].

At baseline and yearly thereafter we collected and processed fasting blood and spot urine samples for subsequent biochemical analyses. Routine biochemical measurements were gauge including fasting blood glucose, total cholesterol, HDL- and LDL-cholesterol; triglycerides, total protein, creatinine and urea nitrogen. Together, with a routine urine exam that included the albumin/creatinine ratio. Serum creatinine test is a wildly use method to check kidney function. Being a normal waste product of the protein metabolism, is use to estimate the eGFR in order to find the correct level of kidney function. Albumin is the most common type of protein found in urine. Higher amounts of albuminuria can be related to damage in the kidney tissue. eGFR derived from serum creatinine and ACR derived from albumin and creatinine from a urine sample represent the most important measures to identify kidney disease [27]. In the present study, serum creatinine-based eGFR was calculated with the CKD-Epi equation [28].

Statistical analysis.

All the statistical tests were 2-tailed and the level of significance was $P < 0.05$. Statistical analyses were carried using R software v3.5.1 ([www.r-project.org], R Development Core Team, 2012). Baseline descriptive data are presented as means ± standard deviations (SDs) for continuous variables and as numbers and percentages (%) for categorical variables, using one-way ANOVA and the $\chi 2$ test for comparisons between groups. We averaged the vitamin K1 dietary intake and the MedDiet adherence 14-point score from baseline to the year before the end of the follow-up in those who did not develop the event or to the last follow-up FFQ before the occurrence of the respective event. Follow-up time was calculated as the time between recruitment and the date of the appeareance of the event, death, end of follow-up or end of the study, whichever came first. Multivariable time-dependent Cox proportional hazard models with robust variance and considering intra-cluster correlations were fitted to assess the association between the average dietary

vitamin K1 intake and the risk of diabetic nephropathy or diabetic retinopathy. Participants who shared their household were defined as a cluster variable included into the Cox models as an independent variable. Hazard Ratios (HRs) and 95% Confidence Intervals (CIs) were calculated considering the first tertile of average vitamin K1 intake as reference. Model 1 was adjusted for sex, age, body mass index (BMI), recruiting center, intervention group, smoking, leisure time physical activity, prevalence of hypertension, prevalence of hypercholesterolemia and use of anticoagulant and insulin. Model 2 was additionally adjusted for average MedDiet adherence (14-point score) and average energy intake. Interaction test for sex and age with vitamin K1 intake were not statistically significant. Likewise, sensitevity analyses stratified by sex, age and BMI categories, as well as for the prevalence of hypertension or dyslipidaemia were performed for the risk of both diabetic nephropathy and retinopathy.

Results

Participants

After a median of 5.9 years of follow-up, we observed a total of 165 new cases of diabetic nephropathy and 70 new cases of diabetic retinopathy. These results are based on a database that included primary end-points events recorded until June 30, 2012.

The baseline characteristics of study participants by baseline tertiles of dietary vitamin K1 intake are shown in Table 1**.** Subjects in the highest tertile of vitamin K1 were likely to be younger and had a lower waist circumference and body weight. Table 2 and Table 3 show the HRs and 95% CIs for the association between tertiles of dietary averaged vitamin K1 intake and the risk of incident DN or DR respectively. After adjusting the models for potential confounders, those participants allocated in the upper tertile of dietary vitamin K1 intake had a significant lower risk of nephropathy than those in the lowest tertile (HR [95%CI]; 0.59 [0.39, 0.90] P for trend= 0.009). However, in contrast, no significant association was observed for the risk of diabetic retinopathy (HR [95%CI]; 1.36 [0.76, 2.43] P for trend= 0.241).

In table 4 and 5, we respectively show sensitivity analyses for the risk of both nephropathy and retinopathy stratified by sex, age and BMI categories, as well as for the prevalence of hypertension or dyslipidaemia. The inverse association between the risk of nephropathy and vitamin K1 intake remained significant in males, in subjects younger than 70 and in subjects with a BMI greater than or equal to 30. Likewise, the association persisted in those subjects with hypertension and dyslipidaemia. In contrast, no significant associations were observed for the retinopathy risk after performing the subgroup analysis.

Discussion

In this prospective analysis of the PREDIMED study, we hypothesized that the dietary intake of vitamin K1 could be associated with a lower risk of diabetic nephropathy and retinopathy, two common microvascular complications of T2D. Higher consumption of vitamin K1 was associated with a reduced risk of diabetic nephropathy but not with diabetic retinopathy. To the best of our knowledge, no previous analysis has assessed the association between vitamin K1 and the risk of microvascular diabetic complications.

In a previous study conducted in streptozotocin-induced rats, the subcutaneous administration of vitamin K for 3 months, showed a protective effect against early-onset diabetic nephopathy. In this in vivo study, the authors also found a significant reduction of blood glucose levels and prevented microalbuminuria. Likewise, the administration of vitamin K reduced the oxidative stress and protected the renal physiology by modulating Ca2+ and Na+/K+-ATPases [19]. This protective effect of vitamin K observed in rats is in line with our results showing a significant reduced risk of diabetic nephropathy in those subjects consuming the highest amounts of dietary vitamin K. Moreover, an epidemiological study conducted in a multi-ethnic population described an inverse association between circulating markers of vitamin K deficiency and decreased eGFR or increased CKD risk, thus suggesting a role of vitamin K on renal function [29]. Even though there are no other studies on humans that can confirm our results, the modulatory role of vitamin K on several mechanisms involved in the pathophysiology of diabetic nephropathy could additionally support our findings. Although is still incompletely understood, multiple factors including hyperglycaemia, hyperinsulinemia, inflammation and oxidative stress are implicated in the development and progression of DN [30,31]. All these factors are beneficially modulated by vitamin K [15–17]. In this sense, it has been proposed that vitamin K-dependent proteins, such as osteocalcin may improve insulin sensitivity and glycaemic status through the suppression of inflammation [32].

Contrary to our hypothesis, in the present study, we failed to found a risk reduction for diabetic retinopathy in subjects with the highest intake of dietary vitamin K. Similar to

diabetic nephropathy, a range of hyperglycaemia-linked pathways, including the accumulation of advanced glycation end-product formation and the activation of protein kinase C (PKC) isoforms [33], have been implicated in the development and the progression of this condition [34]. Many inflammatory cytokines have also found increased in patients with DR [35–37] and increasing evidence points to inflammation as one of the main causative contributor for DR development. A cross-sectional case-control study conducted over 58 DR subjects and 57 matched control diabetic subjects, identified insulin resistance as independent specific marker of proliferative diabetic retinopathy [38]. However, the role of insulin resistance in the development of DR is still unclear. In this sense, in an experimental animal model, the systemic insulin resistance in rats without concomitant hyperglycaemia did not induce any significant change in retinal inflammatory gene expression pattern involved in the disease progression, compared to streptozocin-hyperglycaemic rats, which also displays insulin resistance and an upregulated inflammatory status [39]. In addition, the neurodegeneration produced in the retina occurs in early stages during the progression of DR. In animal models, specifically in diabetic rats, apoptosis of retinal neurons can be observed shortly as one months after induction of diabetes [40]. Therefore, we cannot discard that beneficial effect of dietary vitamin K on inflammation and other mechanisms underlying DR are not strong enough considering this rapid evolution of the microvascular damage related to DR.

The results of our study should be interpreted in the context of its limitations and strengths. First, since we did not measure the circulating concentrations of vitamin K1 the estimated dietary vitamin K1 from FFQ data cannot exactly reflect the circulating vitamin K1 available for the intermediate metabolism. However, a previous study have shown an acceptable correlation between dietary vitamin K1 and its circulating levels [41]. Moreover, we have also used the average for dietary vitamin K1 intake and other nutrients to diminish variability and to account for changes in dietary variables during the follow-up. The FFQ used was validated against 3-day-dietary records in a subset of participants showing a very

good correlation with vegetables and specifically with vitamin K. Secondly, neither nephropathy nor retinopathy were defined as primary outcomes of the study, since the PREDIMED objective was to assess the effect of the MedDiet on cardiovascular risk prevention. Thus, this is a post-doc analysis in which an external adjudication committee confirmed all cases of new onset of nephropathy and retinopathy. Third, despite we have fitted robust Cox models adjusted for potential confounders, we cannot completely discard that other variables could affect the observed associations. Finally, beyond other potential explanations discussed before, we cannot discard that the lack of associations observed between dietary vitamin K1 intake and the risk of diabetic retinopathy be due to a lack of statistical power due to the relative small number of events.

In conclusion, the results of our study suggest that a high dietary intake of vitamin K1 could have a protective effect on the development of diabetic nephropathy but not on retinopathy in an elderly diabetic Mediterranean population. This study adds a new potential health benefit to vitamin K1. Further longitudinal studies and clinical trials need to be carried out in order to confirm these results.

Statement of Authorship: MB had full access to all of the data in the study, taking responsibility for its integrity and the accuracy of the data analysis. MB, MAM-G, DC, RE, MF, EG-G, FA, MF, JMS-L, LS-M, XP, JB, ET, MAM, VZ-M, JS-S: contributed to the conception, design, and implementation of the project. LF: was a member of the outcome adjudication committee. All authors contributed to data collection and analytical procedures. JFG-G: conducted the statistical analysis. LC-B, MB and JS-S: interpreted data, and wrote the manuscript; and all authors: read and approved the final version of the manuscript.

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TABLE 1. Baseline characteristics of the subjects included in the diabetic nephropathy and diabetic retinopathy analysis by tertiles of baseline vitamin K1 dietary intake.

	Diabetic Nephropathy				Diabetic Retinopathy			
Variable[1]	**T1 (n= 695)**	**T2 (n= 695)**	**T3 (n= 695)**	***P*[2]**	**T1 (n= 1169)**	**T2 (n= 1170)**	**T3 (n= 1169)**	***P*[2]**
Women, n (%)	351 (50.5)	359 (51.7)	375 (54.0)	0.423	600 (51.3)	615 (52.6)	630 (53.8)	0.475
Age, y	67.0 ± 6.4	67.2 ± 5.8	66.1 ± 6.0	0.002	67.6 ± 6.5	67.8 ± 6.2	66.9 ± 6.1	0.001
BMI, kg/m^2	30.1 ± 4.3	29.5 ± 3.9	30.0 ± 4.2	0.028	30.2 ± 4.1	29.7 ± 3.9	29.7 ± 4.1	0.002
Waist circumference, cm	101.4 ± 10.0	100.0 ± 10.3	101.0 ± 10.4	0.020	102.1 ± 10.1	100.7 ± 10.4	101.0 ± 10.3	0.007
Leisure-time physical activity, MET-min/d	234.9 ± 251.3	266.8 ± 269.0	260.0 ± 275.5	0.062	218.4 ± 239.9	251.6 ± 252.5	245.9 ± 267.3	0.003
Glucose, mg/dl	145.9 ± 41.4	146.9 ± 45.0	148.5 ± 45.5	0.552	146.0 ± 42.6	147.1 ± 45.6	148.4 ± 45.9	0.456
Cholesterol, mg/dl	202.4 ± 41.4	204.0 ± 38.1	210.2 ± 38.1	0.070	203.1 ± 38.8	206.3 ± 39.1	209.8 ± 38.3	0.050
Triglycerides, mg/dl	151.2 ± 108.8	137.3 ± 75.9	153.4 ± 80.8	0.177	159.3 ± 121.3	141.7 ± 75.3	152.0 ± 79.6	0.049
HDL cholesterol, mg/dl	51.5 ± 13.5	52.9 ± 12.8	53.5 ± 13.0	0.271	50.4 ± 12.9	52.9 ± 13.4	53.1 ± 12.7	0.005
Hypertension, n (%)	497 (71.5)	494 (71.1)	483 (69.5)	0.686	867 (74.2)	869 (74.3)	837 (71.5)	0.238
Hypercholesterolemia, n (%)	396 (57.0)	402 (57.8)	437 (62.9)	0.054	699 (59.8)	666 (56.9)	710 (60.7)	0.154
Smoking status, n (%)				0.875				0.428
Never	424 (61.0)	416 (59.9)	425 (61.2)		708 (60.6)	722 (61.7)	705 (60.3)	
Current	85 (12.2)	95 (13.7)	82 (11.8)		145 (12.4)	147 (12.6)	128 (10.9)	

Former	186 (26.8)	184 (26.4)	188 (27.0)		316 (27.0)	301 (25.7)	337 (28.8)	
Education, n (%)				0.827				0.699
Primary education	545 (78.4)	554 (79.7)	536 (77.1)		940 (80.4)	945 (80.8)	919 (78.6)	
Secondary education	103 (14.8)	98 (14.1)	112 (16.1)		161 (13.8)	155 (13.2)	176 (15.0)	
Higher education	47 (6.8)	43 (6.2)	47 (6.8)		68 (5.8)	70 (6.0)	75 (6.4)	
Intervention group, n (%)				0.079				0.140
Mediterranean diet with EVOO	233 (33.6)	247 (35.6)	244 (35.1)		406 (34.7)	404 (34.5)	434 (37.1)	
Mediterranean diet with nuts	199 (28.6)	233 (33.5)	222 (31.9)		335 (28.7)	392 (33.5)	375 (32.0)	
Control diet	263 (37.8)	215 (30.9)	229 (33.0)		428 (36.6)	374 (32.0)	361 (30.9)	

[1] Means ± SDs or n (%), otherwise indicated. EVOO, extra virgin olive oil; MET, metabolic equivalent of task; T, tertiles.

