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Effects of dietary polyphenol intake on metabolic syndrome, body composition and obesity-related inflammation

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EFFECTS OF DIETARY POLYPHENOL INTAKE ON METABOLIC SYNDROME, BODY COMPOSITION AND OBESITY-RELATED INFLAMMATION.

Doctoral thesis report submitted
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INDEX

ABBREVIATIONS

AMPK: Adenosine monophosphate-activated protein kinase

Apo: Apolipoprotein

BMI: Body mass index

BP: Blood pressure

cAMP: Cyclic adenosine monophosphate

CH: Carbohydrate

CI: Confidence interval

CPT-1: Carnitine palmitoyltransferase 1

CVD: Cardiovascular disease

DNA: Deoxyribonucleic acid

EGCG: Epigallocatechin gallate

EVOO: Extra virgin olive oil

FFQ: Food frequency questionnaire

GLP-1: Glucagon like peptide 1

HDL-c: High density lipoprotein cholesterol

HR: Hazard ratio

IL: Interleukin

LDL-c: Low density lipoprotein cholesterol

MAPK: Mitogen-activated protein kinase

MCP-1: Monocyte chemoattractant protein 1

MD: Mediterranean diet

MetS: Metabolic syndrome

MUFA: Monounsaturated fatty acids

NCDs: Noncommunicable diseases

NK-kb: Nuclear factor kappa B

NO: Nitric oxide

OR: Odds ratio

oxLDL: Oxidized low-density lipoprotein

PAI-1: Plasminogen activator inhibitor type 1

PPAR: Peroxisome proliferator-activated receptor gamma

PREDIMED: PREvención con DIeta MEDiterránea

PUFA: Polyunsaturated fatty acids

RCT: Randomized clinical trial

ROS: Reactive oxygen species

RR: Relative risk

SCFA: Short-chain fatty acids

sICAM-1: Soluble intercellular adhesion molecule-1

sVCAM-1: Soluble vascular cell adhesion molecule-1

T2D: Type 2 diabetes

TG: Triglycerides

TNF-a: Tumor necrosis factor a

TPE: Total polyphenol excretion

TPI: Total polyphenol intake

UCP-1: Uncoupling protein 1

VAT: Visceral adipose tissue

Thesis in the form of a collection of published articles.

This thesis comprises 3 main objectives and 1 secondary objective, 4 published articles, 2 manuscripts and 2 supplementary published articles.

ARTICLE 1:

Castro-Barquero S, Tresserra-Rimbau A, Vitelli-Storelli F, Doménech M, Salas-Salvadó J, Martín-Sánchez V, Rubín-García M, Buil-Cosiales P, Corella D, Fitó M, Romaguera D, Vioque J, Alonso-Gómez ÁM, Wärnberg J, Martínez JA, Serra-Majem L, Tinahones FJ, Lapetra J, Pintó X, Tur JA, Garcia-Rios A, García-Molina L, Delgado-Rodriguez M, Matía-Martín P, Daimiel L, Vidal J, Vázquez C, Cofán M, Romanos-Nanclares A, Becerra-Tomas N, Barragan R, Castañer O, Konieczna J, González-Palacios S, Sorto-Sánchez C, Pérez-López J, Zulet MA, Bautista-Castaño I, Casas R, Gómez-Perez AM, Santos-Lozano JM, Rodríguez-Sanchez MÁ, Julibert A, Martín-Calvo N, Hernández-Alonso P, Sorlí JV, Sanllorente A, Galmés-Panadés AM, Cases-Pérez E, Goicolea-Güemez L, Ruiz-Canela M, Babio N, Hernáez Á, Lamuela-Raventós RM, Estruch R. *Dietary Polyphenol Intake is Associated with HDL-Cholesterol and A Better Profile of other Components of the Metabolic Syndrome: A PREDIMED-Plus Sub-Study*. Nutrients. 2020;12(3):689. doi: 10.3390/nu12030689. IF: 5.719, Q1: Nutrition & Dietetics.

ARTICLE 2:

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SUMMARY

A summary in Catalan of the present doctoral thesis is written below.
A continuació s'escriu un resum de la tesi doctoral en català.

Introducció:

La síndrome metabòlica (MetS) és un agrupament de diferents factors de risc cardiovasculars i cardiometabòlics. Als països desenvolupats, la prevalença de la MetS s'ha incrementat fins al 20-25% en la població adulta, i la seva incidència continua en augment. Les estratègies de salut pública pel tractament i la prevenció de la MetS es centren principalment en la modificació de l'estil de vida. En relació amb la MetS, l'obesitat és una malaltia complexa multifactorial definida per l'excés de teixit adipós, que es produeix a través de la hipertròfia i hiperplàsia dels adipòcits. No obstant, la circumferència de cintura és un millor predictor del risc de la malaltia cardiovascular que l'índex de massa corporal o el pes corporal. D'altra banda, el teixit adipós és un òrgan endocrí que secreta una àmplia varietat d'adipoquines, especialment un excés de teixit adipós visceral s'associa amb una major producció d'aquestes adipoquines inflamatòries, donant lloc a la inflamació sistèmica, resistència a la insulina i diversos trastorns metabòlics relacionats amb l'obesitat. Dins del marc de la Dieta Mediterrània, els compostos fenòlics, també coneguts com a polifenols, es caracteritzen per la presència d'un o més anells aromàtics i unitats estructurals de fenol en les seves molècules. Aquests compostos fenòlics es troben als aliments d'origen vegetal, sent les principals fonts dietètiques, com el cafè, les fruites, el vi negre i els olis vegetals, especialment l'oli d'oliva. A més, una ingesta contínua i prolongada de polifenols està relacionada amb una reducció del risc cardiovascular, com la reducció de pressió arterial, paràmetres d'adipositat, millores en el perfil lipídic i efectes antiinflamatoris.

Objectiu:

L'objectiu d'aquesta tesi doctoral és avaluar l'efecte d'una ingesta de polifenols dietètics elevada, dins d'una intervenció d'estil de vida orientat a la pèrdua de pes, sobre paràmetres d'adipositat i altres factors de risc cardiovascular en pacients amb MetS. Addicionalment, estimar la ingesta dietètica de polifenols en la població espanyola de l'estudi PREDIMED (PRevención con DIeta MEDiterránea) Plus i validar una versió actualitzada d'un qüestionari de freqüència d'aliments semi-quantitatiu.

Resultats:
La ingesta dietètica de polifenols, especialment algunes subclasses, té un impacte en els components de la MetS, especialment sobre les lipoproteïnes d'alta densitat (HDL-c, per l'acrònim en anglès). La ingesta total de polifenols no es va associar amb un millor perfil pel que fa als components de la MetS, excepte el colesterol HDL-c, tot i que els estilbens, lignans i altres polifenols van mostrar una associació inversa amb la pressió arterial, els nivells de glucosa plasmàtica en dejú i els triglicèrids. En el cas de la prevalença de diabetis tipus 2, les catequines, proantocianidines, àcids hidroxibenzoics i lignans es van associar inversament amb la diabetis tipus 2. Aquestes associacions eren diferents en funció del sexe i l'índex de massa corporal. En el cas d'indicadors de control de la diabetis, després d'un any de seguiment, els increments en la ingesta total de polifenols i algunes subclasses es van associar amb millors nivells de glucosa i hemoglobina glicosilada.

En el cas de la composició corporal, la ingesta d'algunes subclasses de polifenols s'associa a millores en diferents paràmetres d'adipositat corporal determinats per absorptiometria de raigs X de doble energia (DXA, per l'acrònim en anglès), principalment sobre el teixit adipós visceral i la massa de greix total després d'un any de seguiment. Addicionalment, quan els participants es van classificar en tertils segons els canvis en teixit adipós visceral després d'un any de seguiment, un augment del contingut de teixit adipós visceral va mostrar un increment significatiu dels nivells de l'inhibidor de l'activador del plasminògen-1 (PAI-1, per l'acrònim en anglès), resistina i leptina. En comparar entre tertils de teixit adipós visceral, es van observar diferències significatives en el nivells d'insulina, PAI-1 i c-pèptid. A més, la validació d'una versió actualitzada del qüestionari de freqüència d'aliments va mostrar una fiabilitat moderada segons l'adherència a la dieta Mediterrània i diferents marcadors biològics.

Conclusions:
En conclusió, la ingesta dietètica de polifenols millora els criteris de la MetS, la prevalença de diabetis tipus 2, indicadors de diabetis i la composició corporal, especialment en el teixit adipós visceral. A més, la reducció del teixit adipós visceral després d'un any de seguiment millora els nivells de paràmetres inflamatoris i adipoquines, principalment els nivells d'insulina, c-pèptid i PAI-1. Per últim, la validació d'eines de recollida d'informació nutricional amb marcadors biològics i l'adherència a patrons dietètics saludables, com la Dieta Mediterrània, permet classificar als individus en funció de la seva ingesta dietètica.

INTRODUCTION

1. Metabolic syndrome

1.1. Definition and epidemiological overview

Metabolic syndrome (MetS) is a clustering of different cardiovascular and cardiometabolic risk factors. In developed countries, MetS prevalence has risen to 20-25% in the adult population, and its incidence is increasing (1–3). In Spain, the MetS prevalence is currently reaching epidemic proportions, affecting approximately 42% of adult men and 32% of adult women (4). Its evolution and prevalence differ according to several parameters, such as age, gender, ethnicity, and socioeconomic status (5,6). Public health strategies for MetS treatment and prevention are mainly focused on lifestyle modification, including dietary and physical activity promotion, while pharmacological interventions should be reserved for next steps if lifestyle intervention are not sufficient to manage MetS components. Alberti *et al*. in 2009 standardized the MetS diagnosis defining it as the presence of three or more of the following five criteria: high waist circumference (men ≥ 102 cm; women ≥ 88 cm); elevated triglycerides (TG) (≥150 mg/dL); low levels of high-density lipoprotein cholesterol (HDL-c) (men < 40 mg/dL; women <50 mg/dL) or dyslipidemia treatment; high levels of blood pressure (BP) (≥130/≥85 mmHg) or antihypertensive treatment; and elevated fasting glucose (≥100 mg/dL) or glucose lowering treatment (7). In addition, MetS increases the risk of type 2 diabetes (T2D) onset and major cardiovascular disease (CVD) events by two-fold and five-fold, respectively, and other chronic diseases, such as cancer, neurogenerative diseases, non-alcoholic fatty liver disease, reproductive, lipid and circulatory disorders, atherosclerosis, and all-cause mortality (8–12).

1.2. Metabolic syndrome risk factors

Recent evidence has demonstrated the association between MetS incidence and prevention with modifiable lifestyle factors such as diet and physical activity. Among the different dietary interventions for MetS treatment and prevention, single-nutrient intervention has several limitations, and dietary advice must be focused on the overall dietary pattern. Recent evidence supports the implementation of healthy food-based dietary interventions instead of calorie or isolated nutrient restriction (13,14). Diet is a key determinant of CVD and CVD risk, not only considering the quality of the diet, also the rest of CVD risk factors are closely determined by dietary habits, such as body weight and BP management, glucose metabolism, among others.

1.3. Pathophysiology of metabolic syndrome: inflammation

By definition, MetS is characterized by an altered oxidative/antioxidant status and low-grade chronic inflammation. Oxidative stress and systemic inflammation are modifiable by lifestyle, mainly physical activity promotion and diet, being excessive energy intake and sedentary behaviors the main contributors of pro-inflammatory cytokines secretion (15–18). Atherosclerosis is an inflammatory disease that contributes to major CVD incidence and mortality. This chronic inflammation observed in atherosclerosis plays a key role in major CVD events, including coronary artery disease, being C-reactive protein, interleukin (IL)-1, IL-6, IL-8, IL-1β, IL-18, monocyte chemoattractant protein 1 (MCP-1), tumor necrosis factor alpha (TNF-α), among others, the main inflammatory markers (19–21). Additionally, these inflammatory mediator secretions may correlate with CVD severity (21). Moreover, oxidative stress has been described as a key factor of atherosclerosis and CVD pathogenesis. Reactive oxygen species (ROS) are mainly produced through mitochondrial activity on mitochondrial respiratory chain via one-electron reduction of molecular oxygen, nitric oxide (NO) synthase and oxidase enzymes such as nicotinamide adenine dinucleotide phosphate oxidases, xanthine oxidase, lipoxygenase, myeloperoxidase, uncoupled endothelial NO synthase, among others (22). Excessive ROS production leads to oxidative stress, promoting cell proliferation and migration, autophagy, endoplasmatic reticulum stress, necrosis, deoxyribonucleic acid (DNA) damage, endothelial dysfunction, and higher levels of oxidized low-density lipoprotein (oxLDL) (23). Moreover, ROS production activates inflammatory response increasing the secretion of inflammatory cytokines such as IL-6, IL-8, TNF-α, and MCP-1, among others (24). Simultaneously, the activation of nuclear factor kappa B (NK-κβ) and nuclear factor erythroid-derived 2-like 2 resulted in a higher production of inflammatory cytokines and inhibition of NO synthase, which NO secretion has an important anti-inflammatory, antihypertensive, and antithrombotic role mediated by its vasodilator and anti-platelet aggregation activity (25).

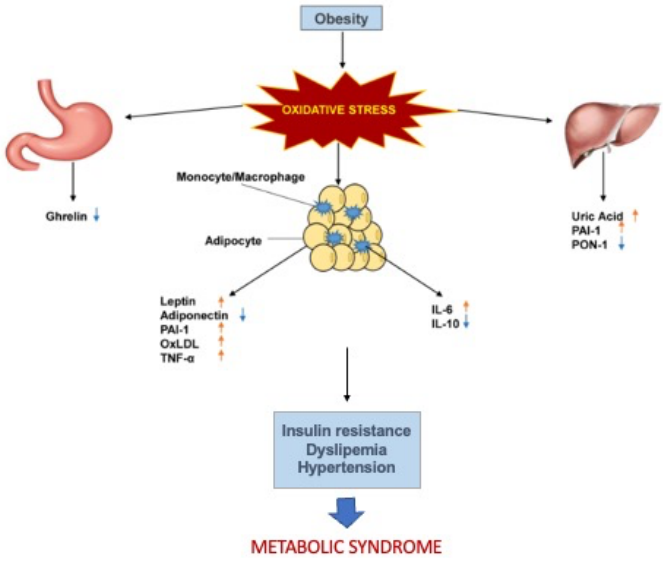

Figure 1: Pathophysiology involved in MetS (Srikanthan K, *et al.* 2016) (26)

2. Body composition and adipose tissue distribution

2.1. Definition of obesity and epidemiological overview

Obesity is a multifactorial complex disease defined by an excess of adipose mass, which occurs through adipocyte hypertrophy and hyperplasia (27). Obesity prevalence is increasing in an alarming rate, affecting over 200 million men and nearly 300 million women all around the world, and it is predicted that global prevalence of obesity will reach 18% in men and 21% in women by 2025 (28,29). Obesity is diagnosed and classified by body mass index (BMI) categories, defining underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obesity stages (≥30 kg/m^2), including morbid obesity (>40 kg/m^2) (30,31). However, in the early 1980s, body fat distribution, measured by the ratio of waist-to-hip circumferences, was stronger correlated with metabolic disturbances and CVD than the BMI (32–35). This ratio enables to estimate if the abdominal perimeter for a given BMI or hip circumference was excessive, indicating excessive abdominal fat (36). While waist circumference is a better predictor for CVD than BMI or body weight, and it may be useful as an index of total adiposity, it cannot differentiate visceral from subcutaneous adiposity (37).