[2] *P* values are based on the difference between tertiles of baseline dietary vitamin K1 intake (ANOVA for the continuous variables and χ^2 test for categorical variables).

TABLE 2. Adjusted HRs of diabetic nephropathy according to tertiles of dietary vitamin K1 intake.[a]

	T1 (n = 695)	T2 (n = 695)	T3 (n = 695)	P-trend
Vitamin K1, μg/d[b]	229 ± 51	344 ± 30	527 ± 126	
Events, % (n)	9.35 (65)	9.07 (63)	5.32 (37)	
Crude model, HR (95% CI)	1 (ref.)	0.91 (0.64, 1.29)	0.53 (0.36, 0.80)	0.001
Model 1, HR (95% CI)[c]	1 (ref.)	0.97 (0.68, 1.40)	0.58 (0.36, 0.88)	0.007
Model 2, HR (95% CI)[d]	1 (ref.)	0.99 (0.67, 1.45)	0.59 (0.39, 0.90)	0.009

[a] Cox regression was used to evaluate the risk of diabetic nephropathy according to tertiles of average dietary vitamin K1 intake.

[b] Means ± SDs

[c] Model 1 was adjusted for sex, age, BMI, recruiting centre, intervention group, smoking, leisure time activity, prevalence of hypertension, prevalence of hypercholesterolemia, use of anticoagulant and use of insulin.

[d] Model 2 was adjusted for previous variables and average Mediterranean Diet adherence (14-point score) and average energy intake.

TABLE 3. Adjusted HRs of diabetic retinopathy according to tertiles of dietary vitamin K1 intake.[a]

	T1 (n = 1170)	T2 (n = 1170)	T3 (n = 1169)	P-trend
Vitamin K1, µg/d[b]	219 ± 53	334 ± 29	519 ± 126	
Events, % (n)	2.00 (23)	1.50 (18)	2.50 (29)	
Crude model, HR (95% CI)	1 (ref.)	0.74 (0.40, 1.37)	1.22 (0.71, 2.10)	0.400
Model 1, HR (95% CI)[c]	1 (ref.)	0.70 (0.38, 1.27)	1.12 (0.63, 1.97)	0.557
Model 2, HR (95% CI)[d]	1 (ref.)	0.81 (0.44, 1.40)	1.36 (0.76, 2.43)	0.241

[a] Cox regression was used to evaluate the risk of diabetic retinopathy according to tertiles of average dietary vitamin K1 intake.

[b] Means ± SDs

[b] Model 1 adjusted for sex, age, BMI, recruiting centre, intervention group, smoking, leisure time activity, prevalence of hypertension, prevalence of hypercholesterolemia, use of anticoagulant and use of insulin.

[c] Model 2 was adjusted for previous variables and average Mediterranean Diet adherence (14-point score) and average energy intake.

Table 4. Subgroup analyses of the incidence of diabetic nephropathy by tertiles of dietary vitamin K1 intake.[a]

	Events/total			HR (95% CI)[b]			
	T1	T2	T3	T1	T2	T3	P-trend
Sex							
Female	25/333	27/334	17/333	1 (Ref.)	0.88 (0.62, 2.08)	0.89 (0.40, 1.58)	0.519
Male	40/361	36/362	20/362	1 (Ref.)	0.88 (0.53, 1.45)	0.48 (0.27, 0.83)	0.009
Age, years							
<70	33/454	34/455	11/455	1 (Ref.)	0.93 (0.55, 1.58)	0.29 (0.14, 0.68)	<0.001
≥70	27/240	36/241	24/240	1 (Ref.)	1.39 (0.80, 2.40)	0.85 (0.48, 1.50)	0.393
BMI, kg/m^2							
<30	35/377	28/378	17/377	1 (Ref.)	0.86 (0.50, 1.48)	0.55 (0.29, 1.02)	0.052
≥30	31/317	36/318	18/318	1 (Ref.)	1.04 (0.60, 1.79)	0.53 (0.28, 0.98)	0.027
Hypertension							
Presence	49/491	57/492	28/491	1 (Ref.)	1.20 (0.78, 1.85)	0.59 (0.36, 0.96)	0.019
Absence	15/203	9/204	7/204	1 (Ref.)	0.55 (0.23, 1.34)	0.47 (0.19, 1.20)	0.147
Dyslipidaemia							
Presence	37/411	32/412	16/412	1 (Ref.)	0.82 (0.49, 1.39)	0.43 (0.22, 0.84)	0.013

Absence	27/283	31/284	22/283	1 (Ref.)	1.22 (0.70, 2.11)	0.83 (0.47, 1.47)	0.427

[a] Cox regression was used to evaluate the risk of diabetic nephropathy according to tertiles of average dietary vitamin K1 intake.

[b] Models adjusted for sex, age, BMI, recruiting centre, intervention group, smoking, leisure time activity, prevalence of hypertension, prevalence of hypercholesterolemia, use of anticoagulant and use of insulin, average Mediterranean Diet adherence (14-point score) and average energy intake. Sex, age, BMI, prevalence of hypertension and prevalence of hypercholesterolemia were removed in each model in which they were the dependent variable.

Table 5. Subgroup analyses of the incidence of diabetic retinopathy by tertiles of dietary vitamin K1 intake.[a]

	Events/total			HR (95% CI)[b]			
	T1	T2	T3	T1	T2	T3	P-trend
Sex							
Female	9/554	6/555	12/555	1 (Ref.)	0.63 (0.22, 1.80)	1.41 (0.57, 3.48)	0.445
Male	14/615	12/615	17/615	1 (Ref.)	0.98 (0.46, 2.06)	1.45 (0.67, 3.14)	0.338
Age, years							
<70	13/718	15/719	19/719	1 (Ref.)	1.26 (0.58, 2.73)	1.63 (0.78, 3.38)	0.186
≥70	9/442	6/445	8/443	1 (Ref.)	0.66 (0.24, 1.85)	1.05 (0.32, 3.48)	0.917
BMI, kg/m^2							
<30	9/627	10/628	16/627	1 (Ref.)	1.18 (0.49, 2.86)	1.68 (0.70, 4.03)	0.238
≥30	9/442	6/445	8/443	1 (Ref.)	0.82 (0.37, 1.82)	0.99 (0.41, 2.39)	0.983
Hypertension							
Presence	8/312	5/312	12/312	1 (Ref.)	0.94 (0.47, 1.89)	1.16 (0.54, 2.47)	0.672
Absence	15/857	13/585	16/627	1 (Ref.)	0.65 (0.19, 2.16)	2.08 (0.80, 5.42)	0.069
Dyslipidaemia							
Presence	15/478	9/478	18/478	1 (Ref.)	1.16 (0.45, 2.99)	1.93 (0.72, 5.19)	0.195

Absence	8/691	9/692	11/692	1 (Ref.)	0.60 (0.26, 1.40)	1.20 (0.57, 2.54)	0.485

[a] Cox regression was used to evaluate the risk of diabetic retinopathy according to tertiles of average dietary vitamin K1 intake.

[b] Models adjusted for sex, age, BMI, recruiting centre, intervention group, smoking, leisure time activity, prevalence of hypertension, prevalence of hypercholesterolemia, use of anticoagulant and use of insulin, average Mediterranean Diet adherence (14-point score) and average energy intake. Sex, age, BMI, prevalence of hypertension and prevalence of hypercholesterolemia were removed in each model in which they were the dependent variable.

CHAPTER 3

Vitamin K dietary intake is associated with cognitive function in an elderly Mediterranean population

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Vitamin K dietary intake is associated with cognitive function in an elderly Mediterranean population.

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ABSTRACT

Background & Aims: In the last years, evidence that dietary vitamin K could have a role in the cognitive response has increased. However, data from large trials are limited. The objective of this study was to assess the association of 2-year changes in the dietary intake of vitamin K with cognitive function measured through scores of cognitive-neuropsychological tests.

Methods: We assessed in 3737 participants of the multicentre PREDIMED-PLUS trial, (47.2% women, age 65.1 ± 4.9 years overweight/obese and with MetS) the adjusted odds ratios of cognitive function decline according to 2-year changes in vitamin K consumption. Participants answered a battery of cognitive function tests (MMSE, WAIS III, Phonology and Semantic verbal fluency test, Trail making test A and B) and FFQ to determine the vitamin K dietary intake.

Results: After adjusting for potential cofounders, the highest increase of dietary vitamin K intake (Median [IQR]; 195.6 µg/d [119.6, 367.2]) were inversely associated with a MMSE score lower than 24 (OR [95%CI]; 0.56 [0.35, 0.98]) compared to those that decreased the intake of vitamin K (Median [IQR]; -104.8 µg/d [-307.2, -53.2]). A significant positive association between changes in dietary vitamin K intake and the semantic verbal fluency test scores (OR [95%CI]; 0.69 [0.53, 0.97]) was found. We did not find any association with the other cognitive function tests assessed in the study.

Conclusion: An increase in the consumption of dietary vitamin K is associated with a better cognitive function scores, independently of recognized risk factors for cognitive decline, in an elderly Mediterranean population at high cardiovascular risk.

Keywords: Vitamin K1 dietary intake, cognitive impairment

INTRODUCTION

Cognitive impairment (CI) is a chronic condition characterized by the loss of memory, concentration and the depletion of the ability to learn new things. It affects the decisions making and in consequence the persons everyday life. Up to one third of cases of cognitive impairment ends in dementia. Although the impairment is less severe than in dementia, it can also result in the inability to live independently. (1) A recent systematic review and meta-analysis estimates that the CI incidence per 1000 person-years was 22.5 for ages 75–79 years, 40.9 for ages 80–84 years, and 60.1 for ages above 85 years. (2) The world health organization (WHO) estimates that by the year 2050 there will be approximately 2 billion individuals over 60 years old, and will become the 22% of the world's population. (3) Because of this, and due to the cost of long-term health care of patients suffering dementias, CI is considered a costly condition for national health systems.

Multiple factors, rather than a single cause, determine the progress of CI. Cardiovascular diseases, hypertension, hypercholesterolemia and overweight or obesity, are related to the development of dementias. (4) Other possible recognized contributors related to these metabolic derangements are insulin resistance, inflammation and oxidative stress. (5) Type 2 diabetes has also been associated with cognitive impairment and dementia. (6,7). In this regards, changes in the brain structure and function may be related to diabetic glycaemic control and duration, although the exact mechanisms underlying cognitive impairment and dementias are not well understood yet. (7)

Either way, modifiable risk factors, like nutrition, may play also a crucial role. A recent review of observational studies and clinical trials that investigated the associations between nutrition and the prevention of CI in humans showed that certain nutrients like folate, flavonoids and vitamin D, but also some food groups such as seafood, vegetables and fruits are inversely related to CI in elderly populations. Somehow, evidence also supports a robust association for healthy dietary patterns such as the Mediterranean diet and cognitive impairment. (8)

In the last years, evidence that vitamin K could have a role in the cognitive function has increased. Historically recognized for its role in blood coagulation, currently, among other actions in brain, vitamin K is also involved in sphingolipid metabolism. Sphingolipids are major components of cell membranes, present in particularly high concentrations in cells

of the central nervous system. Beyond their structural role, they are important players in cellular processes such as proliferation, differentiation, senescence, cell–cell interaction, and transformation. (9) Alterations in sphingolipid metabolism have been associated to neurodegenerative disorders such as Alzheimer and Parkinson diseases, but also to other neurodegenerative aging processes. (10) Beyond of its role in the sphingolipid metabolism, vitamin K has demonstrated beneficial effects on insulin sensitivity and glucose metabolism, decreasing the risk of type 2 diabetes (11–13). In the same line, both its anti-inflammatory (14,15) and anti-oxidant (16) properties could play an important effect decreasing the risk of CI.

A cross-sectional analysis examining vitamin K and cognitive function has found that high dietary phylloquinone intake was associated with better cognition and behaviour among older adults. (17) In the same line, in another cross-sectional analysis, serum phylloquinone concentrations were associated with better verbal episodic memory performances, but not with non-verbal episodic memory, executive functions, and speed of processing. (18)

The present analysis is aimed to assess whether increasing dietary intake of vitamin K was associated with a better cognitive functioning measured through scores of cognitive-neuropsychological tests.

METHODS

The PREDIMED-Plus study design and subjects

The PREDIMED-PLUS study is an ongoing randomized multicentre trial conducted in Spain. Its principal aim is to evaluate the effect of an intensive intervention with an energy reduced Mediterranean diet, physical activity promotion and behavioural support on the cardiovascular events compared to an unrestricted caloric Mediterranean control diet. The study protocol is available at http://predimedplus.com. Participants, recruited in 23 different Spanish centres, are men aged 55–75 years and women aged 60–75 years, with overweight or obesity (body mass index 27–40 kg/m2), who at baseline met at least three components of the metabolic syndrome. (19) The final sample included in this analysis was of 3737 participants. We excluded those participants lacking or without the complete dietary information at the two-year visit at the time of the analysis and those who were outside the predefined values for total energy intake (>4000 or <800 kcal/d in men and >3500 or <500 kcal/d in women).

Dietary Phylloquinone intake

Total energy, macro and micronutrients intake were assessed by a 143-item food frequency questionnaire (FFQ) and estimated using Spanish food composition tables. (20,21) To estimate the dietary intake of vitamin K we used the United States Department of Agriculture (USDA) nutrient database, as this information is not available in the Spanish food composition tables.

Cognitive functioning assessment

At baseline and at 2 years of follow-up, a battery of different tests is administrated to the whole PREDIMED-Plus cohort in order to evaluate changes in the cognitive function. All tests included in the cognitive battery have been standardized for the Spanish population in the age range of the study. The following tests were used:

Mini-mental State Examination (MMSE) is a scored form of cognitive mental functioning. It assesses only the cognitive aspects of mental functions, excluding queries about mood, the form of thinking and abnormal mental experiences. (22) It is widely used due to its practical structure, its availability in several languages and its high levels of sensitivity identifying individuals classified as cognitively impaired. (23)

Wechsler Adult Intelligence Scale III - Digit Span (WAIS III - DS) consist in a two subtest to assess both attention and memory. It requires that the participant repeat sequences of digits of increasing length forward and then in reverse order. DS Forward recalls for attention and short-term memory capacity and DS Backward recall for working memory capacity. (24)

Verbal Fluency Test (VF) assesses both verbal ability and executive control using phonemic and semantic fluency tasks. The phonemic fluency task consists in verbalize, in a minute time, as many words as possible starting with the letter P. In the semantic fluency task, participants have to name, also in 60 seconds, as many different animals as they can without repeating. The final number of words the participant accomplishes corresponds to the total raw score. (25)

Trail Making Test (TMT) is a useful assessment tool to investigate executive function and speed of processing. It consists of two parts, A and B, which have to be performed as rapidly and precisely as possible. In TMT-A, participants are requested to connect consecutive numbers in the correct order drawing a line; and in TMT-B they are asked to connect consecutive numbers and letters in an alternating numeric and alphabetic sequence. The score on each different part is represented by the seconds required to complete the task. (26)

Cognitive impairment was settle for the MMSE with a 24 score cut-off, while for the other test it was defined based on our study population mean score and a -1.5 SD cut-off except for the TMT that the cut-off was +1.5 SD.(27)

Other covariates

At baseline and in each annual visit, trained personnel with calibrated equipment determined different anthropometric measures (i.e. body weight, waist circumference). Baseline routine biochemical analysis including glucose and lipid profile measurements were performed in fasting blood samples. Systolic and diastolic blood pressure are yearly evaluated, using a validated semiautomatic oscillometer (Omron HEM-705CP, Netherlands) after 5 minutes of rest in-between measurements and determined in triplicate. Likewise, the use of different oral medication: anti-hypertensive, anti-diabetic or lipid-lowering medication was recorded. The risk of depression was

assessed by the Beck Depression Inventory–II (BDI-II), a 21-item self-report inventory for measure characteristic attitudes and symptoms of depression. Cut-off values were classified as moderate in-between 20-28 and severe from 29 to 63.(27)

Statistical analysis

Baseline descriptive data are presented as means ± standard deviations (SD) for quantitative variables or as percentages (n) in case of categorical variables. We used multivariate logistic regression models to evaluate the relationship between tertiles of 2-year changes in vitamin K dietary intake and each of the cognitive function tests measured at two years of follow-up (MMSE, WAIS III, Phonology verbal fluency test, Semantic verbal fluency test, Trail making test A and Trail making test B). Models were adjusted for likely confounding variables: sex, age (years), body mass index, smoking habit (actual, former, never), leisure time physical activity (kcal/day) and education (primary, secondary and higher education level), baseline score of evaluated cognition test, prevalence of diabetes (yes/no), hypertension (yes/no) and hypercholesterolemia (yes/no), use of medication (anticoagulants medicines, oral diabetes medicines and insulin), baseline food consumption (dairy, meat, fish, fruit, nuts, legumes, cereals in grams/day), alcohol and alcohol squared in grams per day, and moderate-high risk of depression assessed by the BDI-II. Additionally, models were adjusted for the intervention group and stratified by centre. Vegetables were not included as an adjusting dietary variable since is the major food group source of vitamin K. Participants who shared their household were randomized in the same intervention group and were included as cluster in all models to consider intra-cluster correlations. Furthermore, it was assessed the trend assigning the median score of every tertile of vitamin K intake and using them as continuous variables. Results are presented as odds ratio and 95 % CI. For this analysis, we used the PREDIMED-Plus database of March 12th of 2019.

Possible sex and diabetes interactions with tertiles of changes in vitamin K intake were evaluated using the likelihood ratio test, and no effect modifications ($P>0.05$) were observed. Because the negative biological effect of diabetes on cognition processes is well established, results were also separately analyzed for diabetic and non-diabetic individuals as a secondary analysis. P-values <0.05 were considered significant for all test. Statistical analyses were performed with R software v3.5.1 (www.r-project.org) (R Development Core Team, 2012).

RESULTS

Participants

Of the 3737 participants included in this analysis, 47.2% were women, with a mean age of 65.1 ± 4.9 years; 73% were obese with a mean BMI of 32.4 ± 3.4. Because of the study design, the analysis cohort shows high prevalence of hypertension and hypercholesterolemia. Table 1 shows baseline characteristics of the trial participants by tertiles of 2-year changes in vitamin K dietary intake. Those participants allocated in the highest tertile of change of consumption were slightly younger, mostly men and more likely to have hypertension. Regarding to the cognitive function assessment, they had a higher MMSE score, though the mean differences in-between groups were not clinically relevant. Same tendency is shown for the WAIS III - Digit Span backward recall test results, where higher changes in vitamin K consumption reflect a better performance.

Cognitive impairment and vitamin K association

Adjusted odds ratios of worse performances in the cognitive function test assessed with the MMSE score at 2 years according to 2-year changes in vitamin K consumption are presented in Figure 1. After adjusting for potential cofounders, our results show a positive association between the normal cognition prevalence (MMSE > 24) and a higher increase of vitamin K dietary consumption (OR [95% CI]; 0.56 [0.35, 0.98]).

Regarding the other cognitive function tests, we found no significant associations except for the semantic verbal fluency test (Table 2). Those participants located in the highest tertile of changes in dietary vitamin K had a significant lower likelihood of having a worse performance in the test, compared to those that decrease their consumption (OR [95%CI]; 0.70 [0.49, 0.99] P for trend= 0.043).

Since the presence of type 2 diabetes is associated with cognitive impairment, we explore the differential risks among the diabetic and non-diabetic population (Table 3). After segregating into two groups, our results show that participants in the highest tertile of change in vitamin K dietary intake had a better cognitive function in case of the non-diabetic population (OR [95%CI]; 0.36 [0.24, 0.76] P for trend= 0.005), but this association disappeared in case of diabetic subjects (OR [95%CI]; 1.03 [0.45, 2.36] P for trend= 0.945).

DISCUSSION

In this prospective analysis of the PREDIMED-Plus trial we show that increasing dietary consumption of vitamin K is associated with a better cognitive function assessed by MMSE test in an elderly high cardiovascular risk population. This association was not found in the other considered cognitive function tests, with the exception of the semantic verbal fluency test. Even though, it has been observed that semantic fluency is reduced in patients with mild cognitive impairment (28), this is the first study showing a positive association between vitamin K consumption and the score provided by this test.

Considering the potential effect of different food groups on cognitive performance, it is relevant to highlight that slower rates of cognitive decline have been shown in individuals who consume high amounts of vegetables compared to those that rarely consume. Interestingly those benefits were greatest for green leafy vegetables, a source of dietary phylloquinone, among other beneficial phytochemicals such as folate and flavonoids. (8) There are previous studies linking the dietary intake of vitamin K or vitamin K serum concentrations with cognitive impairment. In the CLIP Study Geriatric Population, the authors observed a positive significant association between dietary phylloquinone intake and the MMSE score, as well as an inverse association with the Frontotemporal Behavioural Rating Scale (FBRS) score. (17) In the same line, but considering performances in the Rey Complex-Figure recalls, higher levels of serum phylloquinone were associated with better performances in this non-verbal episodic memory test. (13) In a cross-sectional study, those participants with serious subjective memory complaint had a lower mean of dietary vitamin K intake compared with participants without memory complaint. (29) Our results not only reaffirm the previous evidence in a larger sample, but also extend these associations to a longitudinal perspective assessment.

Vitamin K may contribute to retard the cognitive impairment through several interconnected mechanisms. There is an important amount of evidence suggesting that this vitamin regulates insulin sensitivity and glucose tolerance. (11,30–32) It is not fully understood how the insulin acts in the brain, and how it can influence the cognitive functions. Craft et al., reported that patients with Alzheimer's disease had higher plasma levels of insulin though lower in cerebrospinal fluid compared to healthy subjects. This difference may be the result of insulin receptor downregulation at the blood-brain level

that could cause a lower insulin delivery in the central nervous system. (33) In relation to this, we failed to find any protective effect in type 2 diabetic subjects when analysed separately. Whether these results are due to a low sample power, or because vitamin K could be not powerful enough once the type 2 diabetes is already set up, deserve further attention.