The adipose tissue is an endocrine organ that secretes a wide variety of inflammatory adipocytokines, such as TNF-α, IL-6, resistin, leptin, and adiponectin. Visceral adiposity is associated with a higher production of these inflammatory adipocytokines, leading to systemic inflammation, insulin resistance, and several obesity-related metabolic disorders (38). This inflammation due to obesity can be reversed by weight loss, which causes a reduction in fat mass and proinflammatory adipokines. In this sense, it has

been postulated that in weight-loss oriented dietary interventions, for each kilogram of body weight lost, visceral adipose tissue (VAT) is reduced by 2-3% (39). Moreover, the intake of foods rich in bioactive compounds such as omega-3 fatty acids and polyphenols have been described to decrease low-degree inflammation (27).

Obesity treatment mainly consists in weight-loss oriented strategies based on dietary recommendations and physical activity promotion. Moderate weight loss (5 to 10% of body weight) has beneficial effects in glycemic control, TG and HDL-c levels, and BP (40). However, lifestyle interventions are not always effective, especially in patients with severe obesity (BMI >35 kg/m^2). In the last decade, some antiobesity drugs have been developed, but their use is limited because of the elevated cost, side effects, and medical contraindications (41). Finally, bariatric surgery is another strategy used for individuals with severe obesity (BMI $\geq$40 kg/m^2) or BMI >35 kg/m^2 with obesity-associated comorbidities. Despite its effectiveness for weight loss and T2D status, irreversible complications related to the surgical procedures may directly affect the quality of life of the patients (42).

Regarding dietary approaches for the treatment of severe obesity, very low caloric ketogenic diets have been postulated as an effective dietary approach with significant beneficial effects on anthropometric and metabolic components (28). However, several side effects have been reported if this restrictive dietary intervention is not monitored, such as dehydration, halitosis, gastrointestinal effects as nausea, vomiting, diarrhea, constipation, hyperuricemia, and changes in lipid profile, such as a decrease in TG and increased low-density lipoprotein cholesterol (LDL-c) (28). In this sense, the European Association for the Study of Obesity recommend the use of very low caloric ketogenic diets for the achievement of targeted weight-loss and then, the implementation of long-term well-balanced healthy lifestyle including dietary and physical activity recommendations.

2.2. Adipose tissue distribution and metabolic syndrome

Adipose tissue is classified according to its morphology, as white, brown, or beige, or by its location, as subcutaneous or visceral. Considering morphology, its appearance also conferred different function to these adipose tissue subtypes. White adipose tissue is the most predominant and is mainly used for energy storage. In the case of brown adipose tissue, the presence of high number of mitochondria's gives it the characteristic brown appearance and uniquely expresses uncoupling protein 1 (UCP-1), which enables heat production. Brown adipose tissue has gained attention as a potential new strategy for the treatment of obesity, but it has some limitations such as the activation of brown tissue

by cold exposure are rapidly reversible and cold exposure as a therapeutic approach is not feasible in the clinical setting (43). For beige adipose tissue, it's the combination of both white and brown adipocytes and there are several physiological stressors that can promote white adipose tissue browning such as cold exposure, bariatric surgery, severe cachexia, exercise, burns, and pharmacological or nutritional components, including thiazolidinediones and β-adrenergic receptor, linoleic acid, short-chain fatty acids (SCFA), green-tea extract and capsaicin (43–50). Adipose tissue is the main storage of energy, and most part of fat storage is allocated in subcutaneous adipose tissue (around 80% of all adipose tissue in healthy lean subjects), which function is mainly thermoregulatory and the mobilization of TG when needed (51). When subcutaneous fat storage capacity is exceeded, either because of a limited capacity to generate new adipocytes, also known as hyperplasia, or limited capacity to grow existing adipocytes, also known as hyperplasia, fat stats to accumulate ectopically outside the subcutaneous fat.

Fat localized outside subcutaneous fat and storage in visceral compartments is also known as VAT, which can be classified according to its location as omental, mesenteric, and retroperitoneal fat. However, some individuals can accumulate substantial amounts of adipose tissue in the abdominal cavity, which has been reported to be associated with metabolic disturbances such as T2D, including insulin resistance, hyperinsulinemia, glucose intolerance; dyslipidemia, including higher levels of LDL-c, low HDL-c, and free fatty acids release into the portal circulation; inflammatory response promoting the secretion of inflammatory cytokines; and an increased risk of thrombosis and endothelial disfunction (52–56). Besides insulin resistance, an excessive VAT accumulation may indicate a dysfunctional adipose tissue without the capacity to effectively store the excessive energy intake resulting from excessive caloric consumption and a sedentary lifestyle. Moreover, the efficiency in fat storage is translated to accumulate the excess of energy in subcutaneous adipose tissue, which is more insulin-sensitive, and it protects from metabolic disturbances associated to obesity. Nonetheless, in cases where adipose tissue is insulin resistant or dysfunctional, the capacity of storage fat would be limited, which is also known as hypertrophic adipose tissue, and this lipid overflow is deposited in other sites, such as liver, muscle, hearth, among others, which is also known as ectopic fat (37). In obese individuals, VAT adipocytes showed a significantly higher degree of hypertrophy than SAT (57). However, there are some factors associated with a preferential VAT accumulation, including smoking, genetic susceptibility, or neuroendocrine alterations due to stress response. Therefore, both factors, including the expansion of VAT and the alteration of neuroendocrine and hormonal secretion, may

promote diabetogenic and atherogenic effects mediated by insulin resistance (37). However, some authors postulated that these metabolic complications could be entirely mediated by the accumulation of fat in the liver, which can be explained by the fact that the liver is the key metabolic organ responsible of the metabolization of both carbohydrate (CH) and lipids (58–60). It must be noted that high liver fat content has been largely associated with abdominal obesity, whose individuals also showed an excessive hepatic gluconeogenesis explained by insulin resistance, where insulin is one of the key metabolic inhibitory hormones of glucose production and this alteration may explain the hyperglycemic state of T2D or MetS individuals (61). Another potential explanation of the excess of VAT could be the activation of hypothalamic-pituitary-adrenal axis, which promote the CH and lipid metabolism by glucocorticoids. Adipocytes have glucocorticoid receptors, but it has been described that visceral adipocyte present more glucocorticoid receptors compared to subcutaneous adipocytes, which means that the metabolic mediation of glucocorticoids may promote the accumulation of fat in the visceral depot together with inducing insulin resistance in the liver and the muscle (62).
Another metabolic active adipose tissue has been described around blood vessels, known as perivascular fat adipose tissue, presents characteristics of both brown and white adipose tissue and plays a key role in vascular homeostasis (63). Perivascular fat adipocytes can secrete several molecules including adipokines, such as leptin, adiponectin, visfatin, resistin, among others, cytokines, such as IL-6, TNF-α, MCP-1, and other compounds associated to vascular function such as angiotensin II and NO (64).

2.3. Endocrine role of adipose tissue

Adipose tissue is not only an energy storage organ, but it also presents several endocrine and paracrine/autocrine systems that secretes several metabolic mediators, such as adipokines, growth factors, cytokines and chemokines, which secretion is dependent on the energetic status of the adipose tissue depots (65). Additionally, adipose tissue has metabolic flexibility to deal with changes in energy balance during the day, by feeding and fasting periods that cause adipose tissue expansion or reduction (66). The individual adaptative response to adipose tissue remodeling is a key-factor in adipose tissue health and the metabolic disturbances associated, which may contribute to the heterogeneity observed among overweight or obese individuals (67,68).
Adipokines are an important mediator of several metabolic pathways including fatty acids oxidation and liponeogenesis, glucose metabolism, such as gluconeogenesis, glucose uptake, insulin signaling and energy expenditure in liver, skeletal muscle, and brain (65). Leptin was discovered in 1994 and is a hormone expressed exclusively by adipocytes,

mainly in white adipose tissue, and plays a key role in body weight management and satiety through the central nervous system (69). Moreover, it is the only identified adipokine with endocrine functions secreted from adipose tissue and exert effects on distant target organs.

The expression of adiponectin, in contrast to leptin and other adipokines, is inversely proportional to adiposity levels and its mainly produced in subcutaneous adipose tissue (70). Adiponectin main function is its insulin sensitivity-promoting factor, but other metabolic improvements are observed mainly mediated by peroxisome proliferator-activated receptor (PPAR)-α, such as decreasing hepatic gluconeogenesis, increase fatty acid oxidation and its expression has been associated with anti-inflammatory effects (71).

Resistin is a hormone secreted by adipose tissue, especially VAT, being plasma levels increased in obese individuals and decreased with weight loss, and its secretion has been associated with insulin resistance (72,73). Additionally, the pro-inflammatory role of resistin has been attributed mainly to its upregulating role for the secretion of other inflammatory cytokines such as TNF-α and IL-6 (74).

Besides adiponectins and hormones, the inflammatory response of excessive adipose tissue is also mediated by the infiltration of macrophages, representing around 60% of all cells in obese adipose tissue (75). However, this macrophage accumulation is mainly produced in VAT, and mediated by the adipocytes production of MCP-1 (76).

Other inflammatory cytokines are increased in obesity, as IL-6 and TNF-α, which are secreted by adipose tissue and its production is limited in lean individuals or during weight loss (77). These cytokines are positively correlated not only with adiposity, also with BMI and insulin resistance (78).

In summary, in obesity, a pro-inflammatory secretion is observed mediated by a lower expression of adiponectin and higher production of cytokines and chemokines, mainly TNF-α, IL-6 and MCP-1. Other hormonal mediation is observed in adipose tissue, such as glucagon like peptide 1 (GLP-1), which receptor have been identified in adipose tissue and its inhibition could modify satiety and insulin secretion (79). The use of GLP-1 receptor antagonist has been associated with weight loss and glucose metabolism improvements, which can provide CVD protection (80).

2.4. Pathophysiology of body fat distribution

Chronic low-grade inflammation induced by obesity state leads to adipose tissue dysfunction, impaired adipogenesis, and recent evidence suggested that obesity-induced inflammation is one of the main causes of insulin resistance (81).

In the case of T2D, there are several mechanisms underlying insulin resistance in overweight or obese subjects, including adipose tissue dysfunction and lipotoxicity, inflammation, mitochondrial dysfunction, hyperinsulinemia, and oxidative stress (82). Adipose tissue dysfunction means the incapacity to expand properly to store excess of energy, which induces ectopic fat deposition in other tissues, usually in tissues involved in glucose metabolism, such as liver, skeletal muscle, heart, and visceral depots, and this event is known as lipotoxicity. Moreover, dysfunctional adipocytes also secrete inflammatory cytokines, while the production of anti-inflammatory mediators, such as adiponectin, are limited (83). This lipotoxicity is one of the main mechanisms of action leading insulin resistance. **Figure 2** shows the effects of unhealthy expansion of adipose tissue and its association with obesity-related metabolic complications. Moreover, these hypertrophic unhealthy adipocytes secrete several paracrine factors, including mainly cytokines, adipokines and hormones, which facilitate preadipocytes and macrophages recruitment (84). In the case of cytokines secretion, proinflammatory cytokines such as TNF-α, IL-6, IL-8 and MCP-1 by hypertrophic adipocyte leads to the promotion of local and systemic inflammation and the recruitment of macrophages and T-cells (85–87). The expression of these cytokines and chemokines has been positively associated with CVD, mainly mediated by the recruitment of macrophages, increasing the expression of endothelial adhesion molecules, and promoting thrombosis (88–91). Furthermore, along with inflammatory cytokines, adipocytes also secrete anti-inflammatory mediators, such as adiponectin, which production is reduced with excessive adipose tissue, especially in obese individuals (92). It has been described that adiponectin expression is inhibited in inflammatory condition, including chronic obesity, and its secretion reduces adipose tissue inflammation by inhibiting the expression of inflammatory mediators such as VCAM, ICAM, E-selectin, TNF-α, and IL-6, improves insulin sensitivity and promote NO synthesis (93–97). Additionally, adiponectin levels are decreased in patients with CVD. White adipose tissue has several cell types, including endothelial cells, fibroblast, preadipocytes, stem cells, among others, with the main objective to promote adipocyte integrity and hormonal response (98). Inversely to adiponectin levels, leptin levels are positively associated with CVD, including major CV events such as myocardial infarction, stroke, coronary heart disease, among others (99,100). Even though some evidence suggest that some overweight individuals could be classified as “metabolically healthy obese”, there is strong evidence from longitudinal studies showing that metabolically healthy obesity is a transition to the unhealthy obesity state (101,102). In this sense, higher accumulation in lower body, especially SAT in gluteofemoral area, has been postulated as metabolically protective, and it is associated with insulin sensitivity,

improvements in lipid profile as decreased plasma TG and increased HDL-c levels, lower lipolysis, lower expression of pro-inflammatory cytokines and elevated leptin and adiponectin levels (103–105). Moreover, gluteofemoral distribution of body fat mass is associated with decreased risk of T2D in lean, overweight or obese individuals (103).

One of the main concerns about MetS or high CVD risk overweight/obese individuals is whether weight loss may represent the most relevant therapeutic target. Weight loss, by itself, does not distinguish between losing fat or lean mass. For this reason, regular physical activity is a key component of lifestyle intervention programs for the management of overweight/obese individuals, and it has shown to avoid muscle mass loss associated with energy restriction, which is an important factor to consider especially in elderly individuals with overweight or obesity (106–108). In this sense, individuals who practice regular vigorous exercise could loss adipose tissue without losing body weight due to the increase in muscle mass (106–108). Additionally, for all the health care providers involved in weight loss programs, individual variation in adipose tissue loss is observed for a given weight loss, which also should be considered in lifestyle interventions.

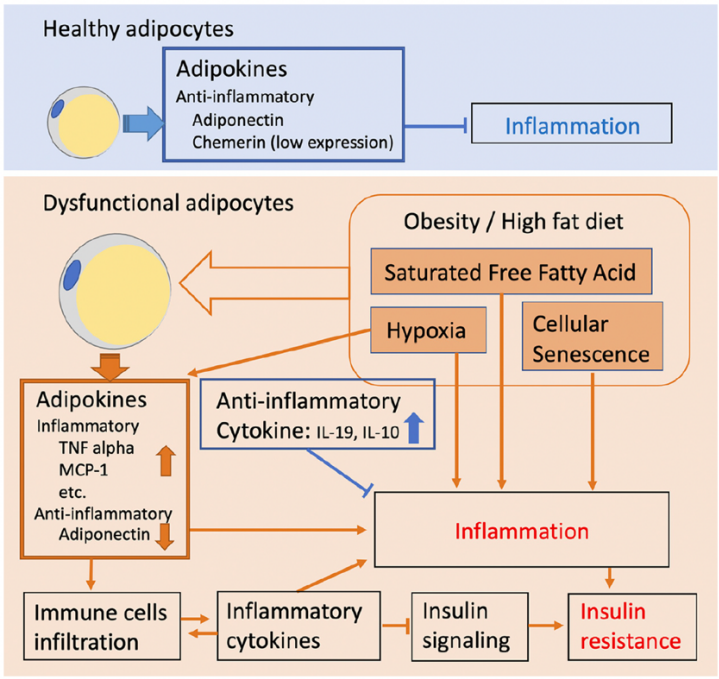

Figure 2: Effects of the expansion of adipose tissue in obesity and its association with obesity-related metabolic complications (Călinoiu LF et al. 2018) (168).