Changes in the circulating levels of adipokines also influence the development of insulin resistance. (34) Our group examined the effect of vitamin K dietary intake on inflammatory risk markers related to insulin resistance and diabetes, finding a significant reduction in inflammatory cytokines on those subjects who increased their dietary vitamin K intake after a 1-year follow-up compared to those who decreased or did not change the intake amount. (35) This reduction in inflammatory cytokines, including TNF-α and IL-6, could explain one of the mechanisms behind the beneficial role of vitamin K on the insulin metabolism. Results from another randomized clinical trial conducted over premenopausal prediabetic women showed that 1000μg of phylloquinone supplementation significantly increased serum adiponectin levels compared with the placebo group. (36) A study aiming to associate vitamin K status, inflammation and cognition found that higher levels of inflammatory markers correlate with cognitive decline. Likewise, significantly higher levels of dietary phylloquinone in those individuals with better cognition compared to those with the poorest function were reported. Additionally, after controlling for other confounding variables, both, dietary and serum phylloquinone levels were significant independent predictors of normal scores in the MMSE, indicating better cognitive function. (37)

The strengths of our study include a large sample size, a longitudinal analysis based on two-year changes in the dietary vitamin K intake and the use of different test to assess various dimensions of the cognitive impairment. In addition, all analyses were controlled for potential confounders that were cautiously recorded by trained professional personnel. On the other hand, our study also has limitations. First, due to the design of the study, our sample only included elderly Mediterranean participants with overweight/obesity and metabolic syndrome; therefore, these results cannot be extended to the general population. Second, the vitamin K intake was estimated using FFQ and not assessed by levels of a plasma circulating marker that take into account the levels of absorption. Either

way, previous reports showed a significant association between dietary phylloquinone intake and plasma phylloquinone. (38)

In conclusion, our findings demonstrated that increasing the consumption of dietary vitamin K is associated with a better cognitive function scores, independently of traditional risk factors for cognitive decline, in an elderly Mediterranean population.

TABLE 1. Baseline characteristics of the 3737 subjects by tertiles of 2-years changes in dietary vitamin K1 intake.

	Vitamin K1 intake (µg/d)[1]			
Variable[2]	T1 (n=1245)	T2 (n=1246)	T3 (n=1246)	*P*[3]
Women, n (%)	625 (50.2)	586 (47.0)	551 (44.2)	0.011
Age, y	65.5 ± 4.9	65.1 ± 4.9	64.9 ± 4.9	0.008
BMI, kg/m^2	32.4 ± 3.4	32.4 ± 3.4	32.4 ± 3.4	0.973
Waist circumference, cm	107.0 ± 9.8	107.2 ± 9.3	107.3 ± 9.3	0.842
Leisure-time physical activity, MET-min/d	392.8 ± 363.8	368.6 ± 326.0	368.8 ± 348.1	0.134
Glucose, mg/dl	116.1 ± 30.3	115.6 ± 29.8	115.7 ± 31.4	0.895
Total cholesterol, mg/dl	195.3 ± 36.3	197.4 ± 36.7	196.7 ± 39.3	0.380
Triglycerides, mg/dl	149.9 ± 76.2	149.8 ± 76.4	148.2 ± 74.3	0.833
HDL cholesterol, mg/dl	48.2 ± 11.2	47.5 ± 11.5	47.8 ± 11.6	0.283
Diabetes, n (%)	450 (36.1)	419 (33.6)	421 (33.8)	0.335
Hypertension, n (%)	1051 (84.4)	1046 (83.9)	1065 (85.5)	0.010
Hypercholesterolemia, n (%)	859 (69.0)	859 (68.9)	857 (68.8)	0.244
Use of anticoagulant medication, n (%)	40 (3.2)	31 (2.5)	41 (3.3)	0.432
Smoking status, n (%)				0.876
Never	571 (45.9)	550 (44.1)	537 (43.1)	
Current	135 (10.8)	155 (12.4)	140 (11.2)	
Former	539 (43.3)	541 (43.5)	569 (45.7)	

Education, n (%)				0.350
Primary education	680 (54.6)	623 (50.0)	626 (50.2)	
Secondary education	315 (25.3)	366 (29.4)	362 (29.1)	
Higher education	250 (20.1)	256 (20.6)	258 (20.7)	
Cognitive functioning assessment, raw scores				
Mini-Mental State Examination Score	28.0 ± 2.0	28.3 ± 1.9	28.2 ± 2.0	0.001
WAIS III - Digit Span - Forward Recall	8.7 ± 2.4	8.9 ± 2.6	8.9 ± 2.5	0.315
WAIS III - Digit Span - Backward Recall	5.0 ± 2.3	5.2 ± 2.3	5.3 ± 2.2	0.042
Phonology Verbal Fluency Test	11.9 ± 4.4	11.8 ± 4.5	12.0 ± 4.4	0.689
Semantic Verbal Fluency Test	15.6 ± 4.7	15.7 ± 4.9	15.6 ± 4.7	0.782
Trail Making Test - A	53.5 ± 29.0	52.5 ± 30.0	51.8 ± 25.1	0.318
Trail Making Test - B	136.7 ± 84.4	134.8 ± 87.9	136.8 ± 88.2	0.811

[1] 2-years changes in vitamin K1 intake (µg/d), median (IQR), T1= -104.8 (-307.2, -53.2), T2= 22.6 (1.1, 50.6) and T3= 195.6 (119.6, 367.2).

[2] Means ± SDs or n (%), otherwise indicated. MET, metabolic equivalent of task; T, tertile.

[2] *P* values are based on the difference between tertiles of 2-years changes in dietary vitamin K1 intake (ANOVA for the continuous variables and χ^2 test for categorical variables).

TABLE 2. Association between changes of dietary Vitamin K1 intake and scores of cognitive function tests at 2-year follow-up[1]

	T1 (n=1245)	T2 (n=1246)	T3 (n=1246)	*P*-trend
2-year changes in Vitamin K1 intake (µg/d), median (IQR)[2]	-104.8 (-307.2, -53.2)	22.6 (1.1, 50.6)	195.6 (119.6, 367.2)	
WAIS III - Digit Span - Forward Recall				
Subjects with scores < 1.5 SD, n	85	96	81	
Crude model	1 (Ref.)	1.13 (0.84, 1.54)	0.94 (0.68, 1.29)	0.616
Full-Adjusted Model[3]	1 (Ref.)	1.07 (0.69, 1.65)	1.02 (0.66, 1.57)	0.966
WAIS III - Digit Span - Backward Recall				
Subjects with scores < 1.5 SD, n	137	142	133	
Crude model	1 (Ref.)	1.04 (0.81, 1.33)	0.95 (0.74, 1.23)	0.681
Fully adjusted model[3]	1 (Ref.)	1.02 (0.71, 1.46)	0.97 (0.68, 1.39)	0.862
Phonology Verbal Fluency Test				
Subjects with scores < 1.5 SD, n	71	84	85	
Crude model	1 (Ref.)	1.19 (0.86, 1.65)	1.21 (0.87, 1.68)	0.278
Fully adjusted model[3]	1 (Ref.)	1.07 (0.74, 1.55)	1.16 (0.79, 1.69)	0.447
Semantic Verbal Fluency Test				
Subjects with scores < 1.5 SD, n	100	99	75	
Crude model	1 (Ref.)	0.98 (0.74, 1.31)	0.73 (0.54, 0.99)	0.044
Fully adjusted model[3]	1 (Ref.)	0.93 (0.66, 1.29)	0.70 (0.49, 0.99)	0.043

Trail Making Test - A				
Subjects with scores > 1.5 SD, n	83	71	63	
Crude model	1 (Ref.)	0.84 (0.61, 1.17)	0.74 (0.53, 1.04)	0.089
Fully adjusted model[3]	1 (Ref.)	0.78 (0.52, 1.16)	0.78 (0.51, 1.15)	0.223
Trail Making Test - B				
Subjects with scores > 1.5 SD, n	122	119	126	
Crude model	1 (Ref.)	0.97 (0.74, 1.27)	1.03 (0.79, 1.35)	0.777
Fully adjusted model[3]	1 (Ref.)	0.91 (0.67, 1.25)	0.95 (0.69, 1.30)	0.779

[1] Logistic regression was used to evaluate the risk of lower scores of cognitive function tests according to tertiles of 2-year changes of dietary Vitamin K1 intake.

[2] Total intakes changes are expressed as median and interquartile range (IQR).

[3] Fully adjusted model was adjusted for sex, age, BMI, smoking habit, leisure time activity and education, prevalence of diabetes, hypertension and hypercholesterolemia, use of medication (anticoagulants, oral antidiabetic drugs, and insulin), baseline food intake (dairy, meat, fish, fruit, nuts, legumes, cereals), alcohol and alcohol squared in grams per day, and moderate-high risk of depression assessed by the BDI-II. Baseline scores of each questionnaire were included in each model in which they were the dependent variable. In addition was adjusted for intervention group and stratified by center.

TABLE 3. Association between changes of dietary Vitamin K1 intake and MMSE scores in the diabetic and non-diabetic populations.[1]

	T1	T2	T3	*P*-trend
Diabetic population[2], n	430	430	430	
MMSE score < 24, n	23	16	19	
Crude model	1 (Ref.)	0.68 (0.35, 1.30)	0.82 (0.44, 1.54)	0.582
Fully adjusted model[2]	1 (Ref.)	1.05 (0.44, 2.46)	1.03 (0.45, 2.36)	0.945
Non-diabetic population[3], n	815	816	816	
MMSE score < 24, n	38	26	17	
Crude model	1 (Ref.)	0.67 (0.40, 1.11)	0.43 (0.24, 0.76)	0.004
Fully adjusted model[2]	1 (Ref.)	0.54 (0.34, 1.02)	0.36 (0.21, 0.73)	0.005

[1] Logistic regression was used to evaluate the risk of lower scores of MMSE at 2-years follow-up according to tertiles of changes in dietary Vitamin K1 intake.

[2] Fully adjusted Model was adjusted for sex, age, BMI, smoking habit, leisure time activity and education, baseline score of MMSE, prevalence of hypertension and hypercholesterolemia, use of medication (anticoagulants medicines, oral diabetes medicines, and insulin), baseline food intake (dairy, meat, fish, fruit, nuts, legumes, cereals), alcohol and alcohol squared in grams per day, and moderate-high risk of depression assessed by the BDI-II. In addition was adjusted for intervention group and stratified by centre.

[3] Fully adjusted Model was adjusted by the same variables as in the previous model without use of oral antidiabetic drugs and insulin.

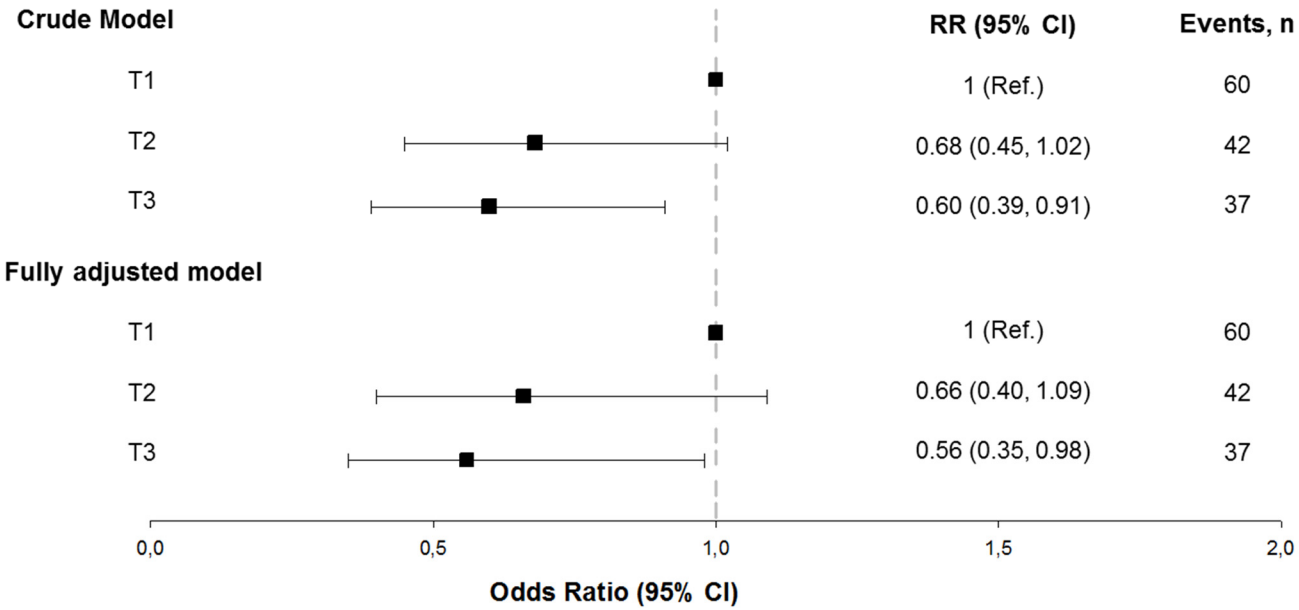

Figure 1. Odds ratios of cognitive impairment (MMSE scores <24) at 2-year follow-up by tertiles of changes in dietary Vitamin K1 intake.

Figure legend. Logistic regression analysis of cognitive impairment (MMSE scores <24 at 2 years follow-up) by tertiles of 2-year changes in dietary vitamin K1 intake. n= 1245 in T1, n= 1246 in T2 and T3. Median (IQR) of 2-years changes in vitamin K1 intake (µg/d), T1= -104.8 (-307.2, -53.2), T2= 22.6 (1.1, 50.6) and T3= 195.6 (119.6, 367.2). The model was adjusted for sex, age, BMI, smoking habit, leisure time activity and education, baseline score of MMSE, prevalence of diabetes, hypertension and hypercholesterolemia, use of medication (anticoagulants, oral antidiabetic drugs, and insulin), baseline food intake (dairy, meat, fish, fruit, nuts, legumes, cereals), alcohol and alcohol squared in grams per day, and moderate-high risk of depression assessed by the BDI-II. The model was additionally adjusted for intervention group and stratified by centre.

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VITAMIN K & HEALTHY AGEING
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CHAPTER 4

Dietary Phylloquinone intake is associated with a lower risk of dementia in an elderly Mediterranean population

- Publication status: Under preparation

UNIVERSITAT ROVIRA I VIRGILI
VITAMIN K & HEALTHY AGEING
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Dietary Phylloquinone intake is associated with a lower risk of dementia in an elderly Mediterranean population

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Short running head: Phylloquinone intake and Dementia

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

ABSTRACT

Background: Dementia is a progressive deterioration in the cognition, function and behaviour. The most common cause of dementia is the Alzheimer Disease (AD), approximately 70% of the cases. The mechanism underlying the neuropathological changes remains unclear. Diet seems to be one of the modifiable factors associated. Vitamin K1 have shown anti-inflammatory and anti-oxidant action in different studies as well as an important action in the brain, where it participates in sphingolipid synthesis.

Objective: To examine the association between dietary phylloquinone intake and the risk of dementia in an elderly Mediterranean population.

Methods: A prospective analysis was conducted in 7216 participants from the PREvención con DIeta MEDiterránea (PREDIMED) Study. Hazard ratios (HRs) and 95% confidence intervals (CIs) for dementia incidence were estimated with multivariable Cox proportional hazards models. Dietary phylloquinone intake was evaluated using a validated food frequency questionnaire. The time to the dementia event was calculated as the time between recruitment and the date of the appearance of the event, the time to the last visit of the follow-up, date of death, or the end of the study.

Results: After a median of 5.9 years follow-up we documented a total of 121 new cases of dementia. Subjects in the highest tertile of dietary phylloquinone intake had a significant lower risk of dementia than those in the lowest tertile HR [95%CI]; 0.53 [0.30, 0.96] P for trend= 0.104) after adjusting for potential confounders.

Conclusion: High intake of dietary phylloquinone is associated with a reduced risk of dementia in an elderly Mediterranean population.

INTRODUCTION

Dementia is a growing public health problem characterized by the progressive loss of cognitive functioning, including the memory and language function, and other behavioural abilities beyond what might be expected from normal ageing. (1) The prevalence of dementia is estimated in up to 50 million cases around the world, being consider one of the major causes of disability and dependency among older people. This number will double in the next twenty years, with bigger increases in developing countries. The causes of dementia can vary depending on the changes that takes place in brain, but Alzheimer's disease (AD) is the most common cause of dementia is adults, accounting for approximately 70% of the cases. (1,2) The expression of this neuropathology results from the sum of several risk factors, some non-modifiable such as age and genetics, and some others related to lifestyle and environmental factors. This could explain why several non-communicable chronic diseases highly related to the lifestyle, such as obesity, type 2 diabetes, hypertension or cardiovascular diseases shown an increased risk of cognitive decline or dementia in numerous population-based studies. (3,4)

However, the exact mechanism underlying the neuropathological changes remains unclear. Oxidative stress and inflammation maybe trigger or reinforced by the presence of metabolic disorders, are thought to play a pivotal role in precipitating neurodegenerative diseases. (5,6) More recently, insulin resistance has also been considered as a risk factor per se, potentially inducing a bioenergetic shift in peripheral and central nervous system energy metabolism. (7)

Consequently, as supported by different observational studies, antioxidant-rich foods, such as fruits and vegetables, and those foods with a demonstrated insulin down lowering effects, may have a protective effect from cognitive decline and AD. In contrast, diets rich in saturated and trans fats, and deficient in some micronutrients such as vitamins B or D are considered deleterious nutritional factors. On the other hand, there is evidence that a healthy dietetic pattern, high in antioxidants and polyunsaturated fatty acids, such as the Mediterranean Diet, is associated with a reduce incidence of AD. (8–10)

Phylloquinone is a fat-soluble vitamin, also known as vitamin K1, primarily found in green leafy vegetables and olive, soy and canola oils. Historically, it has been

associated with the blood clotting cascade and the bone mineralization (11), but recently different studies have also shown it anti-inflammatory (12,13) and anti-oxidant action.(14) It also has an important action in the brain, where it participates in sphingolipid synthesis. (10) Our group have also observed that vitamin K inhibit the production of inflammatory cytokines, associated with insulin response. (15) Presse et al. observed that patients with early stages of AD were found to have significantly lower vitamin K intakes than age and sex matched healthy participants. (16) Later, the same author founded a positive association between serum Phylloquinone and performances in verbal episodic memory suggesting a specific role of this vitamin in memory consolidation. (17)

Going through the previous evidence we hypothesize that dietary phylloquinone intake is associated with a decreased risk of dementia among an elderly Mediterranean population at high cardiovascular risk.

METHODS

Participants

A total of 7.447 high cardiovascular risk individuals, men between 55 and 80 years and women between 60 and 80 years were included. Although free of cardiovascular disease (CVD) at enrolment, they had either type 2 diabetes mellitus (T2DM) or three or more of the following cardiovascular risk factors: hypertension, smoking, high LDL-cholesterol, hypertriglyceridemia, low HDL-cholesterol (≤ 40 mg/dL), overweight or obesity or family history of premature cardiovascular disease. Exclusion criteria were the presence of severe medical conditions impairing the ability of the person to participate in a nutrition intervention study, alcohol or drug abuse, body mass index (BMI) ≥40 kg/m2, and allergy or intolerance to olive oil or nuts. All participants included in the study provided written informed consent according to a protocol approved by the institutional review boards of all the recruiting centers.

Study Design

PREDIMED is a large multicentre, randomized, parallel-group controlled clinical trial. Participants included in the trial were randomized to one of the three intervention groups: Mediterranean Diet (MedDiet) supplemented with extra-virgin olive oil (EVOO), MedDiet supplemented with mixed nuts, or advice on a low-fat diet (control diet) with the main objective of assess the effect of the Mediterranean diet on the primary prevention of cardiovascular disease (http://www.predimed.es). (18) Same randomization sequence was used in all 11 sites, although 425 participants who shared a household were assigned to the same intervention group. Also, 467 participants of one of the recruiting centres were allocated by clusters instead of using individual randomization.

Vitamin K1 dietary intake

Total energy, nutrient and micronutrient intake were assessed by a 137-item semi-quantitative validated food frequency questionnaire. (19) The information was gathered annually in face-to-face interviews with the participants performed by trained dietitians. Energy and nutrient intake were estimated using Spanish food

composition tables. (20,21) Vitamin K1 intake was estimated using the United States Department of Agriculture (USDA) nutrient database as this information is not available in the Spanish food composition tables. Dietary phylloquinone intake reproducibility was estimated by Pearson correlation (r=0.755) with an interclass correlation coefficient of 0.860 (p<0.001) (unpublished data).

Dementia events

Alzheimer's disease and other dementias events were previously specified as secondary outcome of the PREDIMED Trial and externally confirmed by an independent adjudication committee, whose members were blinded to the dietary intervention and to the participants' dietary behaviours. Alzheimer's disease was defined by the NINCDS-ADRA criteria. For other dementias: DLB consortium for dementia with Lewy bodies, NINDS-AIREN criteria for vascular dementia, consensus criteria for frontotemporal lobar degeneration and INPH guidelines for normal pressure hydrocephalus. (22–26)

Biochemical and anthropometrical measurement assessment.

Fasting blood samples were collected and processed for subsequent biochemical analysis. Anthropometric measures such as weight and waist circumference were determined by trained personnel with calibrated equipment at baseline and at each annual visit during the follow-up. Blood pressure was measured in triplicate using a validated semiautomatic oscillometer and the mean was recorded. Physical activity was assessed by the validated Spanish version of the Minnesota Leisure-Time Physical Activity questionnaire. It was administered at each visit. (27) APOE genotype was determined by genomic DNA extracted from buffy coats. Carriers of the risk genotype (ε4 allele) were grouped. The common APOE polymorphism was genotyped on a 7900HT Sequence Detection System (Applied Biosystems, ABI; Foster City, CA, USA) using fluorescent allelic discrimination TaqMan assays. For genotyping, two APOE SNPs were selected: rs429358 (Cys11Arg) and rs7412 (Arg158Cys) according to the NCBI SNP database. (28) Genotype frequencies did not deviate from Hardy-Weinberg equilibrium expectations.

Statistical analysis

All the statistical tests were 2-tailed and the level of significance was $P < 0.05$. Statistical analyses were carried using SPSS 19.0 for windows (SPSS Inc., Chicago, IL, USA) and STATA 14 (StataCorp, College Station, TX). Baseline descriptive data are presented as means ± standard deviations (SDs) for continuous variables and as numbers and percentages (%) for categorical variables using one-way ANOVA and the $\chi 2$ test for comparisons between groups. Multivariable Cox proportional hazard models were fitted to assess the association between dietary vitamin K1 intake and the risk of dementia. Participants who were outside the predefined values for total energy intake (>4000 or <800 kcal/d in men and >3500 or <500 kcal/d in women) were excluded from the analysis. Dietary food groups were adjusted for total energy intake using the residuals method. (29) We averaged food consumption from the baseline to the end of the follow-up or to the last follow-up FFQ before the occurrence of dementia event. Follow-up time was calculated as the time between recruitment and the date of the appearance of the event, death, end of follow-up or end of the study, whichever came first. We analysed the relationship between the vitamin K1 intake and the risk of dementia disease with a crude Cox regression model with robust variance and other three multivariate Cox regression models with robust variance and considering intra-cluster correlations. The participants who shared their household was defined as a cluster variable and was included in all models as an adjustment variable. Hazard Ratios (HRs) and 95% Confidence Intervals (CIs) were calculated considering the first tertile of phylloquinone intake as reference. The different models were adjusted for potential confounders. Model 1 was adjusted for sex, age, body mass index (BMI), recruiting centre, intervention group, smoking, leisure time activity and education. Model 2 was additionally adjusted for diabetes, hypertension and hypercholesterolemia at baseline; for use of anticoagulant and the presence of the allele E4 in the APOE structure. The final model, was also adjusted for dietary variables in average energy-adjusted tertiles, alcohol and alcohol squared in grams per day consumption.