3. The Mediterranean Diet, and metabolic syndrome

3.1. The Mediterranean diet

The Mediterranean diet (MD) refers to the dietary pattern, culture and culinary techniques adhered to countries and populations living in the Mediterranean Sea basin (109). This dietary pattern has stimulated a great deal of scientific evidence, demonstrating the potential health benefits associated with adherence to MD, and the primary and secondary prevention of many health outcomes, including CVD, T2D, and MetS. Additionally, the MD has been recognized by the UNESCO as an Intangible Cultural Heritage of Humanity (110) and the 2015–2020 American Dietary guidelines referred to the MD as an example of a healthy dietary pattern (14). Unfortunately, MD adherence is not characteristic of Mediterranean countries today (111). A pioneer epidemiological study evaluating MD benefits was conducted by Keys in the 60s. The Seven Countries Study was an ecological, international investigation that enrolled nearly 13,000 men in seven countries (Greece, Italy, Japan, Finland, United States, the Netherlands, and the former Yugoslavia) (112). This study pointed out the protective effect of MD on CVD, highlighting the role of dietary fat profile on lipid profile.

The MD is a plant-based diet characterized by a high intake of vegetables including leafy green vegetables, fruits, whole-grain cereals, pulses, legumes, nuts, and extra virgin olive oil (EVOO) (cold pressed) as the main dietary sources of fat. Moreover, classical recipes are seasoned with sauces such as sofrito, whose main ingredients are olive oil, tomato, garlic, onion, or leek. Most of the typical foods of the MD are rich in phenolic compounds and carotenoids, such as naringenin, hydroxytyrosol, lycopene and β-carotene (113). Moderate alcohol intake of fermented alcoholic beverages such as red wine, mainly during meals, is also characteristic of the MD, which also comprises a low to moderate intake of fish and poultry, and low consumption of red meat, butter, sweets, pastries, and soft drinks (11,114,115). The traditional MD is a high fat and low-CH dietary pattern, which provides a 35–45% of total daily energy intake from fat, about 15% from protein, and 40–45% energy from CH (11,115). However, the profile of this fat is mainly from monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), being the main food sources of total fat intake EVOO and nuts.

EVOO is one of the key foods of the MD and it's the main contributor of MUFAs in MD countries. Oleic acid is the major component of EVOO, and many studies have linked MUFA intake to improvements in insulin resistance, one of the main risk factors for MetS, blood lipid profile, and to a reduction in both systolic and diastolic BP levels (11,116,117). EVOO is also rich in polyphenols, which present anti-inflammatory and antioxidant

effects and its intake has been associated with improvements in lipid profile and endothelial function (118).

Besides the beneficial effects of unsaturated fats, the whole dietary pattern, characterized by high intake of fruits and vegetables together with moderate red wine consumption, provides wide nutritional components, such as antioxidant vitamins (vitamin C, E, and β-carotene), phytochemicals (such as polyphenols), folates and minerals, which may exert beneficial effects (118,119). The protective mechanisms of MD against CVD are summarized in **Figure 3**.

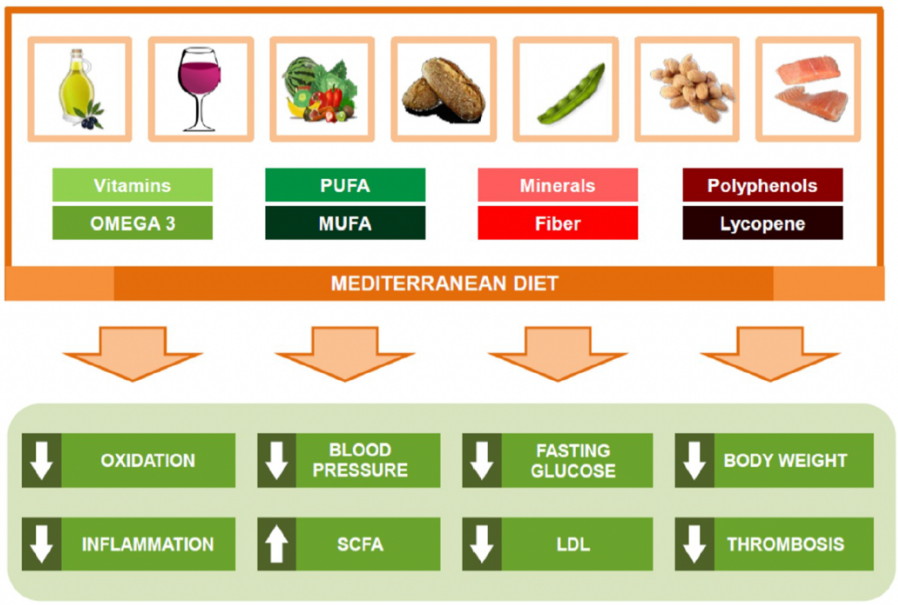

Figure 3: Protective mechanisms of the Mediterranean diet against cardiovascular disease (Casas R, *et al.* 2018) (120)

Franquesa *et al*. concluded that there is strong evidence about the effect of the MD on obesity and MetS prevention in healthy or high-CVD risk individuals, as well as on mortality risk in overweight or obese individuals (121). As previously mentioned, a meta-analysis of 12 cross-sectional and prospective cohorts showed that higher adherence to the MD was associated with a 19% lower risk of developing MetS (Relative risk (RR): 0.81 (95% confidence interval (CI) 0.71 to 0.92)), and MetS individual components, such as waist circumference and BP, were also improved (RR: 0.82 (95% CI 0.70 to 0.96); RR: 0.87 (95% CI 0.77 to 0.97), respectively) (122). The CARDIA, the Coronary Artery Risk Development in Young Adults study, is a prospective study including 4,713 individuals which evaluated the evolution of CVD risk factors in adult population in the

United States (123). They observed a lower incidence of MetS in individuals with a higher adherence to the MD (Hazard ratio (HR): 0.67 (95% CI 0.49 to 0.90)) compared to those with lower adherence, showing a linear trend according to the five score categories (*p* for trend = 0.005) (124). Kesse-Guyot *et al*. conducted a prospective 6-year follow-up study with 3,232 subjects from the SU.VI.MAX study to evaluate the association between different MD adherence scores and the incidence of MetS (125). They found that participants with higher adherence showed a 53% lower risk compared to the lowest tertile of the MD score (odds ratio (OR): 0.47 (95% CI 0.32 to 0.69) and 0.50 (95% CI 0.32 to 0.77 for each MD score) (125). In addition, MD adherence scores were associated with improvements in some individual criteria of MetS, such as waist-circumference, BP, TG, and HDL-c levels (125). Moreover, lower MetS prevalence was observed in Korean adults with medium to high MD adherence (OR: 0.73 (95% CI 0.56 to 0.96) and 0.64 (95% CI 0.46 to 0.89), respectively) (126).

The MD has reported an anti-inflammatory effect mainly mediated by the improvements in the circulating levels of several pro-inflammatory mediators, such as adhesion molecules (soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), E- and P-selectin), cytokines (IL-1, IL-6, IL-8, IL-12p70, c-reactive protein and TNF-α), chemokines (MCP-1, RANTES, macrophage inflammatory protein, among others), and molecules related to atheroma plaque vulnerability after three months, one, three and five years of follow-up in participants who followed a MD supplemented with EVOO and nuts (127–132). Aligned with the anti-inflammatory properties reported, epigenetic studies found that following a MD supplemented with EVOO or nuts influence the methylation status of peripheral white blood cell genes (48,49). These methylation modifications were observed mainly in genes related to metabolism, T2D, and inflammation. However, anti-inflammatory properties of MD have been also attributed to phenolic compounds.

Other preventive effects have been attributed to MD, which adherence has been inversely associated with the incidence of CVD and mortality, as well as cancer and degenerative diseases (114,135). In the case of CVD, MD is associated with clinically meaningful reductions in the risk of developing the main CVD outcomes, including coronary heart disease and stroke (136). In a prospective cohort study with 25,994 healthy women from the US Women's Health Study, Ahmad *et al*. observed an inverse association between the highest MD adherence score and the incidence of CVD compared to the lowest score (HR: 0.72 (95% CI 0.61 to 0.86), *p* for trend <0.001) (136). MD is, by definition, a plant-based diet, which are defined by the exclusion of some or all animal products and promotes a reduction in animal-source food intake along with an

increase in plant-based food intake (137,138). Finally, the replacement of some animal-derived foods implies the intake restriction of some potential harmful components mainly present in red and processed meat, such as excessive sodium, heme iron, nitrates and nitrites, which intake has been associated with CVD and MetS outcomes (138–140).

In the case of T2D, the pathophysiology is mainly mediated by a progressive loss in insulin secretion by pancreatic β-cells together with insulin resistance by several tissues involved in glucose metabolism such as skeletal muscle, liver, and adipose tissue (141). The presence of T2D is a risk factor for developing chronic complications including low-grade inflammation and atherogenesis (141). **Figure 4** shows T2D pathophysiology and its role in low-grade inflammatory response. Among T2D associated metabolic alterations, dyslipidemia, especially high TG, and low HDL-c levels, are considered crucial in the T2D physiopathology (142).

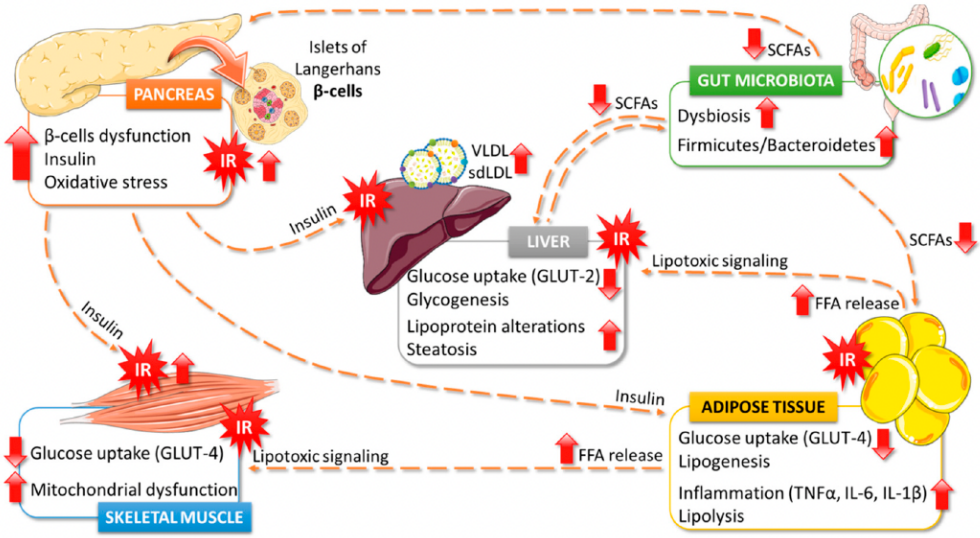

Figure 4: Type 2 diabetes mellitus pathophysiology and its role with low-grade inflammatory response (Bocanegra A, *et al.* 2021) (143).

3.2. The Prevención con DIeta MEDiterránea Plus (PREDIMED-Plus) study

The PREDIMED-Plus is an on-going 6-year multicentric randomized clinical trial (RCT) assessing the effect of a weight-loss oriented energy-reduced MD and physical activity promotion on the primary prevention of hard CV clinical events. A total of 6,874 subjects were recruited and randomized in the 23 recruiting centers from September 2013 to November 2016. Eligible participants were men (aged 55–75 years) and women (aged 60–75 years) with overweight or obesity who met at least three components of MetS at baseline. The study protocol and methods have been published elsewhere (144). Briefly,

participants randomized in the intervention arm were advice to follow an energy-restricted MD accompanied by physical activity promotion and behavioral support. Energy reduction was approximately 30% (around 600 kcal/day) of baseline energy requirements considering metabolic rate and level of physical activity. Moreover, trained dietitians conducted monthly individual interviews and group sessions during the first year of intervention and, from the second year of intervention, trimestral individual interviews and monthly group sessions were performed. Participants allocated in the control arm have one individual interview and two group session per year, following dietary advice based on the traditional MD used in the PREDIMED trial and usual care practices in the Spanish National Health System (145).

Dietary intake was assessed by the 143-item food frequency questionnaire (FFQ), which is an updated version of the FFQ used in the PREDIMED trial (146). Additionally, an updated version of the MD adherence score was designed to assess energy-restriction (147). This updated energy-restricted MD adherence score include 5 items from the original 14-points MD adherence score (lean meat instead of red meat; servings of vegetables per day; pieces of fruit per day; servings of pulses per week; servings of fish/seafood per week), 1 item was included in another item (use of EVOO for cooking, salad dressings, and spreads), and 4 new items were included based on CH restriction (added sugar; servings of white bread per day; servings of whole grain bread or cereals per week; servings of refined cereals per week). Energy-restricted MD score items and criteria were described in **Table 1**.

Question	Criteria for 1 point
Do you use only extra-virgin olive oil for cooking, salad dressing, and spreads?	Yes
How many fruits units (including natural fruit juices) do you consume per day?	≥3 units per day
How many servings of vegetables/salad do you consume per day? (1 serving 200g, considering side dishes as half a serving)	≥2 (≥1 portion raw or in a salad) servings per day
How many servings of white bread do you consume per day? (1 serving: 75g)	<1 per day
How many times per week do you consume whole-grain cereals and pasta?	≥5 per week
How many servings of red meat, hamburgers, or meat products (ham, sausage, etc.) do you consume per week? (1 serving: 100-150g)	<1 serving per week
How many servings of butter, margarine or cream do you consume per day? (1 serving: 12g)	<1 serving per week
How many sugary beverages or sugar-sweetened fruit juices do you consume per week?	<1 per week
How many serving of legumes do you consume per week (1 serving: 150g)	≥3 servings per week
How many servings of fish or shellfish do you consume per week? (1 serving: 100-150g of fish or 4-6 units or 200g of shellfish)	≥3 servings per week
How many times per week do you consume commercial sweets or pastries (not homemade), such as cakes, cookies, sponge cake or custard?	<3 per week
How servings of nuts (including peanuts) do you consume per week? (1 serving: 30g)	≥3 servings per week
Do you preferentially consume chicken, turkey, or rabbit instead of beef, pork hamburgers or sausages?	Yes
How many times per week do you consume vegetables, pasta, rice, or other dishes seasoned with sofrito (sauce made with tomato and onion, leek or garlic and simmered in olive oil)?	≥2 per week
Do you avoid adding sugar to beverages (coffee, tea)?	Yes
How many times per week do you consume non-whole grain pasta or white rice?	<3 per week
How many glasses of wine do you drink per day? (1 glass: 200 mL)	2-3 for men; 1-2 for women

Table 1: Adapted from Schröder H, *et al*. 2021.

Salas-Salvadó *et al*. evaluated the effect of PREDIMED-Plus intervention on weight loss and CVD risk factors in 626 participants of the trial after 12-months of follow-up (148).

Participants allocated in intervention group lost and average of 3.2kg vs 0.7kg in the control arm (mean difference: -2.5kg (95% CI -3.1 to -1.9), *p*-between group <0.001). Moreover, interim-analysis of the PREDIMED-Plus trial showed significant and clinically meaningful improvements in MD adherence and anthropometric parameters in the intervention arm compared to the control group (149). Energy-restricted MD score was significantly improved in intervention arm (mean increase 4.7 points (95% CI, 4.6 to 4.8), between-group difference: 2.2 points (95% CI, 2.2 to 2.4), $p<0.001$). Significant improvements were observed also in several CVD factors in intervention arm compared to control, such as waist circumference, fasting glucose, TG, and HDL-c levels (all $p<0.002$) and inflammatory and adipokines concentration, including leptin, IL-18, MCP-1 (all $p<0.05$).

A subsample of participants underwent DXA scans for body composition assessment in 7 out of the 23 recruiting centers. Konieczna J, *et al*. compare the use of different body adiposity indicators to evaluate CVD risk in PREDIMED-Plus participants (150). Among adiposity indicators, traditional anthropometric parameters such as BMI, waist circumference, waist-to-hip ratio, and waist-to-height ratio were tested as well as DXA scan derived parameters. Significant correlations were observed in both sexes among DXA-derived VAT measurements (total VAT, VAT/total fat mass ratio, and VAT/subcutaneous fat ratio), and MetS criteria, including HbA1c, glucose abnormalities, TG, and HDL-c (all $p<0.001$). Thus, DXA body composition parameters are better predictors of CVD and metabolic disturbances compared to traditional anthropometric measurements.

4. Phenolic compounds

4.1. Definition and classification

Phenolic compounds, also known as polyphenols, are characterized by the presence of one or more aromatic rings and phenol structural units in its molecules. These polyphenols are plant-derived molecules, mainly secondary metabolites of plants, and the characteristics of these phenolic structures underline biological, metabolic, and chemical properties.

Polyphenols are ingested as conjugated forms with acid-alcohol or glucosides, which are deglycosylated, becoming aglycone form of polyphenols, and absorbed across the intestinal epithelium. Only a small portion of dietary polyphenols are absorbed, and the absorption rate depends on the molecular structure, gut microbiota, culinary technique, among other factors (151). Moreover, after absorption, polyphenols are metabolized first in the small intestine and later in the liver, and the conjugated metabolites obtained are

mainly methylated, sulfated and glucuronidated products. Nevertheless, only 5-10% of phenolic intake is incorporated into plasma (68), while the remaining 90-95% undergoes fermentation by microbiota. Thus, the low bioavailability of polyphenols implies difficulties to guarantee the required plasma levels or optimal dietary intake to ensure the potential health benefits related to their intake.

More than 500 different polyphenols have been described in different food sources, especially in fruits, vegetables, seeds, vegetal oils, beverages such as tea, coffee, and fermented alcoholic beverages, species, cocoa and their derives (153). The intake of phenolic compounds and their main food sources depends on dietary patterns, gender, socio-economic factors, and region. Interestingly, the main food sources of polyphenols in Mediterranean countries (including Spain, Greece, Italy, and the south of France), are coffee, fruits, red wine, and vegetable oils, especially olive oil, whereas in non-Mediterranean countries, coffee, tea, fruits, and wine are the main food sources (154). The differences observed in the phenolic-rich foods intake are translated to a different profile of polyphenol subclasses intake. Mediterranean countries show the highest intake of flavonoids and stilbenes whereas non-Mediterranean countries show the highest intake of phenolic acids because of the high intake of coffee and tea.

Polyphenols are classified by their molecular structure, including flavonoids, which are characterized by C6-C3-C6 skeleton, and nonflavonoids compounds. According to the published literature, polyphenols are classified into five subclasses, including flavonoids and four subclasses of nonflavonoids: phenolic acids, stilbenes, lignans and other polyphenols (**Figure 5**). This classification of polyphenols is based on their chemical structures and functions (153).

4.1.1. Flavonoids

The main dietary sources of flavonoids are fruits, vegetables, nuts, and beverages such as tea, and red wine. The dietary intake of flavonoids varies from very few to several hundred milligrams, based on food choices, as well as cultural dietary habits (155). Flavonoids can be divided into 9 subgroups: anthocyanins, flavones, flavanones, flavonols, flavan-3-ols or flavanols (including its polymeric forms, also known as proanthocyanidins), isoflavonoids, chalcones, dihydrochalcones, and dihydroflavonols (156). There is enough scientific evidence about the inverse association between flavonoids intake and CVD risk (157).

Anthocyanins are a subclass of flavonoids that are responsible for the dark color of certain fruits and vegetables such as cherries, berries, grapes, and eggplants (158). In the case of flavones, such as apigenin and luteolin, the main food sources are vegetables

and fruits. Flavanones are a flavonoid subclass specific of citrus fruits, and the most studied compounds are hesperetin and naringenin. The consumption of fresh fruits, as well as natural and industrially extracted citrus fruit juices are the main sources of flavanones (159,160). Flavonols are one of the most representative subclass of flavonoids intake since they are widely distributed flavonoids in nature. They are also known as the most active compounds within the flavonoids group (161). The most common food sources of flavonols are cocoa, tea, wine, fruits and vegetables, spices, among others (162). Flavanols, or also known as flavan-3-ols, include catechins, proanthocyanidins and theaflavins. The average range of flavan-3-ol intake in the European diet has been reported to be between 180 mg/day to 600 mg/day, and the main food sources are fruits, especially berries, apples, grapes, and tea (163). Finally, isoflavones are found mainly in legume plants and significant amounts of isoflavones can be found in soybean and its products (164).

4.1.2. Phenolic acids

Phenolic acids are known to have antioxidant properties that can be translated to health benefits in the human body. Phenolic acids have aromatic phenolic rings that can donate an electron and transfer a hydrogen atom to free radicals (81). The potential mechanisms of action involved in the observed health benefits are its action as free-radical scavengers and reducing agents. Additionally, phenolic acids in general and hydroxybenzoic acids can activate some endogenous antioxidant pathways to increase antioxidant enzymes levels (166). Phenolic acids can be classified into two major subclasses: benzoic acids that contain seven carbon atoms, and cinnamic acids that contain nine carbon atoms. The most well-known derivatives of cinnamic acids include caffeic acid, ferulic acid, isoferulic acid, and p-hydroxycinnamic acid (167). The antioxidant capacity of phenolic acids was studied by Sevgi *et al*. showing that ferulic acid, compared to other abundant phenolic acids (such as caffeic, chlorogenic, cinnamic, gallic, p-hydroxybenzoic, syringic, *p*-coumaric and vanillic acids) has the highest antioxidant activity (165). Ferulic acid is mainly found in cereals, such as wheat, barley, oats, and rye (168), especially in whole grain products.

4.1.3. Stilbenes

Stilbenes are nonflavonoid phenolic compounds, characterized by the presence of 1,2-diphenylethylene nucleus (169). These polyphenols are found in a vast number of plants and their main function is natural defense. Although there are more than 400 natural stilbenes, its presence is limited to a heterogeneous group of plant families, as stilbene

synthase is not ubiquitously expressed in all of them. The intake of stilbenes is low because only a few foods are food sources of this type of phenolic compounds (e.g., some types of berries, grapes, red wine, and peanuts) (86). The main sources of stilbenes in diet are wine, which represents a 98.4% of stilbenes intake, grape, berries, and grape juice by 1.6%, whereas peanuts and other berries content is lower than 0.01% (154). The best characterized and studied stilbene is resveratrol (3,5,40-trihydroxy-trans-stilbene), which is a phytoalexin mainly found in the skin of grapes (171). To date, 20 metabolites of resveratrol have been identified in significant quantities.

4.1.4. Lignans

The main food source of lignans are fiber-rich foods, such as cereals, fruits, and vegetables (172). The lignan content in foods usually does not exceed 2 mg/100 g (around 1–1.6 mg per day) (173). Nevertheless, the levels of these bioactive compounds are high in flaxseed (335 mg/100 g) and sesame (373 mg/100 g), as well as rye and wheat brans in minor proportions (174,175). Lignans, also known as “phytohormones” due to their power to simulate hormone activity, have been reported as “functional foods” because of their potential health benefits for humans (176,177).

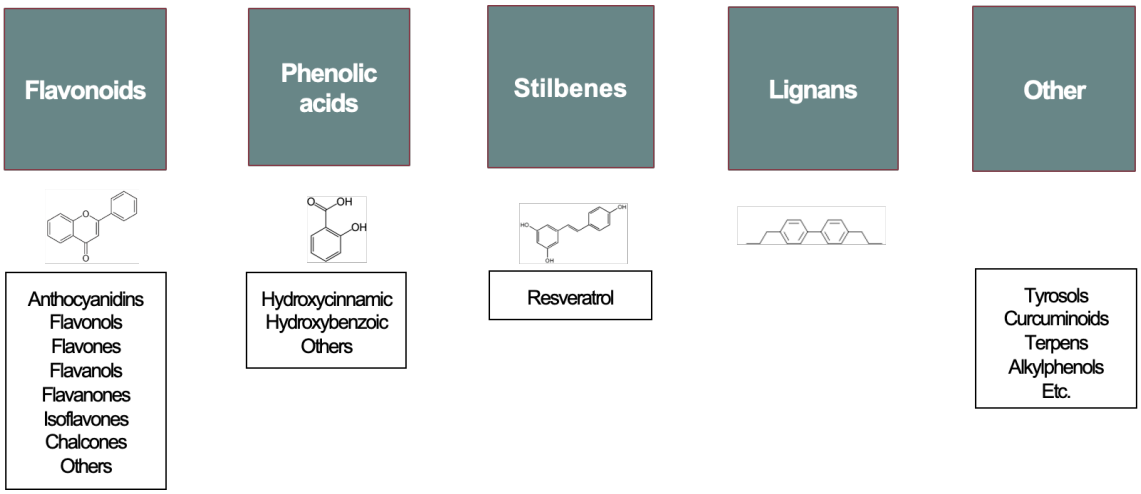

Figure 5: Main polyphenol classes and subclasses according to Phenol-Explorer database (http://phenol-explorer.eu/)

4.2. Health effects of dietary polyphenol intake

There is a large body of evidence attributing several health benefits to dietary polyphenol intake, including anti-obesogenic, anti-diabetic, anti-hypertensive, anti-inflammatory, and improving lipid profile (178).

Several mechanism have been described about the effect of dietary polyphenols delaying atherosclerosis progression including regulation of signaling and transcription pathways, such as NK-κβ, antioxidant activity, prevention of leukocyte migration and infiltration inside the vascular wall, reduction of adhesion molecules and pro-inflammatory cytokines levels, increase NO production, and improvements in CVD risk factor, such as lipid profile, coagulation activity, endothelial function, among others (179,180).

In the case of hypertension, high-flavanol cocoa supplementation decreased diastolic BP (-1.6 mmHg) and systolic BP (-1.2 mmHg) (181). In this sense, a meta-analysis of 23 RCT observed a beneficial effect of chocolate and cocoa products on endothelial function, which is also related to BP management (182). The mechanisms underlying anti-hypertensive effects of dietary polyphenols are the improvements in endothelial dilatory function and increase the NO production. Although the reduction of BP levels is modest, small changes in BP levels are clinically significant. Indeed, 2 mmHg reduction of diastolic BP levels was associated with a reduction of the risk of stroke and coronary heart disease by 15 and 6% respectively (183).

Catechin polyphenol consumption with green tea supplementation, has been associated with lipid profile improvements, mainly in LDL-c, while no significant effects were observed for HDL-c levels (184–186). Moreover, supplementation with other flavonoids, such as isoflavones, flavones or flavanones showed improvements in lipid profile (187–191). The potential mechanisms underlying lipid profile health benefits are mainly at intestinal level, where polyphenols reduce lipid availability, cholesterol absorption, chylomicron secretion and inhibiting pancreatic lipases (192,193).

For glucose metabolism, insulin sensitivity improvements have been observed in polyphenol-enriched diets without changes in body weight, suggesting a direct effect of polyphenols on insulin (193,194). The potential mechanisms underlying anti-diabetic effects of polyphenols are inhibiting glucose absorption in the intestine via the sodium-dependent glucose transporter 1; glucotoxicity protection of the pancreatic β-cells, and improving glucose uptake via GLUT4, which is translated to a suppression of glucose release from liver storage (195).

4.2.1. Flavonoids

The intake of flavonoids has been associated with a lower risk of ischemic stroke, and recent evidence suggest that individuals prone to atherosclerosis or higher CVD risk, including smokers or high alcohol drinkers, should benefit the most from higher flavonoid intake (196–198). Interestingly, in a study with 55,169 participants from The Danish Diet,

Cancer and Health Study, it was found a significant association among total flavonoid and some flavonoid subclasses intake and lower ischemic stroke risk (198). Clinical studies have also shown the protective effects of flavonoids, such as antioxidant, anti-proliferative, and anti-inflammatory effects (199). Regarding the anti-inflammatory effects of flavonoid intake, Gould *et al*. showed that both green tea and isolated soy protein lowered LDL-c by approximately 0.2 mmol/L (200). This reduction in LDL-c results in a 6% decrease in both coronary heart disease related events and CVD mortality. Cocoa and chocolate have shown to lower systolic BP by 5.9 mmHg, which would result in a reduction in CVD mortality risk by 5% (201). When it comes to the antioxidant effects of flavonoids, there are plenty of *in vitro* evidence supporting the antioxidant properties of these bioactive compounds. However, the *in vivo* studies available have shown controversial results and indicate the need for further research (202).

In a meta-analysis of fourteen prospective cohort studies (203), the association between the intake of different subclasses of flavonoids and the risk of CVD was measured. The analysis performed were mostly related to the intake of flavonols. The results showed a significant inverse association between flavonol intake and CVD risk (RR: 0.89 (95% CI 0.84 to 0.94); *p* for trend=0.001). In addition, the association between the intake of flavones, flavan-3-ols, and flavanones with CVD risk was also measured. The results showed that the consumption of these three subgroups was associated with a decreased risk in CVD. The measured RR for CVD in the case of flavones was 0.88 (95% CI 0.82 to 0.96; *p*=0.003), for flavan-3-ols were 0.87 (95% CI 0.80 to 0.95; *p*=0.002), and for flavanones were 0.87 (95% CI 0.80 to 0.95; *p*=0.002). All these results enhance the recommendation and promotion of a high consumption of flavonoid-rich foods.