RESULTS

Participants

From the total of 7447 PREDIMED participants, 7216 were included in this analysis. Those with extremes of total energy intake (n=153), or the ones that had incomplete dietary data at baseline (n=78) were excluded. For a median of 5.9 years of follow-up we observed a total of 121 new cases of dementia. These results are based on a database that included primary end-points events recorded until June 30, 2012.

Study participants baseline characteristics, by tertiles of cumulative energy-adjusted phylloquinone intake, are shown in Table 1. Those Individuals included in the highest tertile of phylloquinone intake had a lower BMI, lower waist circumference and were more physically active. The prevalence of hypertension was lower in individuals in the upper tertile although they were likely to have hypercholesterolemia and higher blood glucose levels.

Dementia and Phylloquinone association

Table 2 displays the HRs and 95% CIs for dementia, according to tertiles of cumulative energy-adjusted dietary phylloquinone intake. Those subjects included on the upper tertile of dietary phylloquinone intake had a significant lower risk of dementia than those in the lowest tertile (HR [95%CI]; 0.51 [0.27, 0.97] P for trend= 0.043) after adjusting the model for potential confounders. Similarly, the Nelson-Aalen estimates of incidence of dementia and the number of participants at risk by tertiles of averaged energy-adjusted dietary phylloquinone intake are shown in Figure 1. The graphic clearly illustrates how the third tertile separates from the other two, demonstrating the lower incidence in this group.

A sensitivity analysis was conducted where the events of dementia developed during the first year of study were not considered. The protective effect of phylloquinone on the third tertile of consumption continues: (HR [95%CI]; 0.54 [0.29, 1.00] P for trend= 0.047).

DISCUSSION

The present study provides important evidence of positive association between vitamin K1 consumption and a lower risk of dementia in an elderly Mediterranean population, particularly since is the first to evaluate risk on a longitudinal design. Specifically, our results show that those participants with higher dietary Vitamin K1 intake had a significant reduced risk for dementia compared to the lowest tertile of consumption after 6 years follow-up.

Similar results were found in a cross-sectional analysis, participants with AD showed significantly lower vitamin K dietary intakes matched to a control group.(16) In the same line, evidence describes a positive correlation between serum vitamin K concentration with better Mini-Mental State Examination scores in a study assessing cognitive functions of AD female patients. (30) Similar findings can be visualized about cognitive function in healthy older adults, where those with higher serum phylloquinone concentration were associated with better verbal episodic memory performances. (31) Furthermore, in a cross-sectional study among geriatric patients, was found a positive relation between use of antagonist of vitamin K with a more frequent cognitive impairment frequency. (32)

Several mechanisms could explain the potential role of vitamin K on dementia. Oxidative stress is commonly related with brain aging. Particularly in AD patients, as consequence of the presence of amyloid beta plaques and increased inflammatory process, oxidative stress is abnormally increased. (33) Additionally, the presence of the APOE4 allele implicated in AD, is also associated with higher oxidative stress. (34,35) Dietary antioxidants, such as vitamin K, could contribute to counterbalance this oxidative stress. (36) Observational studies have seen a decreased risk of cognitive decline and dementia in individuals with high consumption of fruits and vegetables, natural antioxidant enrich food. (37–39) The same with dietary patterns such as the Mediterranean diet, which have shown beneficial effects through decreasing the general oxidative stress status. (40)

Diabetes and insulin resistance are highly associated with an increased risk of dementia. A meta-analysis estimated that abnormal glucose or insulin levels can

increase the risk of AD by a 63%.(4) In this sense, insulin resistance was associated with reduce cerebral glucose metabolic rate in several brain areas of pre-diabetic and diabetic patients, similar to AD-like patterns. (41) Dietary phylloquinone intake was related with an improvement of cytokines and other markers linked to insulin resistance and diabetes. (15) There is evidence suggesting that vitamin K plays a beneficial role on glucose and insulin metabolism, decreasing plasma glucose levels in both animals and humans and delaying insulin response to glucose infusion. (42,43) In the same line, Mediterranean diet can also contribute to lower insulin values through its high antioxidants content, such as phylloquinone and its low glycaemic index due its high complex carbohydrates input. (44)

Another big contributing factor is inflammation. Although normal brain aging develops with low-grade inflammation, in AD can be observed a release of proinflammatory cytokines due the accumulation of Ab (45). The present study population is specially characterized by chronic inflammation due to its high cardio-metabolic risk, which can also exacerbate the brain inflammation as described by Holmes et al. (46). Metabolic syndrome, present in several of the participants, may also contribute with detrimental effects on cognition. (47)

Some limitations may be considered while interpreting the results from this study. Firstly, we cannot discard some errors in the estimation of the vitamin K intake since we extracted data from a FFQ and calculated the consumption from a food composition database due to the lack of plasma Phylloquinone levels measurements. To attenuate these limitations, the FFQ was validated against 3-day-dietary records in a subset of participants showing a very good correlation with vegetables (r=0.70) (48) and specifically with vitamin K (r=0.860, unpublished data). Even though this estimation cannot be an exact reflection of the real consumption, as a plasma level, although significant high association between this two was previously demonstrated (49). Secondly, these findings cannot be extrapolated to all type of population since the present study was conducted in a specific group: an elderly Mediterranean high cardiovascular risk. Some strengths are important to be acknowledge, such as the longitudinal design and the use of repeated validated FFQ measurements all through the follow-up. In addition, although the dementia cases

are not Predimed study's primary outcomes, the accurate assessment of the events by a neurologist-led Adjudication Committee whose members were blinded to the allocated intervention cannot be disregard.

In conclusion, the results of this study suggest that a high intake of dietary phylloquinone could have a protective role on dementia in an elderly Mediterranean population. Further longitudinal studies, maybe measuring plasma phylloquinone concentration, are necessary to confirm the results obtain in the present study.

TABLE 1. Baseline characteristics of 7216 PREDIMED participants by tertiles of baseline dietary phylloquinone intake.

Variable[1]	Energy-adjusted baseline phylloquinone intake (µg/d)			
	T1	T2	T3	*P*[2]
Women, n (%)	1283 (53.3)	1388 (57.7)	1474 (61.3)	< 0.001
Age, y	67.1 ± 6.4	67.2 ± 6.1	66.8 ± 6.0	0.060
BMI, kg/m^2	30.2 ± 3.8	29.9 ± 3.7	29.9 ± 3.9	0.008
Waist circumference, cm	101.4 ± 10.0	100.0 ± 10.4	100.0 ± 10.6	< 0.001
Leisure-time physical activity, MET-min/d	215.3 ± 231.1	234.7 ± 237.3	243.0 ± 247.3	< 0.001
Glucose, mg/dl	120.4 ± 39.0	121.6 ± 41.1	124.8 ± 43.7	0.001
Cholesterol, mg/dl	211.2 ± 38.0	216.9 ± 44.2	217.4 ± 40.6	0.002
Triglycerides, mg/dl	149.8 ± 98.9	142.2 ± 76.4	146.4 ± 85.5	0.232
HDL cholesterol, mg/dl	51.9 ± 13.3	53.7 ± 14.5	53.9 ± 13.3	0.002
Hypertension, n (%)	1993 (82.9)	2025 (84.2)	1952 (81.2)	0.022
Hypercholesterolemia, n (%)	1746 (72.6)	1704 (70.8)	1762 (73.3)	0.148
Smoking status, n (%)				< 0.001
Never	1404 (58.4)	1505 (62.6)	1529 (63.6)	
Current	387 (16.1)	330 (13.7)	287 (11.9)	
Former	614 (25.5)	571 (23.7)	589 (24.5)	
Education, n (%)				0.672
Primary education	1853 (77.0)	1872 (77.8)	1879 (78.1)	
Secondary education	386 (16.1)	359 (14.9)	351 (14.6)	
Higher education	166 (6.9)	175 (7.3)	175 (7.3)	
Intervention group, n (%)				0.001
Mediterranean diet with EVOO	799 (33.2)	830 (34.5)	845 (35.1)	
Mediterranean diet with nuts	734 (30.5)	806 (33.5)	820 (34.1)	
Control diet	872 (36.3)	770 (32.0)	740 (30.8)	
Dementia incidence, n	53	36	32	0.043

[1] Means ± SDs or n (%), otherwise indicated. n= 2405 in T1, n= 2406 in T2 and n=2405 in T3. EVOO, extra virgin olive oil; MET, metabolic equivalent of task; T, tertile.

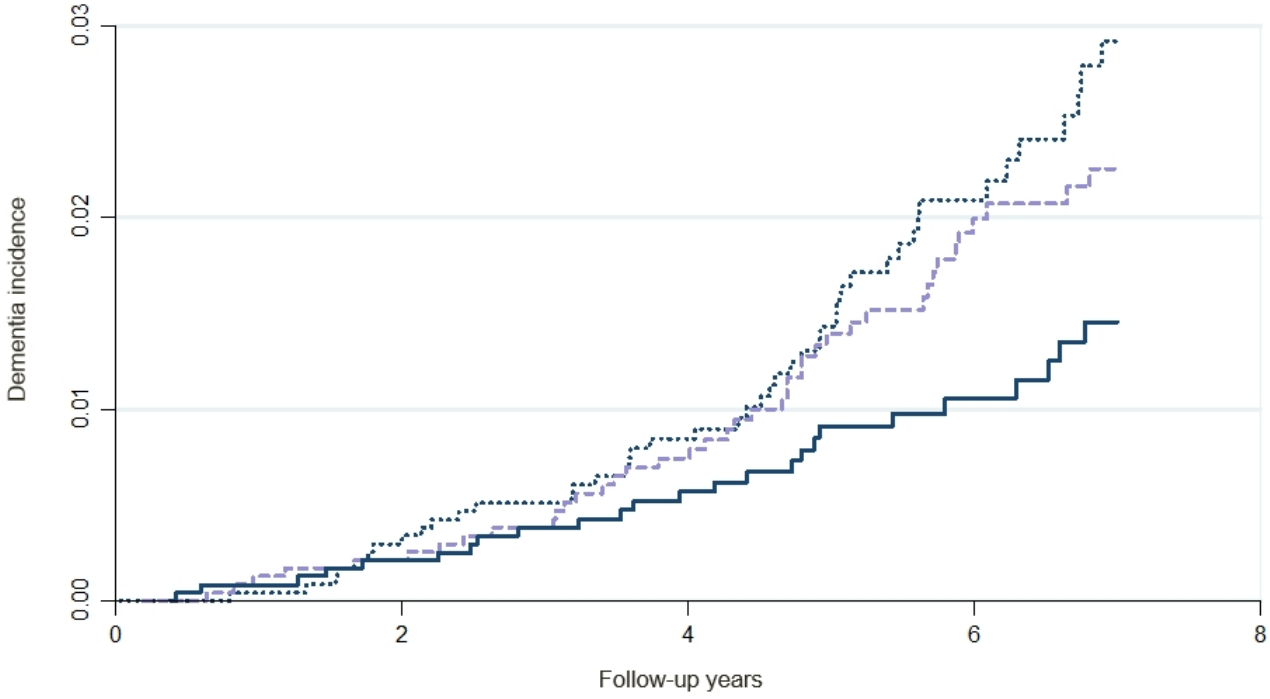
[2] *P* values are based on the difference between tertiles of average energy-adjusted dietary phylloquinone intake (ANOVA for the continuous variables and χ^2 test for categorical variables).