4.2.2. Phenolic acids

The anti-inflammatory activity of phenolic acids is mediated by the reduction of the synthesis of pro-inflammatory cytokines at the intestine level, especially IL-8 and TNF-α (204,205). Another interesting potential health benefit of phenolic acids is the antimicrobial effect due to their antibacterial activity (206), especially caffeic, ferulic and *p*-coumaric acids. It is important to point out that, like other phenolic compounds, phenolic acids are metabolized by bacteria, especially *Lactobacillus fermentum* (207). The antibacterial activity is modified by the chemical structure of hydroxybenzoic acids, which decreases with a higher number of hydroxyl groups (208). This potential benefit can be extrapolated to food industry for the use of these compounds as food preservatives.

There is a variety of evidence available on the potential health benefits of *p*-coumaric and caffeic acid, mediated by its antioxidant and antimicrobial properties (209). There is also evidence available on ferulic acid about its antioxidant effect related to skin cells, protecting them against ultraviolet rays (210). The peroxidation of human LDL-c was used in order to measure the free radical induced damage of cell membranes. The results demonstrated that hydroxycinnamic acid derivatives may be effective antioxidants against LDL-c peroxidation. An experiment on T2D rats showed a significant decrease in serum glucose levels in diabetic rats compared to baseline with the supplementation of gallic acid, concluding that gallic acid, a *p*-coumaric acid, may have antidiabetic effects (211).

In another study, the effects of caffeic and cinnamic acid on insulin sensitivity was measured (212). An increase in insulin sensitivity means better glucose uptake, as well as an enhancement in glucose utilization. Experimental studies were also performed to evaluate the antidiabetic effects of phenolic acids. An experiment on db/db mice, which is an obese genetically mutated mice without leptin receptor, was designed in order to evaluate the antidiabetic effects of caffeic acid (213). The potential mechanisms underlying antidiabetic effects of phenolic acids are a result of the modulative effects of these compounds on GLUT. Phenolic acids intake enhances GLUT2 expression in the pancreatic beta cells and increase the expression and promotion of GLUT4 translocation via PI3K/Art and AMP-activated protein kinase pathways (213,214). The inhibition of alpha-glucosidase and alpha-amylase, which are the two main enzymes that digest dietary CH to glucose, is one of the well-known properties of phenolic acids. Recent human studies have demonstrated that apple and berry juices improve short-term glycemic control (195). Another potential mechanism leaded by phenolic acids is the increase in the secretion of glucose-dependent insulinotropic polypeptide and GLP-1, which facilitates insulin response (215).

4.2.3. Stilbenes

Several studies have reported beneficial health effects of resveratrol although its bioavailability is low. These benefits could probably be explained by its own metabolites (glucuronide and/or sulfate conjugates or dihydroresveratrol conjugated or not), which have also been associated with biological activities (170). Zamora-Ros *et al*. reported that the average intake of stilbenes, as the sum of resveratrol and piceid (glucosilated form), was 0.933 mg per person and per day, after analyzing the dietary data from 41,440 individuals from Spain (216). Nevertheless, other authors have pointed that daily intake might be up to 4 mg/person (217). Variables such as sex (men showed higher dietary

intake of stilbenes), age (higher intake with age), education level or lifestyle, influence in the estimated intake values (216). In the last 20 years, resveratrol has been extensively studied due to its chemopreventive, antiobesity, antidiabetic, and neuroprotective properties, as well as its regulatory effect on cell proliferation, angiogenesis, inflammation, mitochondrial activity, or redox status, among others (169,170). Moreover, resveratrol has been associated to health benefits on CVD (French paradox) (216) and cancer because of anticancer activity, linked to the inhibition of cyclooxygenase 2 (169). There is extensive scientific evidence that the intake of different stilbenes, such as pinosylvin, piceatannol, and resveratrol, can mediate the production of inflammatory cytokines, such as TNF-α, IL-1 and IL-6, as well as insulin resistance by the inhibition of extracellular signal-regulated kinase, c-Jun N-terminal kinase, and NF-kb (169). Others proposed mechanism are the inhibition of ROS production by inhibiting activator protein, mitogen-activated protein kinase (MAPK), as well as, cyclooxygenase enzymes, sirtuin 1, and Toll-like-receptor pathways, which might explain the protective effect exerted by stilbenes against the appearance of diseases such as obesity, cancer, CVD and neurodegenerative (169,218). Animal studies have also reported that resveratrol may lead to a body-fat reduction, increasing the expression of UCP and therefore, thermogenesis. Several meta-analyses have also suggested that resveratrol supplementation is associated with significant reductions of BP. So, Liu *et al*. reported an improvement of systolic BP (-11.90 mmHg) after a daily intake of resveratrol higher than 150 mg, while this effect was not observed for lower doses (219). Similar conclusions were reported by Hausenblas *et al*. where the dose of resveratrol ranged from 10 to 5000 mg per day, and Feringa *et al.,* where the total daily doses ranged from 150 mg to 2000 mg (220,221). In the last-mentioned meta-analysis, authors also reported a significant reduction in systolic BP (-1.54 mmHg, p=0.02) and heart rate (-1.42 bpm, p=0.01), although no changes were observed for diastolic BP, lipid profile and CRP concentrations (221). Finally, a meta-analysis performed by Fogacci *et al*. suggested that resveratrol could be used as a nutraceutical, with health benefits on BP, when is administered in high daily doses ($\geq$300 mg per day) in diabetic patients (222). Significant improvement of HbA1c concentrations, total cholesterol, LDL-c, and systolic BP, after daily administration ranging from 250 mg to 1 g of resveratrol to patients with T2D were observed (223,224). Additionally, resveratrol does not seem to have any effect on HDL-c levels or apolipoprotein (Apo) A concentrations according to data reported by different interventional studies, although it may reduce TG levels (225–227).

Resveratrol has also been shown to promote endothelial function which may help to prevent atherosclerosis and CVD. Carrizzo *et al*. also observed that resveratrol improves

vascular function in patients with hypertension and dyslipidemia by modulating NO metabolism (228). Similar results were reported in other studies, showing improvements in endothelial function after a daily consumption of 300 mg or 75 mg of resveratrol in 24 hypertensive adults or 28 healthy obese, respectively (229,230). Nonetheless, no effects on lipid oxidation, adiponectin, leptin, insulin, body composition, SIRT1, adenosine monophosphate-activated protein kinase (AMPK) pathways or inflammatory biomarkers (CRP, MCP-1, TNF-α) were observed after the daily consumption of 500 mg of resveratrol for 4-weeks in healthy obese men (231).

To date, scientific evidence showed that the effects of resveratrol on inflammation are very inconclusive and have a great variability. According to Poulsen *et al*. the dose and the study population could partially explain the lack of beneficial effects of resveratrol on inflammatory biomarkers (232). Additionally, the heterogeneity among the trials (inflammatory status, sample size, sources of resveratrol, length of the intervention, baseline characteristics of study participants, etc.), could also explain the inconsistence observed in relation to the anti-inflammatory effects of resveratrol.

4.2.4. Lignans

There is large scientific evidence about the beneficial effects of a lignan-rich foods diet to control noncommunicable chronic diseases (NCDs), such as MetS, hypertension, T2D and CVD (233–237). Also, lignans might present anti-carcinogenic effects, according to data showed by experimental evidence in animals and humans, but results to date are controversial (238–241). Although lignan supplementation have also been associated with improvement on inflammatory markers (242,243) and other cardiovascular risk factors such as plasma total cholesterol, LDL-c, and high BP (244–246), other clinical trials have reported inconsistent results (247). Witkowska *et al*. did not find in individual lignan intake of secoisolariciresinol, pinoresinol, and matairesinol any association with the prevalence of CVD or CVD risk factors (247). Authors only found a reduction of 30% hypercholesterolemia in postmenopausal women after lariciresinol intake.

A meta-analysis of 28 RCTs with 1,548 participants found significant reductions in total- and LDL-c by 0.10 mmol/L and 0.08 mmol/L, respectively (248). The cholesterol-lowering effects were apparently higher in postmenopausal women and volunteers with high cholesterol levels at baseline (248). No changes were observed in HDL-c or TG levels. Moreover, in another meta-analysis (15 RCTs, 1,302 participants) performed by Ursoniu *et al*. reported significant reductions in both systolic (-2.85 mmHg, p=0.027) and diastolic BP (-2.39 mmHg, p=0.001) after supplementation with different types of flaxseed products (249). Besides, authors reported greater effect on both systolic and diastolic

BP when the intervention time was equal or higher than 12 weeks (-3.10 and -2.62 mmHg, respectively). Nevertheless, in a recent meta-analysis did not find significant changes in total, LDL-c and HDL-c observed after sesame consumption, except for TG levels (-0.24 mmol/L, p<0.001) (250). The hypocholesterolemic effects of lignans are unclear and more trials are required. Several observational evidence that supports that dietary lignans can exert a cardiovascular benefit. Tresserra-Rimbau *et al*. showed significant associations between stilbenes and lignans intake with lower all-cause mortality risk (251). This result was aligned with CVD risk, and significant associations were found between highest intake of lignans and a significant reduction of cardiovascular risk by 49% (252). To date, the underlying mechanisms are still unknown. However, among others potential beneficial effects, lignans may exert anti-inflammatory, antioxidant, and antitumor functions (253–256). Focusing on inflammation, flaxseed [rich in α-linolenic acid and lignans (ranged 0.2–13.3 mg/g)] is emerging as a functional food to reduce the risk of CVD (257).

4.3. Intake estimation and main food sources

The beneficial effects of polyphenols depend on the amount ingested and its absorption. Thus, to study the possible benefits of polyphenols in humans, it is necessary to know the total polyphenol content of the foods and their exact polyphenolic profile. Typically, polyphenol intake was evaluated using the data extracted from FFQ. Recently, polyphenol intake has been measured using the analysis of several biomarkers of intake, mainly phase II enzyme-conjugated polyphenol metabolites, which are present in bloodstream, urine, and fecal samples. Unfortunately, there are thousands of potential biomarkers of polyphenol intake and there is no consensus yet (258).

The intake of dietary polyphenols and the main food sources depends on the dietary pattern and the foods of each region, as described in **Table 2.** Nevertheless, the profile of polyphenols subclasses and the main food sources is very different according to Mediterranean and non-Mediterranean countries. In Mediterranean countries, dietary polyphenol intake come mainly from coffee, fruits (the main source of flavonoids, representing 45% of the intake), wine, and vegetables oils (representing 26% of lignans intake), whereas in the non-Mediterranean countries, polyphenols come from coffee, tea, and wine (40.9%, 17.4%, and 4.6% of total polyphenols, respectively) (154). In the case of the MD, individuals with the highest MD adherence showed a higher intake of flavonoids, with fruits, vegetables, and red wine as the main food sources (259).

Mediterranean Area	Polyphenol Subclass (% of TPI)[a]	Main Food Sources (% TPI)
Spain, Greece, Italy, and south of France (258)	Phenolic acids (49), flavonoids (45), other polyphenols (0.6), stilbenes, and lignans (<0.7)	Coffee (36), fruits (25), red wine (10)
France (154)	Phenolic acids (54), flavonoids (42)	Coffee (44), tea (7), apples (7), red wine (6)
Spain	Flavonoids (54), phenolic acids (37), other polyphenols (8.7), stilbenes, and lignans (<0.3)	Coffee (18), oranges (16), apples (12), olives and olive oil (11), red wine (6)
Sicily (Italy) (260)	Phenolic acids (53), flavonoids (37), lignans (0.4), stilbenes (0.3)	Nuts (28), coffee (7), red wine (6), tea (5)

Table 2. Profile of the dietary polyphenol subclasses intake among the Mediterranean countries. TPI: Total polyphenol intake. [a]Dietary polyphenol intake was determine by the Phenol-Explorer Database (http://phenol-explorer.eu/) for all the areas described.

In the case of the PREDIMED study, where the main key foods of MD were EVOO and nuts, EVOO and olives provide around 11% of the total polyphenol intake. The phenolic profile of EVOO and olives are unique, with 98% of the polyphenol content being classified as “other phenolic acids” and “other polyphenols” subclasses. This characteristic phenolic profile has resulted in health benefits, a claim which has been recognized by the European Food Safety Authority (EFSA) (261).

Furthermore, food intake and the variety of food consumption represent more than 25,000 compounds, which most of them are metabolized creating new compounds (262). The food metabolome is highly variable, but it constitutes a high valuable information source for dietary exposure and allows the identification of potential foods, nutrients or bioactive compound that influence health status (263). The application of metabolomics or dietary biomarkers is more objective than the traditional nutritional epidemiology tools, which are mainly self-reported methods with inherent random and systematic errors, such as recall bias and portion sizes (264). Several biomarkers of dietary intake have been identified in epidemiological studies. These biomarkers have been measured mainly in plasma, serum samples or derivates as red blood cells, and urine. At present, some food biomarkers have been used as biomarkers of food intake, which are summarized in **Table 3**. Moreover, other biomarkers obtained from the metabolization of

food components or endogenous metabolites altered by specific nutrients exposure should be considered as potential food intake biomarkers. However, dietary biomarkers have several limitations, including the difficulty and cost of sample collection, and the production or chemical alteration of the biomarker due to several conditions, including health status, age, sex, interaction with genetic factors, or lifestyle factors such as smoking or alcohol intake (265,266).

Food category and food	Biomarkers
Fruits (total)	4-*O*-Methylgallic acid, β-cryptoxanthin, carotenoids, flavonoids, gallic acids, hesperetin, isorhamnetin, kaempferol, lutein, lycopene, naringenin, phloretin, vitamin A, vitamin C, zeaxanthin.
Apple	Kaempferol, isorhamnetin, *m*-coumaric acid, phloretin
Orange	Caffeic acid, hesperetin, proline betaine
Grapefruit	Naringenin
Citrus fruit	Ascorbic acid, β-cryptoxanthin, hesperetin, naringerin, proline betaine, vitamin A, zeaxanthin
Vegetables (total)	Ascorbic acid, α-carotene, β-carotene, β-cryptoxanthin, carotenoids (mix), enterolactone, lutein, lycopene
Carrot	α-Carotene
Tomato	Carotenoids (mix), lycopene, lutein
Vegetables, leafy	Ascorbic acid, β-carotene, carotenoids (mix)
Vegetables, root	Ascorbic acid, α-carotene, β-carotene
Fruits and vegetables (total)	α-Carotene, apigenin, ascorbic acid, β-carotene, β-cryptoxanthin, carotenoids (mix), eriodictyol, flavonoids (mix), hesperetin, hippuric acid, lutein, lycopene, naringenin, phloretin, phytoene, zeaxanthin
Cereal products	
Whole-grain rye	5-Heptadecylresorcinol, 5-pentacosylresorcinol, 5-tricosylresorcinol
Whole-grain wheat	5-Heneicosylresorcinol, 5-tricosylresorcinol, alkylresorcinols (mix)
Whole-grain cereals (total)	5-Heneicosylresorcinol, 3,5-dihydroxybenzoic acid, 3-(3,5-dihydroxyphenyl)-1-propanoic acid, 5-pentacosylresorcinol, 5-tricosylresorcinol, alkylresorcinols (mix)
Seeds	
Soy products	Daidzein, genistein, isoflavones (mix), *O*-desmethylangolensin
Meats	
Meat	1-Hydroxypyrene glucuronide, 1-methylhistidine
Meat, beef	Pentadecylic acid
Animal products (total)	1-Methylhistidine, 3-methylhistidine, margaric acid, pentadecylic acid, phytanic acid
Dairy products (total)	
Milk, dairy products	Iodine, margaric acid, pentadecylic acid, phytanic acid
Fish	
Fatty	DHA, EPA, long-chain ω-3 PUFAs, polychlorinated biphenyl toxic equivalents, pentachlorodibenzofuran, polychlorinated biphenyl 126, polychlorinated biphenyl 153, ω-3 PUFAs
Lean	Long-chain ω-3 PUFAs
Beverages (nonalcoholic)	
Tea	4-*O*-Methylgallic acid, gallic acid, kaempferol
Coffee	Chlorogenic acid
Alcoholic beverages (total)	5-Hydroxytryptophol/5-hydroxyindole-3-acetic acid, carbohydrate-deficient transferrin, ethyl glucuronide, γ-glutamyltransferase, aspartate aminotransferase, alanine aminotransferase
Wine	4-*O*-Methylgallic acid, caffeic acid, gallic acid, resveratrol metabolites

Table 3: Biomarkers of food consumption or food group. Adapted from Scalbert *et al.* 2014 (263).

In the case of polyphenols, it is important to mention that phenolic compounds are not essential for basic metabolism and are treated as xenobiotics. For this reason, more than 200 phase I/II metabolites have been identified after the intake of polyphenol-rich foods (153). Moreover, gut microbiota plays a key role in polyphenol metabolization, which metabolites could be useful as potential polyphenol intake biomarkers. However, it requires biological samples including urine, plasma, or other samples, such as fecal, and the cost of the analytical methodology for its identification is higher compared to dietary questionnaires (267). Additionally, high inter- and intra-individual differences in absorption, metabolism and excretion of dietary polyphenols should be considered, as well as the average lifetime of the specific compound or metabolite in the biospecimens (268).

4.4. Food analysis and food composition databases

Dietary polyphenol exposure in nutritional studies is challenging, and its intake has been assessed using dietary questionnaire, including FFQ and dietary recalls, or nutritional biomarkers. Dietary questionnaires are easy to administer, inexpensive and wide nutritional data could be estimated, including dietary patterns, key foods, nutrients, and bioactive compounds, such as polyphenols (269). The choice of a particular food questionnaire depends on the study population, the specific food or nutrient of interest and available resources. However, dietary questionnaires are self-reported and susceptible to random and reporting error, including portion size, and avoiding reporting dietary intake of unhealthy foods. Regarding polyphenol databases, Phenol-Explorer is the most comprehensive available open-access database on polyphenol content in habitual foods, which includes all known polyphenol metabolites (270). Phenolic compound databases have limitations, with several missing food items or unknown values, and changes in phenolic composition after cooking processes are not included.
The differences observed between dietary polyphenol intake between study populations are due to the inclusion or exclusion of polyphenol-rich foods in FFQ, the study population characteristics and the food matrix, where polyphenol-rich foods and other nutritional components of the specific food could modify the health benefits (271,272). An example could be processed foods, such as polyphenol-rich juice, which composition includes high sugar content and, consequently, the nutritional quality is reduced. Although FFQ are limited and its use ascertained the frequency of polyphenol rich foods

intake while specific key-foods for some polyphenols may not be captured, such as spices intake for curcuminoids, its use is widely extended in epidemiological studies for assessing long-term dietary intake. However, a retrospective FFQ with specific food and beverages items and each frequency of consumption (e.g., never, monthly, weekly, daily, etc.) allows to classify individuals into consumption categories.

4.5. Bioavailability

The effect of gut microbiota should be considered, as it metabolizes part of the dietary polyphenols, and its metabolism can modify their absorption, bioavailability, and biological activity. It has been estimated that only 5-10% of dietary polyphenol intake is absorbed in the small intestine, while the rest of polyphenols may accumulate in the intestine lumen and are metabolized by the gut microbiota (273). Polyphenol substrates accumulation in the lumen may modulate the microbiota composition, which could influence health status (274–277). The interindividual variability in gut microbiota, which determines polyphenol absorption, can explain the variety of health effects that are found in the literature, such as its role in energy and CH metabolization, its interaction with immune system, appetite control, and alterations in gut microbiota, also known as dysbiosis, has been associated with CVD, obesity, and cancer (193,195,197).

Food composition complexity is a limitation to evaluate the bioactivity of a specific polyphenol. Moreover, more beneficial effects are observed with a dietary polyphenol-rich pattern than with a single molecule, such as supplements. This is probably mediated by improvements in chemical stability within the food matrix and provide additive or synergic effects among specific polyphenol compounds (193). Nevertheless, it is difficult to establish the most effective dosage, food matrix and supplementation composition.

In the case of flavonoids, its bioavailability is very low, and it varies amongst the different flavonoid subclasses (279). After their absorption, these compounds are conjugated to sulphate and methyl groups in the gut mucosa (280). The ones that reach the colon, are metabolized by the microbiota. Suggested methods to increase the bioavailability of flavonoids in the human body include the use of absorption enhancers, novel delivery systems, changing the absorption site from large to the small intestine, and improving metabolic stability. With all the scientific evidence available regarding the anti-inflammatory properties of flavonoids, they have great therapeutic potential to become anti-inflammatory drugs (281). However, this would only become possible if the bioavailability can be enhanced. Flavonoid supplementation with the goal of increasing bioavailability is not a possibility, mainly because there is not yet a recommended dietary intake of these bioactive compounds in order to ensure desired levels of intake.

5. Role of dietary polyphenol intake

A continuous and prolonged polyphenol intake is related to BP and adiposity lowering effects, improvements in lipid profile, and anti-inflammatory effects, which all may act as CVD protectors (252).

5.1. Inflammation

Dietary bioactive compounds, such as polyphenols, exert anti-inflammatory effects by suppressing pro-inflammatory adipokine production or increasing the anti-inflammatory adipokines. Specifically, polyphenol-mediated signaling pathways associated with obesity-related inflammation include prostaglandin E2, AMPK, NF-κB, PPAR-γ and MAPK (27). These pathways are summarized in **Figure 6**.

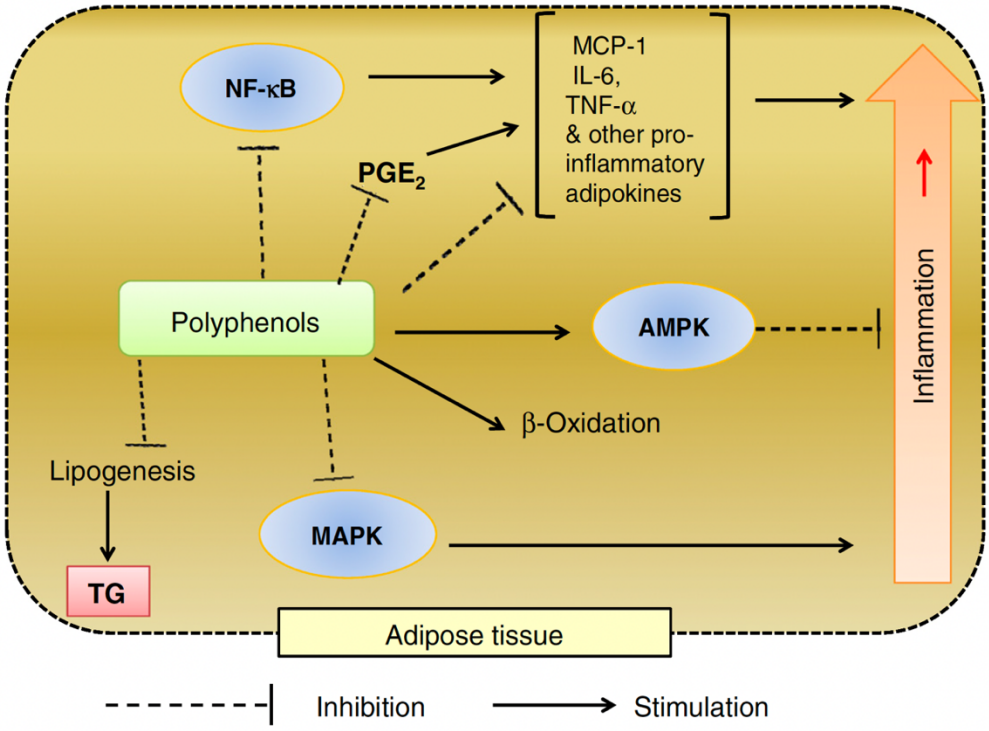

Figure 6: Anti-inflammatory effects of polyphenols on adipose tissue. (Siriwardhana N, *et al.* 2013) (27).

The anti-inflammatory properties of polyphenols have been also studied in the PREDIMED study (282). Total polyphenol excretion (TPE) in urine was analyzed in 1,139 participants from the three-intervention arm. Authors observed an increase in TPE excretion in participants randomized in the MD groups, which was associated with a reduction in several inflammatory parameters (sVCAM-1, sICAM-1, IL-6, MCP-1, and TNF-α), compared to baseline levels suggesting a dose-dependent anti-inflammatory effect.

Moreover, polyphenol-rich dietary interventions, such as the PREDIMED study, may influence the expression of key genes involved in inflammatory response, thrombosis, foam cell formation and atherosclerosis (283).

Excessive VAT is characterized by chronic low-grade inflammation, and it is well established that higher secretion of pro-inflammatory mediators is observed in individuals with higher VAT depots, which increase the risk of CVD. Thus, lifestyle modifications, including improvements in physical activity levels may influence body adiposity, including VAT, being one of the best anti-inflammatory strategies for patients with MetS.

Regarding flavonoids, quercetin is one of the most widely available compounds found in fruits, vegetables, tea, red wine, species, among other foods. The anti-inflammatory effects of quercetin have been reported for both adipocytes and macrophages mediated by NF-κB activation and reducing the infiltration of macrophages to adipose tissue (284).

Moreover, isoflavones are diphenolic compounds with estrogenic and antioxidant activity. Dietary isoflavones intake has been associated with several health benefits, such lipid profile improvements, lowering of adiposity levels, insulin sensitivity, lower incidence of some types of cancer, reduction of the main side-effects of menopause and osteoporosis (285,286). In fact, Food and Drug Administration approved a health claim for soy, the main food source of isoflavones, and 25g of soy protein, which provide approximately 50 mg of isoflavones, may reduce heart disease risk. The potential mechanism of action of isoflavones intake are mediated by estrogen-like receptors activation and PPAR-γ, which may modulate insulin sensitivity and adipocyte growth (287,288).

Resveratrol intake or supplementation may exert an anti-inflammatory effect, specifically in obesity-related inflammation by inhibiting NF-κB and activating sirtuin-1 (289). Overall, resveratrol reduced lipogenesis and the expression of inflammatory adipokines such as resistin, while in adipocytes, resveratrol increases adiponectin levels (290,291).

5.2. Adipogenesis and adipose tissue metabolism

5.2.1. Epidemiological and experimental data

Evidence for polyphenols' effect on obesity and weight control in humans is inconsistent due to the heterogeneity among study design, study populations, intervention period, and polyphenol supplements. An intervention clinical trial with polyphenol-enriched foods, such as an apple juice, showed a significant reduction in total fat mass but not in body weight, BMI, or waist circumference (292). However, a double-blinded,

randomized, parallel clinical trial conducted in 17 participants with obesity and type 1 diabetes (BMI between 30.1 and 33.3 kg/m^2) with a supplement of 370 mg of polyphenols (mg of gallic acid equivalent) obtained from grapefruit, grape, green tea, and other food extracts, showed a significant reduction in body weight, BMI, and waist and hip circumference compared with a placebo group after 12 weeks of intervention (293). Moreover, only few studies have studied the relationship between total polyphenol intake (TPI) from diet and weight control. Guo *et al*. analyzed the association between body weight and TPE using a urine biomarker in a population at high CVD risk in a long-term study. After five years of follow-up, they showed an inverse association between total TPE and BMI, body weight, and waist circumference (294).

Similarly, a study conducted in the Mediterranean area demonstrated that higher dietary intake of flavonoids was inversely associated with an excess of body weight and obesity (295). Some studies conducted in non-Mediterranean areas have shown an effect of polyphenol intake on weight control, but other clinical trials did not find any relationship between polyphenol intake and weight loss or changes in body composition. A longitudinal study from a Netherlands cohort that included 4,280 participants aged 55–69 years over 14 years of follow-up showed an association between a higher flavonoid intake and a lower increase in BMI in women (p<0.05) (296). Within the flavonoids, catechins are related to benefits on anthropometric parameters and body composition. More evidence that includes some studies with green tea extracts rich in catechins, epigallocatechin gallate (EGCG), showed a significant reduction in body weight, waist circumference, body fat mass, and visceral and subcutaneous fat (297). Based on a meta-analysis of 11 studies, Hursel *et al.* concluded that catechin or an EGCG–caffeine mixture contained in green tea had a minimal effect on weight loss and weight loss maintenance (297). Therefore, the clinical significance of the small changes seen in the body composition parameters indicates that green tea has no significant effect on weight loss and weight loss maintenance (298).

Resveratrol also showed potential antiobesity effects by inhibiting adipocyte differentiation and decreasing proliferation, mediated by adipocyte apoptosis, and decreasing lipogenesis, promoting lipolysis and β-oxidation (296). However, evidence about the effect of resveratrol intake on weight loss and weight loss maintenance is limited and the effects only seem to be achieved through dietary supplementation. Tome-Carneiro *et al*. performed several randomized, parallel, dose–response, placebo-controlled studies with a grape supplement rich in resveratrol and other grape polyphenols (299,300). The effects were statistically significant for CVD risk factors, including reduction in LDL-c, oxLDL, and thrombogenic plasminogen activator inhibitor

type 1 (PAI-1), and an increase in adiponectin and anti-inflammatory cytokines levels. However, they were not significant for adiposity parameters. Thus, the antiobesity potential and the optimal dose of resveratrol needs to be studied.

Even though the spice turmeric is not a characteristic food of the MD, curcumin, a yellow-colored polyphenol from the curcuminoids subclass, is known for its health benefits such as anti-inflammatory, anticarcinogenesis, antiobesity, antiangiogenesis, and antioxidant activities (301). The antiobesity properties of curcumin are similar to resveratrol's, inhibiting adipocyte differentiation, lipogenesis, reducing proinflammatory cytokines' synthesis in the adipose tissue, and promoting β-oxidation (301). Like resveratrol, clinical trials investigating the antiobesity properties of curcumin are limited. Ramirez-Bosca reported improvements in serum lipid profile through an increase in HDL-c and Apo A, as well as a decrease in LDL-c, ApoB, and the ApoB/ApoA ratio (302) with a daily supplement dose of 10 mg of a curcumin extract over 30 days.