TABLE 2. Adjusted HRs of dementia according to tertiles of dietary phylloquinone intake. [1]

Variable	T1 (n=2405)	T2 (n=2406)	T3 (n=2405)	*P*-trend
Dementia incidence, n	49	45	27	
Phylloquinone (µg/d), median (IQR)[2]	229.1 (190.7, 261.8)	331.4 (309.0, 355.0)	465.4 (416.1, 555.5)	
Cases/Person-years, n	10855	11081	10891	
Crude model	1 (Ref.)	0.81 (0.54, 1.22)	0.51 (0.32, 0.82)	0.005
Model 1[3]	1 (Ref.)	0.81 (0.53, 1.24)	0.56 (0.32, 0.98)	0.044
Model 2[4]	1 (Ref.)	0.82 (0.54, 1.26)	0.57 (0.33, 0.98)	0.049
Model 3[5]	1 (Ref.)	0.80 (0.49, 1.27)	0.51 (0.27, 0.97)	0.043

[1] Cox regression was used to evaluate the risk of dementia according to tertile of average dietary energy-adjusted total dietary phylloquinone intake.
[2] Total intakes are expressed as median and interquartile range (IQR).
[3] Model 1 was adjusted for sex, age, BMI, recruiting center, intervention group, smoking (never, current, past), leisure time activity (MET/d) and education (primary education, secondary education, higher education).
[4] Model 2 was additionally adjusted for diabetes at baseline, hypertension and hypercholesterolemia at baseline, use of anticoagulant and presence of the allele E4 in the APOE structure.
[5] Model 3 was additionally adjusted for dietary variables in average energy-adjusted tertiles (vegetables, fruits, legumes, cereals, meat, dairy, fish, olive oil and nuts), alcohol and alcohol squared in grams per day.

Figure 1. Nelson-Aalen estimates of incidence of Dementia by tertiles of phylloquinone intake.



Participants at risk, n								
	Year 1	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Year 6**	**Year 7**	**Year 8**
T1	2404	2384	2342	2234	1907	1479	1080	658
T2	2405	2385	2385	2292	2018	1656	1354	966
T3	2403	2396	2396	2281	2013	1586	1211	845

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F. DISCUSSION

UNIVERSITAT ROVIRA I VIRGILI
VITAMIN K & HEALTHY AGEING
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1. GENERAL DISCUSSION

Throughout this doctoral thesis, I attempt to answer the research question of whether there is an association between vitamin K1 dietary intake and certain age-related health conditions. The results derived from this work, showed that a high dietary consumption of vitamin K1 was positively associated with a reduced risk of cataracts, a lower risk of diabetic nephropathy incidence, a better cognitive function and a decreased risk of dementia in an elderly Mediterranean population.

In the last years, vitamin K has emerged as a multifunctional micronutrient, implicated in several aspects of health beyond coagulation, including a protective effect in the ageing process and age-related diseases, rising a potential future use of this vitamin in clinical practice. Misbalances in insulin metabolism, inflammation and oxidation processes are suggested to underlay these diseases. Results shown this Doctoral Thesis provides some support for the new properties attributed to vitamin K1.

Even though the exact underlying mechanisms of action are still unknown, because of vitamin K recent proposed roles as an anti-inflammatory and antioxidant agent independent of GGCX action, this vitamin has been suggested as a modulator in pathological processes associated with old age. The development of a pro-inflammatory status and the presence of multiple stressors like ROS, DNA damage and telomere loss set off a pro-inflammatory senescence-associated secretory phenotype (SASP). This phenotype, which involves high levels of cytokines, has been described as a promoter of local and systemic inflammation, and a risk factor for morbidity and mortality during the ageing process[145]. Decreased vitamin K levels have been associated to increased aging processes and age-related disorders, by interfering with the γ-carboxylation of VKDPs such as Gas6 and GRP involved in apoptosis and pathological calcification, and by modulating inflammation, oxidative stress and mitochondrial dysfunction independently of its activity as co-factor for GGCX[54] (Figure 6).

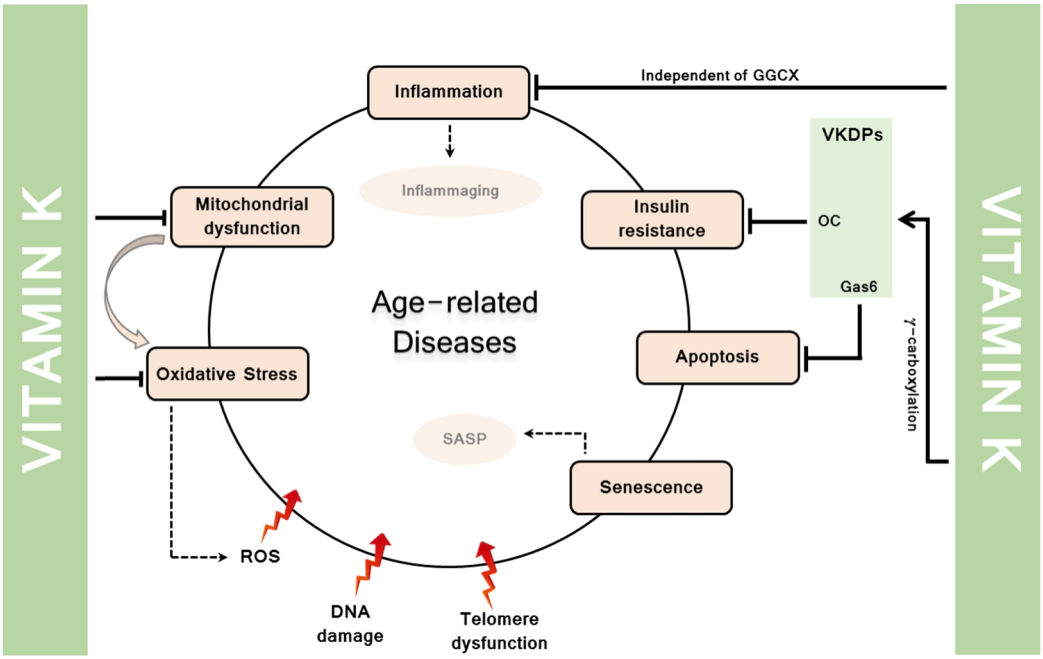

Figure 6. Possible associations of Vitamin K on the development of age-related diseases. Modified from Simes et al. (2019)[54].

All these mechanisms have been somehow associated with cataracts, type 2 diabetes and related complications, cognition and dementia.

Our findings concerning the incidence of cataracts showed a reduced risk associated with a high intake of dietary vitamin K1. Previous findings in induced diabetic cataracts in Wistar rats, showed that vitamin K1 inhibited the cataract formation modulating the calcium homeostasis in the lens and lowering the glycaemic levels through its action on the pancreas[86]. Recently, results from the same group, propose that vitamin K1 may prevent the cataract formation by inhibiting lens aldose reductase 2 (ALR2) activity[146]. Aldose reductase inhibitors (ARIs) have been previously associated with better management of major diabetic complications such as cataracts, retinopathy and nephropathy[147]. Even though epidemiological studies have already related a lower risk of cataracts with a higher consumption of antioxidant nutrients and dietary compounds, thus far, none has been able to find a link with dietary vitamin K1 consumption. Therefore, the

protective effect observed in our results helps to reinforce all these new approaches in animals extending them to the human population.

Regarding the diabetic complications studied in the present work, results showed in those subjects with the highest consumption of vitamin K1, a significant reduced risk of diabetic nephropathy. There are several factors modulated by vitamin K that are directly related to the development and progression of the diabetic nephropathy, such as chronic hyperglycaemia, hyperinsulinemia, inflammation and oxidative stress that may contribute to this finding[148,149]. An inverse association between circulating markers of vitamin K deficiency and decreased eGFR has been previously studied, suggesting a potential implication of vitamin K on the renal function[150]. Another study, in this case performed in diabetic induced rats, showed that the administration of vitamin K significantly protected against diabetic nephropathy, reducing the oxidative stress and the levels of blood glucose, and preventing microalbuminuria. Additionally, they observed a protection of the renal physiology as the administration of vitamin K modulated the Ca2+ and Na+/K+-ATPases[95]. On the other hand, the recently proposed role of vitamin K1 as an aldose reductase inhibitor[146] may also be a possible explanation for these results, since the potential benefits of ARIs in the prevention and development of diabetic nephropathy in patients with T2D have been previously described[147].

Moreover, results derived from this work also showed that vitamin K1 dietary consumption is associated with the cognitive function and the risk of all types of dementia in an elderly population. After several studies observed a link between cognitive impairment and the use of vitamin K antagonists (VKAs) as oral anticoagulants, the interest in the association between this micronutrient and cognitive decline has significantly increased in the last years. Different studies have searched for this association; however, the possible link between vitamin K and cognitive function among older adults is still under debate. A cross-sectional study, conducted on a cohort of elderly subjects free of cognitive impairment, found higher serum phylloquinone levels in those participants who

performed better in verbal episodic memory assessed by specific tests[119]. These results are supported by two other studies in geriatric patients where those participants with higher dietary vitamin K consumption had less severe subjective memory complaint and better cognition[105,151]. In the same line, MMSE scores associated with cognitive impairment among an elderly Irish population significantly correlated with the lowest dietary vitamin K consumption[152]. However, no association was observed between desphospho-uncarboxylated matrix Gla protein (dp-ucMGP) and cognitive functions in the only study to the date with a prospective design. Nonetheless, this longitudinal study with a six-year follow-up, used only one single indirect measurement of vitamin K status that could limit the proposed results[153].

Furthermore, only one cross-sectional study has assessed the link between Alzheimer's disease and vitamin K. This small sample study, of 31 AD patients and 31 healthy controls, has found that vitamin K intakes were significantly lower in those participants with AD compared to cognitively intact control subjects[118]. In addition, it has been observed that vitamin K may prevent the β-amyloid induced brain cells apoptosis through the activation of Gas-6, a VKDP associated with cerebral homeostasis[154]. The deposition of β-amyloid peptides are responsible for the neuronal death that defines the disease[108].

The pro-inflammatory state and the oxidative stress, proper of the ageing process, is highly associated with the cognitive decline and later development of dementia in the elderly. The antioxidant properties of vitamin K have been observed to have an effect in nervous cells, specifically in cultured neurons and oligodendrocytes, preventing cell death caused by oxidative stress[155]. Additionally, vitamin K has been linked to several processes of the central nervous system metabolism. Specifically in the sphingolipids metabolism, vitamin K acts as an inductor of the synthesis of sphingolipids, essentials parts of the nervous system cell membrane and linked to the neural proliferation and differentiation[103].

In summary, the results presented in the different studies that take part of this Doctoral Thesis support the emerging evidence of the beneficial health effects of vitamin K1, particularly in age-related diseases.

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2. STRENGTHS AND LIMITATIONS

The results of this Doctoral Thesis should be interpreted in the context of its limitations and strengths.

Firstly, we cannot discard some errors in the estimation of the vitamin K1 intake. Since we lack measurements of circulating levels of phylloquinone in plasma, the dietary intake was estimated using data provided by FFQs. In addition, because none of the Spanish food composition tables included the vitamin K contents, we used the USDA food-composition database to estimate these intakes. Yet, a previous study showed a good correlation between dietary vitamin K intake and its circulating levels. Moreover, we used validated FFQs that showed an intraclass correlation coefficient of 0.81 for vegetables, the main source of dietary vitamin K1.

Second, due to the observational nature of the PREDIMED and the PREDIMED-plus studies design, results can only be interpreted as associations, lacking a cause and effect relationship. In the same line, we also cannot discard that other variables could affect the observed associations, despite of controlling the different models for a large number of potential confounders.