Evidence from *in vitro* and experimental models suggests the potential effects of polyphenols on obesity, obesity-related inflammation, and other metabolic disorders. These studies showed significant reduction of body weight by increasing basal metabolic rate, increasing β-oxidation, lowering TG synthesis, and improving insulin sensitivity. Individuals with obesity have reported to be more dependent on glucose oxidation rather that fat oxidation (303).

5.2.2. Mechanism involved

The mechanisms involved in weight loss where polyphenols may have a role are inducing satiety; stimulating energy expenditure by inducing thermogenesis in brown adipose tissue; modulating adipose tissue by inhibiting adipocyte differentiation and promoting adipocyte apoptosis; modulating lipolysis; and activating β-oxidation (304).

Catechins, mainly green tea EGCC, promote β-oxidation by regulating the expression in adipose tissue of PPAR-γ and fatty acid synthase, while increasing the levels of carnitine palmitoyltransferase 1 (CPT-1), a protein that facilitates the transport of fatty acids to the mitochondria, which is a limiting step for β-oxidation (218). Relative to metabolic disorders, an *in vitro* study about the effect of white tea EGCG showed improvements in cellular glucose metabolism mediated by glucose transporters and a potential hypocholesterolemic effect stimulating LDL-c receptor binding activity (306). In the case of resveratrol, its involvement in regulating β-oxidation has been studied by increasing AMPK activity through preventing the degradation of intracellular cyclic adenosine monophosphate (cAMP) (307). The AMPK function is to regulate glucose transport and fatty acid metabolism. Therefore, its activation may lead to fatty acid oxidation and

suppression of hepatic gluconeogenesis as well as improvements in insulin sensitivity. Other studies revealed that resveratrol could mediate the expression of PPAR-γ or promote β-oxidation by inhibiting the synthesis of malonyl-CoA (308), which is a precursor and promoter of fatty acid synthesis. Curcumin contains polyphenols, and there is substantial evidence about its effectiveness in stimulating β-oxidation, inhibiting fatty acid synthesis, and decreasing fat storage (304). The molecular pathways are similar to EGCG in the upregulation of UCP-1, but also entail the reduction of lipid biosynthesis by the downregulation of fatty acid synthesis enzymes (309). Within the flavonoids, anthocyanins have been reported as having a role as antiobesity agents. Anthocyanins are widely found in fruits, such as apples with peel, strawberries, blueberries, blackberries, and blood oranges. To induce fatty acid oxidation, the postulated pathways are the modulation of AMPK synthesis and regulation of the expression of genes participating in β-oxidation (310). Regarding EVOO polyphenols, tyrosol derivates, such as oleuropein, are involved in energy metabolism and adiposity (311), reducing the expression of PPAR-γ, compromising adipocyte differentiation, and improving insulin sensitivity (312). Another interesting mechanism studied by Oi-Kano *et al.* in experimental models showed an increase in UCP-1 expression, which translates to the formation of "beige" adipose tissue, leading to a decrease of VAT (313). Hydroxytyrosol and its derivatives constitute around 90% of the total polyphenol content of EVOO (314). *In vitro* studies reported that hydroxytyrosol downregulates the expression of PPAR-γ and -α, which is translated to a reduction in adipocyte size (315). Additionally, an increase in AMPK and lipase (hormone-sensitive and phosphorylated lipase) was observed in adipocytes exposed to hydroxytyrosol (316). Furthermore, these effects were not reported to have an impact on body weight and adiposity in humans (316). There are several mechanisms of action involved and each polyphenol presents different pathways, as shown in **Figure 7**.

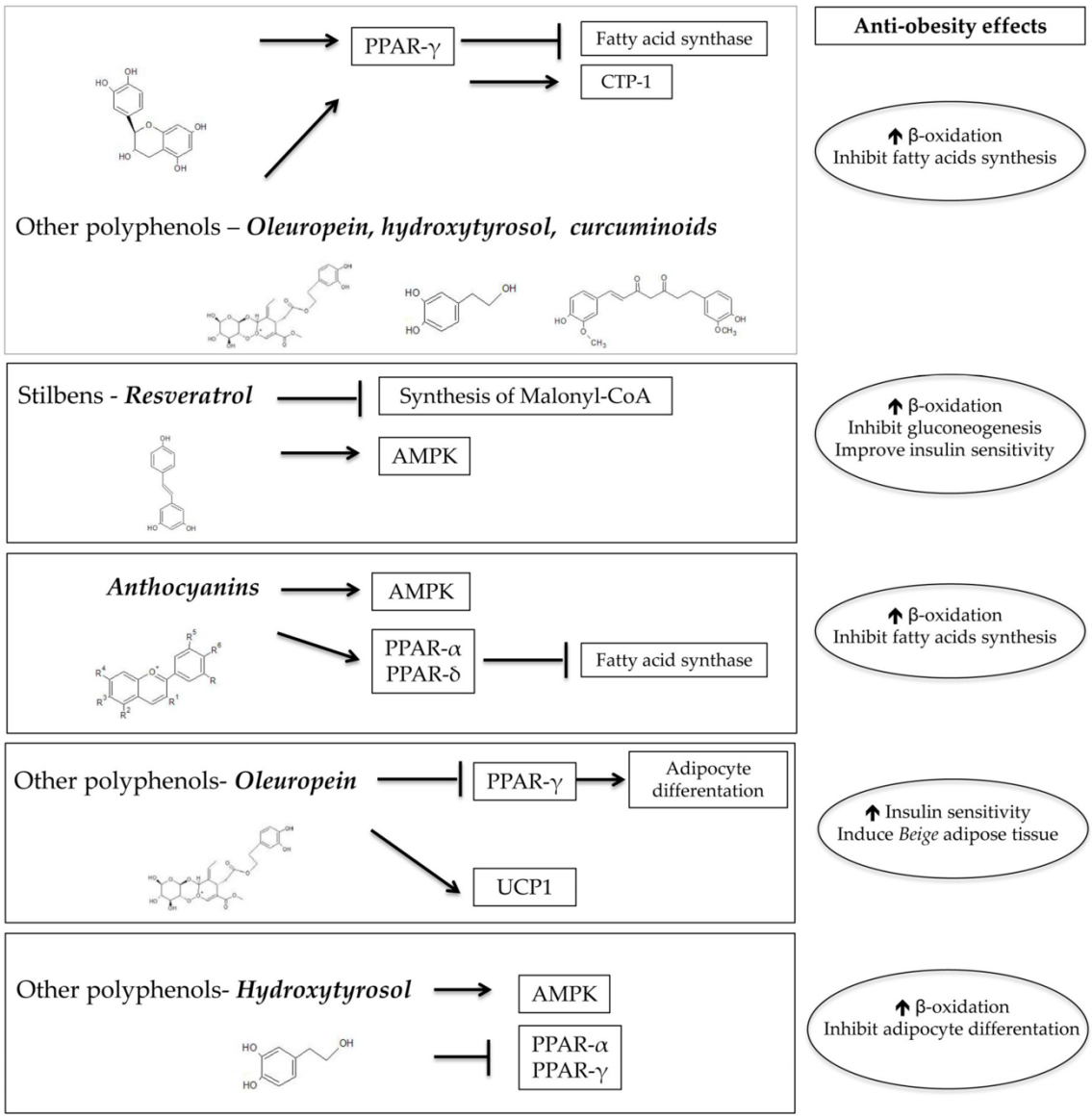

Figure 7: Molecular mechanisms of polyphenols involved in obesity. (Castro-Barquero S *et al.* 2018) (320).

5.3. Modulation of gut microbiota

Microbiota has been linked to intestinal health, the immune system and bioactivation and metabolism of nutrients, such as vitamins B and K and bioactive compounds. Recent clinical studies suggest a correlation between elevated plasma trimethylamine N-oxide, which is produced by gut bacteria metabolism and dietary components, such as L-carnitine, betaine, and choline, and a higher risk of T2D, hypertension, and atherosclerosis (318–320). Therefore, it has been well studied that diet affects the composition and activity of gut microbiota and situations of gut microbiota dysbiosis may be involved in the development of CVD. Additionally, dysbiosis has been associated with obesity in humans and microbial imbalance can be reversed with weight loss (321).

SCFAs are formed from the fermentation of oligosaccharides, proteins, and peptides (322), with the main SCFA products being acetate, propionate, and butyrate. The contribution of SCFA products against obesity has been linked to decreasing weight gain by preventing fat accumulation (49,323,324). Adipocytes express high levels of SFCA receptors, and its production may play a key role in adipose tissue homeostasis (325). Fernandes *et al*. showed that obese subjects present higher SCFA products in stool samples than lean subjects because of the differences in their colonic fermentation (326). The before-mentioned SCFA main products display different mechanisms to induce satiety: butyrate acts on intestinal cells, increasing GLP-1 production (327), and propionate increases intestinal gluconeogenesis (49), both pathways leading to improvements in glucose homeostasis and increasing satiety. Nevertheless, the main tool to balance the gut microbiota is diet. This notion is promoting the use of prebiotics, which are mainly dietary components such as nondigestible CH. Other dietary compounds not absorbed by the small intestine, such as polyphenols, are accumulated in the large intestine, thus being exposed to the enzymatic activities of the gut microbiota (328).

During the last decade, the role of gut microbiota has been demonstrated, especially in the pathophysiology of T2D, mainly due to its role in CH metabolism (143). In this sense, it has been reported that individuals with obesity, T2D or MetS present a higher *Firmicutes/Bacteroidetes* ratio, characterized by fewer SCFA production, mainly butyrate (329–331). Moreover, the potential mechanisms underlying the CVD risk associated to dysbiosis may be the decrease in the intestinal barrier integrity, leading to inflammatory response, alteration in bile acid and cholesterol metabolism, and limited production of beneficial microbiota products such as SCFA (43,332).

Regarding microbiota composition, some studies have shown significant effects of MD adherence and microbiota composition, especially in some microbial species (333–335). The influence of MD on microbial function could be mainly attributed to its high-fiber content, which leads to SCFA production, and its production plays a key-role in host metabolism (336).

HYPOTHESIS

The pro-inflammatory response observed in obesity can be reversed mainly by weight loss, but some bioactive compounds found in foods, such as omega-3 fatty acids and polyphenols have been described to decrease low-grade inflammation. Previous epidemiological studies have investigated the association between dietary polyphenol intake and MetS components and T2D, in healthy populations or those at high risk of CVD, but to our knowledge, there are no previous studies on these associations in subjects previously diagnosed with MetS.

Moreover, recent evidence suggests that weight-loss, even small amounts of body weight, are associated with improvements in health. The benefits of dietary polyphenol associated with body composition include the activation of lipolysis and β-oxidation, inhibit lipogenesis and adipocyte proliferation, and exert a prebiotic effect for gut microbiota. Therefore, an increase in dietary polyphenol intake might be associated with improvements in body composition parameters in overweight or obese population with MetS.

It is well established that VAT is associated with CVD and metabolic disturbances, inducing chronic low-grade systemic inflammation mediated by macrophage infiltration and the secretion of several proinflammatory cytokines. In this sense, a reduction in VAT may improve inflammatory and adipokines levels greater than weight loss. Thus, a decrease in VAT might be associated with greater improvements on obesity-related inflammatory and adipokines concentration in older overweight or obese individuals with MetS.

Finally, dietary intake assessment in epidemiological studies is complex due to the measurement errors. Food frequency questionnaire (FFQ) is the dietary assessment tool used for polyphenol and dietary intake estimation in several epidemiological studies, and its validation for a specific study population, with objective measurements of nutrients and key-food intake, is needed. In summary, an updated version of the FFQ may show reliability and validity according to biological markers and MD adherence.

OBJECTIVES

The general objective of this thesis project is to evaluate the effect of a high dietary polyphenol intake, included in an intensive weight-loss-oriented lifestyle intervention program, on adiposity parameters and other cardiovascular risk factors in patients with MetS.

Main specific objectives:

1. To describe dietary polyphenol intake and the main food sources in participants with MetS.
2. To assess whether dietary intake of some polyphenol sub-classes in participants with MetS is associated with:
    2.1. MetS components, including levels of BP, fasting glucose, HDL-c, TG, and waist circumference.
    2.2. T2D prevalence and control parameters, including fasting glucose and HbA1c levels.
    2.3. Body composition parameters measured by DXA scan, including total fat mass (g) and total body fat (%), and regional adiposity, as trunk, leg, android, gynoid, subcutaneous and visceral fat mass (g).
3. To assess whether changes in VAT are associated with obesity-related inflammatory markers and adipokines concentration, including insulin, glucagon, IL-6, visfatin, ghrelin, GLP-1, TNF-α, MCP-1, PAI-1, resistin, C-peptide, leptin, adipsin and adiponectin.

Secondary objectives:

1. Development and validation of an updated version of the FFQ to estimate dietary intake according to food and nutrient consumption, biological markers, and MD adherence.

RESULTS

Manuscript I

Title: "Dietary Polyphenol Intake is Associated with HDL-Cholesterol and A Better Profile of other Components of the Metabolic Syndrome: A PREDIMED-Plus Sub-Study"

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MDPI

Article

Dietary Polyphenol Intake is Associated with HDL-Cholesterol and A Better Profile of Other Components of the Metabolic Syndrome: A PREDIMED-Plus Sub-Study

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Abstract: Dietary polyphenol intake is associated with improvement of metabolic disturbances. The aims of the present study are to describe dietary polyphenol intake in a population with metabolic syndrome (MetS) and to examine the association between polyphenol intake and the components of MetS. This cross-sectional analysis involved 6633 men and women included in the PREDIMED (PREvención con DIeta MEDiterranea-Plus) study. The polyphenol content of foods was estimated from the Phenol-Explorer 3.6 database. The mean of total polyphenol intake was 846 ± 318 mg/day. Except for stilbenes, women had higher polyphenol intake than men. Total polyphenol intake was higher in older participants (>70 years of age) compared to their younger counterparts. Participants with body mass index (BMI) >35 kg/m^2 reported lower total polyphenol, flavonoid, and stilbene intake than those with lower BMI. Total polyphenol intake was not associated with a better profile concerning MetS components, except for high-density lipoprotein cholesterol (HDL-c), although stilbenes, lignans, and other polyphenols showed an inverse association with blood pressure, fasting plasma glucose, and triglycerides. A direct association with HDL-c was found for all subclasses except lignans and phenolic acids. To conclude, in participants with MetS, higher intake of several polyphenol subclasses was associated with a better profile of MetS components, especially HDL-c.

Keywords: polyphenols; metabolic syndrome; Mediterranean diet; glignans; stilbenes; HDL-cholesterol

1. Introduction

Polyphenols are plant-derived molecules characterized by the presence of one or more aromatic rings and attached hydroxyl groups [1]. They are classified into five subclasses according to their chemical structure, including flavonoids and nonflavonoids subclasses defined as phenolic acids, stilbenes, lignans, and other polyphenols. These bioactive compounds are responsible for some health and sensory properties of foods, such as bitterness, astringency, and antioxidant capacity. The intake of phenolic compounds and their food sources is highly variable and depends on dietary patterns, sex, socioeconomic factors, and the native foods of each region [2]. The Mediterranean diet (MedDiet) is characterized by a high intake of phenolic compounds because MedDiet interventions promote the intake of phenolic rich and plant-based products, such as legumes, vegetables, fruits, nuts and wholegrain cereals, and promote the use of extra virgin olive oil as the main source of fat. It has been suggested that phenolic compounds are partly responsible for the beneficial effects attributed to the MedDiet [3].