Third, because the study was conducted in elderly subjects at high cardiovascular risk living in a Mediterranean country, our findings cannot be extrapolated to other elderly healthier populations.

Fourth, all the results presented in this work are part of post-hoc analyses since neither of our outcomes were part of the primary objective of the studies. However, the assessment of the different events was objective and accurate, confirmed by an End-point Adjudication Committee, whose members were blinded to the treatment and updated the information once a year.

On the other hand, this work has several other strengths that deserve to be mentioned. Both studies analysed in this thesis have large sample size and longitudinal design. PREDIMED have a long follow-up that allowed the use of repeated dietary measurements.

G. CONCLUSIONS

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The main conclusion derived from this Doctoral Thesis is that a high dietary vitamin K1 intake is associated with healthy ageing through decreasing the risk of different age-related diseases among an elderly Mediterranean population at high cardiovascular risk.

1. High intake of dietary phylloquinone is associated with a reduced risk of cataracts.

2. High dietary intake of vitamin K1 could have a protective effect on the development of diabetic nephropathy but not on retinopathy.

3. Increasing the consumption of dietary vitamin K1 is associated with better cognitive function scores, independently of several traditional risk factors for cognitive decline.

4. High intake of dietary phylloquinone is associated with a reduced risk of dementia.

The findings of this investigation complement those of previous studies that support the potential protective role of vitamin K in the ageing process and age-related conditions. The potential new roles that involves vitamin K in misbalances in insulin metabolism, inflammation and oxidation processes are proposed to underlay these diseases. Further prospective epidemiologic studies and randomized clinical trials measuring vitamin K through plasma phylloquinone concentration, are warranted to confirm these results.

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H. FUTURE INSIGHTS

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The demographic transition headed for an older society is becoming a challenge for the global social economy, especially for the public health care systems. Evidence, as the results presented in this work, supporting different promoters of health on age-related conditions would contribute to the design of effective nutritional strategies to improve the quality of life towards a heathier ageing process.

It has been strongly suggested that vitamin K has a beneficial effect on several ageing conditions. However, the current accessible evidence are in its majority observational studies and only a reduce number of randomized clinical trials are available. Our analyses extend the healthy proprieties of vitamin K in two large prospective studies in a post-hoc analysis. Therefore, future research in the field should evaluate the effect of this vitamin through long-term randomized clinical trials specifically designed for this objective in order to reduce bias and examine cause-effect relationship. Additionally, the vitamin K intake assessment should be also measured through biomarkers of its intake, in order to minimize errors in the estimation that can occur while using other dietary assessment tools.

Regardless of the existing evidence, the mechanisms implicated in these novel functions as an antioxidant and anti-inflammatory agent are not fully understood, and it will be interesting that future investigations would focus on metabolic and molecular pathways related to oxidation and inflammation.

Going through the available evidence, a knowledge gap concerning the adequate doses of vitamin K is noticeable. The lack of sufficient evidence does not allow to stablish RDAs and the current recommendations are expressed in AIs. Nevertheless, there is no documented toxicity reported for individuals that overdose these recommendations, and higher doses seem to be promoting improvements in the prognosis of the different conditions. Establishing a tolerable upper intake level may be necessary in order to do intervention studies with diet supplements and in a possible future, implementing its use in clinical practice.

Future works need also to focus on what happens with the menaquinones, which cannot be properly estimated since the dietary intake is usually low, the current knowledge of food content is deficient and we ignore the fraction produced by our gut microbiota. Animal models have shown a potential benefit of these types of vitamin K and it is a field that needs to be explore. The new insights regarding the biochemical signalling that takes place between the gut microbiome and the central nervous system and its implications with a diversity of conditions opens new possible therapeutic strategies that need to be explored.

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J. APPENDICE

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María Lucía Camacho Barcia

1. Scientific contributions

1.1 Scientific articles included in this doctoral thesis

Camacho-Barcia ML, Bulló M, García-Gavilán JF, Ruiz-Canela M, Corella D, Estruch R, Fitó M, García-Layana A, Arós F, Fiol M, Lapetra J, Serra-Majem L, Pintó X, García-Arellano A, Vinyoles E, Sorli JV, Salas-Salvadó J. *Association of dietary vitamin k1 intake with the incidence of cataract surgery in an adult Mediterranean population: A secondary analysis of a randomized clinical trial.* JAMA Ophthalmol. 2017 Jun 1;135(6):657-661. doi:10.1001/jamaophthalmol.2017.1076.

Camacho-Barcia L, Díaz-López A, García-Gavilán J, Ruiz-Canela M, Sorli JV, Castañer O, Estruch R, Ros E, Gea A, Forga LL, Fitó M, Casas M, Gómez-Gracia E, Arós F, Fiol M, Santos-Lozano JM, Serra-Majem LL, Pintó X, Salas-Salvadó J, Bulló M. *Dietary vitamin K1 intake is associated with a lower risk of diabetic nephropathy but not diabetic retinopathy.* Submitted: CLINICAL NUTRITION.

Camacho-Barcia L, García-Gavilán J, Martínez-González MA, Fernandez-Aranda F, Galié S, Corella D, Cuenca A, Romaguera D, Vioque J, Alonso-Gómez AM, Wärnberg J, Martínez JA, Serra-Majem LL, Estruch R, Bernal-López MR, Lapetra J, Pintó X, Tur JA, Garcia-Rios A, Bueno-Cavanillas A, Delgado-Rodríguez M, Matía-Martín P, Daimiel L, Martín-Sánchez V, Vidal J, Vázquez C, Ros E, Ruiz Canela M, Sorlí JV, de la Torre R, Konieczna J, Oncina-Cánovas A, Tojal-Sierra L, Pérez-López J, Abete I, Sánchez-Villegas A, Casas R, Muñoz-Garach A, Santos-Lozano JM, Bouzas C, Razquin C, Martínez-Lacruz R, Castañer O, Yañez AM, Valls-Enguix R, Belló-Mora MC, Basterra-Gortari J, Basora J, Salas-Salvadó J, Bulló M. *Vitamin K dietary intake is associated with cognitive function in an elderly Mediterranean population.* Ready to submit.

1.2 Other scientific articles

Hernández-Alonso P, García-Gavilán J, **Camacho-Barcia L**, Sjödin A, Hansen TT, Harrold J, Salas-Salvadó J, Halford JCG, Canudas S, Bulló M. *Plasma metabolites associated with homeostatic model assessment of insulin resistance: metabolite-model design and external validation*. Sci Rep. 2019 Sep 25;9(1):13895. doi: 10.1038/s41598-019-50260-7.

Hansen TT, Hjorth MF, Sandby K, Andersen SV, Astrup A, Ritz C, Bulló M, **Camacho-Barcia ML**, García-Gavilán JF, Salas-Salvadó J, Harrold JA, Halford JCG, Sjödin A. *Predictors of successful weight loss with relative maintenance of fat-free mass in individuals with overweight and obesity on an 8-week low-energy diet.* Br J Nutr. 2019 Aug 28;122(4):468-479. doi: 10.1017/S0007114519001296. Epub 2019 Jun 27.

Papandreou C, **Camacho-Barcia L**, García-Gavilán J, Hansen TT, Hjorth MF, Halford JCG, Salas-Salvadó J, Sjödin A, Bulló M. *Circulating metabolites associated with objectively measured sleep duration and sleep variability in overweight/obese participants: a metabolomics approach within the SATIN study.* Sleep. 2019 May 1;42(5). pii: zsz030. doi: 10.1093/sleep/zsz030.

García-Gavilán JF, Bulló M, **Camacho-Barcia L**, Rosique-Esteban N, Hernández-Alonso P, Basora J, Martínez-González MA, Estruch R, Fitó M, Salas-Salvadó J. *Higher dietary glycemic index and glycemic load values increase the risk of osteoporotic fracture in the PREDIMED-Reus trial.* Am J Clin Nutr. 2018 Jun 1;107(6):1035-1042. doi:10.1093/ajcn/nqy043.

Camacho-Barcia L, Bulló M, Garcia-Gavilán JF, Martínez-González MA, Corella D, Estruch R, Fitó M, Gómez-García E, Arós F, Fiol M, Santos-Lozano JM, Serra-Majem L, Pintó X, Basora J, Toledo E, Muñoz MA, Zanon-Moreno V, García-Layana A, Salas-Salvadó J. *Dairy products intake and the risk of incident cataracts surgery in an elderly Mediterranean population: Results from*

the PREDIMED study. Eur J Nutr. 2018 Mar 27. doi: 10.1007/s00394-018-1647-8.

Hernández-Alonso P, **Camacho-Barcia L**, Bulló M, Salas-Salvadó J. *Nuts and Dried Fruits: An Update of Their Beneficial Effects on Type 2 Diabetes.* Nutrients. 2017 Jun 28;9(7). pii: E673. doi: 10.3390/nu9070673.

1.3 Non-indexed articles

Vold Andersen S, Sandby K, Fiil Hjortha M, Kellebjerg Korndala S, Ritza C, Sjödina A, Halford JCG, Mead BM, Christiansen P, Harrold JA, **Camacho-Barcia L,** García-Gavilán JF, Salas-Salvadó J, Bulló M, Toft Hansen T. *No effects on appetite or body weight in weight-reduced individuals of foods containing components previously shown to reduce appetite - Results from the SATIN (Satiety Innovation) study.* Obesity Medicine. 2020 March; 17: 100188. https://doi.org/10.1016/j.obmed.2020.100188

Toft Hansen T, Mead BR, García-Gavilán JF, Korndal SK, Harrold JA, **Camacho-Barcia L**, Ritz C, Christiansen P, Salas-Salvadó J, Hjorth MF, Blundell J, Bulló M, Halford JCG, Sjödin A. *Is reduction in appetite beneficial for body weight management in the context of overweight and obesity? Yes, according to the SATIN (Satiety Innovation) study.* J Nutr Sci. 2019: (8),e39. doi:10.1017/jns.2019.36

Camacho-Barcia L, Salas-Salvadó J. Nut consumption and mortality: new data from USA. NUTFRUIT. Ed 71, vol 2, p. 36-37. July 2017.

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2. Participation in national and international conferences

Congress: 2nd IISPV SCIENTIFIC SESSION "Crossroads of metabolism and inflammation"
Date and place: November 12th, 2019. Reus, Spain
Title: Dietary vitamin K1 intake is associated with a lower risk of diabetic nephropathy but not diabetic retinopathy
Format: Oral presentation

Congress: XIV SEEDO Conference - Spanish Society for the Obesity Research (SEEDO)
Date and place: March 14 to 16, 2018. Lleida, Spain
Title: Dairy products intake and the risk of incident cataracts in an elderly Mediterranean population
Format: Poster presentation

Congress: 35th International Symposium on Diabetes and Nutrition - Diabetes and Nutrition Study Group (DNSG)
Date and place: June 19 to 22, 2017. Skagen, Denmark
Title: Dairy products intake and the risk of incident cataracts in an elderly Mediterranean population
Format: Poster presentation

Congress: 24th European Congress on Obesity (ECO2017) - The European Association for the Study of Obesity (EASO)
Date and place: May 17 to 20, 2017. Porto, Portugal
Title: Dietary phylloquinone consumption and the risk of incident cataracts in an elderly Mediterranean population
Format: Poster presentation

Congress: XIII SEEDO Conference – Spanish Society for the Obesity Research (SEEDO)

Date and place: March 15 to 17, 2017. Seville, Spain

Title: Association of dietary vitamin K1 intake with the incidence of cataract surgery in an adult Mediterranean population: a secondary analysis of a randomized clinical trial.

Format: Poster presentation

3. Mobility

Institution: Faculty of Medicine and Health, School of Psychology, Human Appetite Research Unit, University of Leeds. (England, UK)

Objective: To become familiar with the theoretical and methodological underpinnings of work conducted in a human appetite laboratory.

Supervisor: Prof. Graham Finlayson

Length: 3 months, from September to December 2018.

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