The metabolic syndrome (MetS) is defined as a cluster of metabolic disturbances, which include impaired glucose metabolism, elevated blood pressure, and low level of HDL-c, dyslipidemia, and abdominal obesity [4]. Sedentary lifestyle, smoking, and unbalanced diets are well-known modifiable risk factors for MetS, and lifestyle interventions in those areas, especially dietary interventions based on the MedDiet [3–6], might improve this condition. Considering the chronic low-grade inflammation and oxidative stress observed in MetS, polyphenols are good candidates to improve the condition because of their antioxidant and anti-inflammatory properties [7]. Moreover, several epidemiological studies have observed a negative association between polyphenol intake and MetS rates [8]. Regarding MetS components, an adequate intake of phenolic compounds has been shown to improve lipid profile and insulin resistance, and decrease blood pressure levels and body weight [8,9].

Despite the fact that phenol-rich dietary patterns are effective in improving some MetS components, there is no single phenolic compound or extract able to improve all the components of MetS [10]. Nevertheless, given the complexity of MetS and the heterogeneity of polyphenols, more large randomized trials with MetS patients are needed to evaluate the effect of polyphenol intake in reducing MetS complications, and whether intake of the different polyphenol subclasses could be associated with improvements in MetS components, because each subtype has different absorption and metabolism [11].

Therefore, the aims of our study were firstly to describe polyphenol intake in 6633 participants with MetS from the PREvención con DIeta MEDiterranea-Plus (PREDIMED-Plus) trial and to identify the main food sources of polyphenols in those participants, and secondly to examine whether higher intakes of some polyphenol sub-classes are associated with MetS components in this population.

2. Materials and Methods

2.1. Design of the Study

A cross-sectional analysis of the baseline data of participants included in the PREvención con DIeta MEDiterranea-Plus (PREDIMED-Plus) study was performed. The profile of the cohort, recruiting methods, and data collection processes have been described elsewhere [12] and on the website http://predimedplus.com. The study protocol was approved by the 23 recruiting centers Institutional Review Boards and registered in 2014 at the International Standard Randomized Controlled Trial Number registry (http://www.isrctn.com/ISRCTN89898870). All participants provided written informed consent before joining the study.

2.2. Participants

A total of 6874 subjects were recruited and randomized in the 23 recruiting centers between September 2013 and December 2016. Primary care medical doctors from primary care centers of the National Health System assessed potential participants for eligibility. Eligible participants were men (aged 55–75 years) and women (aged 60–75 years) with overweight or obesity (body mass index [BMI] ≥27 and <40 kg/m^2) and at least three components of MetS according to the comprehensive definition of the International Diabetes Federation; National Heart, Lung, and Blood Institute; and American Heart Association (2009) [4]. Exclusion criteria were documented history of cardiovascular diseases (CVD), having a long-term illness, drug or alcohol use disorder, a BMI of 40 or higher, a history of allergy or intolerance to extra virgin olive oil or nuts, malignant cancer, inability to follow the recommended diet or physical activity program, history of surgical procedures for weight loss, and obesity of known endocrine disease (except for treated hypothyroidism). Of the total sample of 6874 randomized participants, 241 participants were excluded from the current analysis (Figure 1): 53 without food-frequency questionnaire (FFQ) data at baseline, and 188 participants who reported energy intake values outside the predefined limits (<3347 kJ [800 kcal]/day or >17,573 kJ [4000 kcal]/day for men; <2510 kJ [500 kcal]/day or >14,644 kJ [3500 kcal]/day for women) [13].

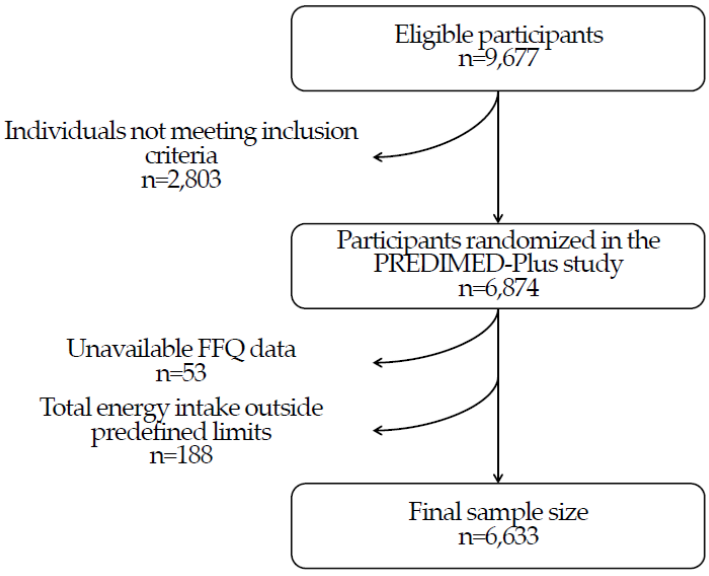

Figure 1. Flowchart of the participants.

2.3. Estimation of Dietary Polyphenol Intake

The total dietary polyphenol intake and polyphenol subclasses were obtained at baseline by the 143-item FFQs used in the PREDIMED-Plus study. As described elsewhere [14], dietary polyphenol intake was estimated following these steps: (1) All foods from the FFQ with no polyphenol content, or only traces, were excluded; (2) recipes were calculated according to their ingredients and portions using traditional MedDiet recipes; (3) when an item from the FFQ included several foods (e.g., oranges and tangerines), the proportion of intake was calculated according to data available in the national survey; (4) no retention or yield factors were used to correct weight changes during cooking because this was already taken into account in the FFQ; (5) the polyphenol content in 100 g of each food item was obtained from the Phenol-Explorer database (version 3.6) [15]; (6) finally, the individual polyphenol intake from each food was calculated by multiplying the content of each polyphenol by the daily consumption of each food. Total polyphenol intake was calculated as the sum of all individual polyphenol intakes from the food sources reported in the FFQ.

The data used to calculate polyphenol intake was obtained by chromatography of all the phenolic compounds, except proanthocyanidins, the content of which was obtained by normal-phase high-performance liquid chromatography. In the case of lignans and phenolic acids in certain foods (i.e., swiss chard, chickpeas, plums, and strawberry jam), data corresponding to chromatography after hydrolysis was also collected, since these treatments are needed to release phenolic compounds that could otherwise not be analyzed. Total and polyphenol subclass intakes were adjusted for energy intake (kcal/day) using the residual method [13].

2.4. Measurements and Outcome Assessment

Data on age, sex, educational levels, anthropometric measurements, dietary habits and lifestyle were collected at baseline. Anthropometric measurements were measured according to the PREDIMED-Plus protocol. Weight was recorded with participants in light clothing without shoes or accessories using a high-quality calibrated scale. Height was measured with a wall-mounted stadiometer. Waist circumference was measured midway between the lowest rib and the iliac crest. The BMI was calculated as weight (kg) divided by the square of height (m^2).

Physical activity and sedentary behaviors were evaluated using the validated Regicor Short Physical Activity Questionnaire [16] and the validated Spanish version of the Nurses' Health Study questionnaire [17], respectively.

Information related to sociodemographic and lifestyle habits, individual and family medical history, smoking status, medical conditions, and medication use was evaluated using self-reported questionnaires. Sociodemographic and lifestyle variables were categorized as follows: age (three categories: <65, 65–70, or >70 years), educational level (three categories: primary, secondary, or high school), physical activity level (three categories: low, moderate, or high), BMI (three categories: 27.0–29.9, 30.0–34.9, or ≥35 kg/m^2), and smoking status (three categories: never, former, or current smoker).

Blood samples were collected after overnight fasting. Biochemical analyses were performed to determine plasma glucose (mg/dL), glycated hemoglobin (%), HDL-c (mg/dL), and triglyceride (mg/dL) levels using standard laboratory enzymatic methods. Low-density lipoprotein cholesterol (LDL-c; mg/dL) was calculated using the Friedewald formula whenever triglyceride levels were less than 300 mg/dL. Blood pressure measurements were obtained after the participant had rested for five minutes. Each measurement was obtained with a validated semiautomatic oscillometer (Omron HEM-705CP), ensuring the use of the proper cuff size for each participant.

2.5. Statistical Analysis

Descriptive statistics were used to define the baseline characteristics of the participants. The database used was the PREDIMED-Plus baseline database generated in September 2018.

Continuous variables are expressed as mean ± SD. Categorical variables are expressed as number (*n*) and percentage (%). Comparisons among quartiles of dietary polyphenol intake used the Pearson chi square test (χ^2) for categorical variables or one-way ANOVA for continuous variables. The associations between dietary polyphenol intake and MetS components were analyzed by linear regression models to determine differences between quartiles of polyphenol subclass intake. The results of the regression models are expressed as unstandardized β-coefficients. For regression models, polyphenol and polyphenol subclasses are expressed as quartiles of energy-adjusted dietary intake. We used robust variance estimators to account for intra-cluster correlation in all linear models, considering members of the same household as a cluster. All regression models were adjusted for potential confounders. Model 1 was adjusted for sex, age, recruiting center, and members of the same household. Model 2 was additionally adjusted for physical activity level, BMI (except for waist circumference criteria), smoking status, and educational level. We additionally adjusted for anti-diabetic treatment when assessing glycemia and antihypertensive treatments when assessing blood pressure. Lastly, model 3 was additionally adjusted for total energy intake (continuous, kcal/day), saturated fatty acids (g/day), and distilled drinks alcohol intake (g/day). In model 3, the analysis of glycemia was additionally adjusted for dietary simple sugar intake (g/day), whereas the analysis of systolic and diastolic blood pressures was also adjusted for dietary sodium intake (mg/day). The normality of the continuous outcomes and standardized residuals was assessed with the Shapiro–Wilk test. Values are shown as 95% confidence interval (CI) and significance for all statistical tests was based on bilateral contrast set at $p < 0.05$. The P value for linear trends was computed by fitting a continuous variable that assigned the median value for each quartile in regression models. The descriptive analyses shown in Tables 1–3 were performed using SPSS software version 22.0 (Chicago, IL, USA) and the regression analysis was performed using Stata software version 16 (StataCorp LP, College Station, TX, USA).

Table 1. Baseline characteristic of participants by quartiles of total polyphenol intake.

	Q1 (<623, 3 mg/d)	Q2 (623.4–799.4 mg/d)	Q3 (799.5–1019.2 mg/d)	Q4 (>1019.3 mg/d)	*p*	*p for Linear Trend*
n	1658	1658	1660	1657		
Age, years	65.2 ± 4.90	64.8 ± 4.87	65.0 ± 4.87	64.9 ± 4.98	0.10	0.19
Women, *n (%)*	894 (53.9)	845 (51.0)	785 (47.3)	685 (41.3)	<0.001	<0.001
Family history of CVD [1], *n (%)*	659 (39.7)	698 (42.1)	662 (39.9)	678 (40.9)	0.48	0.81
Current smokers, *n (%)*	197 (11.9)	205 (12.4)	203 (12.2)	216 (13.0)	0.78	0.36
Former smokers, *n (%)*	647 (39.0)	695 (41.9)	728 (43.9)	800 (48.3)	<0.001	<0.001
BMI, kg/m^2	32.6 ± 3.46	32.6 ± 3.49	32.6 ± 3.51	32.3 ± 3.31	0.03	0.02
Waist circumference, cm	107.0 ± 9.76	107.4 ± 9.70	107.8 ± 9.75	107.8 ± 9.36	0.06	0.01
Body weight, kg	85.2 ± 12.8	86.2 ± 12.8	87.3 ± 13.3	87.5 ± 12.8	<0.001	<0.001
Glucose, mg/dL	113.4 ± 28.9	113.9 ± 31.0	113.9 ± 29.0	113.0 ± 27.6	0.78	0.71
Glycated hemoglobin, %	6.10 ± 0.88	6.22 ± 2.58	6.25 ± 3.53	6.10 ± 0.88	0.15	0.85
Total-cholesterol, mg/dL	196 ± 38.4	197 ± 37.7	196 ± 37.0	198 ± 42.8	0.59	0.57
HDL-cholesterol, mg/dL	47.6 ± 11.5	48.2 ± 11.7	48.7 ± 12.2	47.9 ± 11.9	0.06	0.32
Medications, *n (%)*						
Antihypertensive agents	1272 (76.7)	1285 (77.5)	1294 (77.9)	1304 (78.7)	0.48	0.46
Colesterol-lowering agents	862 (52.0)	846 (51.0)	858 (51.7)	842 (50.8)	0.97	0.52
Insulin	84 (5.07)	98 (5.91)	67 (4.04)	63 (3.80)	0.01	0.01
Metformin	380 (22.9)	404 (24.4)	383 (23.1)	347 (20.9)	0.13	0.12
Other hypoglycemic drugs	324 (19.5)	331 (20.0)	327 (19.7)	303 (18.3)	0.62	0.35
Aspirin or antiplatelet drugs	246 (14.8)	272 (16.4)	249 (15.0)	271 (16.3)	0.26	0.61
NSAIDS	534 (32.2)	469 (28.3)	484 (29.2)	446 (26.9)	0.01	0.01
Vitamins and minerals	210 (12.7)	184 (11.1)	220 (13.3)	183 (11.0)	0.19	0.11
Sedative or tranquilliser agents	417 (25.1)	416 (25.1)	389 (23.4)	392 (23.7)	0.85	0.31
Hormonal treatment (only women)	42 (2.53)	41 (2.47)	33 (1.99)	38 (2.29)	0.924	0.935
Educational level, *n (%)*					<0.001	<0.001
Primary school	887 (53.6)	854 (51.5)	805 (48.5)	719 (43.4)		
Secondary school	468 (28.3)	467 (28.2)	497 (30.0)	481 (29.0)		
University and other studies	301 (18.2)	337 (20.3)	356 (21.5)	456 (27.5)		

[1] Cardiovascular diseases (CVD), body mass index (BMI), high-density lipoprotein-cholesterol (HDL-c) and nonsteroidal anti-inflammatory drugs (NSAIDs). Continue variables are expressed as mean (± SD). Categorical variables are expressed as number (*n*) and percentage (%). Comparisons among quartiles of dietary polyphenol intake with Pearson's chi square test for categorical variables or one-way ANOVA for continuous variables. For glycated hemoglobine parameter, 9% of participants had no values available. The P value for linear trend was computed by fitting a continuous variable that assigned the median value for each quartile in regression models.

Table 2. Contribution (%) of polyphenol subclasses to total polyphenol intake and food sources.

Polyphenol Subclasses	Contribution, Mean (mg/d) ± SD, (%)	Polyphenol Contribution as Aglycones, Mean (mg/d) ± SD, (%)	Food Sources (% of Contribution)
Total polyphenols	846 ± 318	620.9 ± 273.5	
Flavonoids	491 ± 253, (58.0)	406.3 ± 237.2 (65.44)	
▪ Anthocyanins	43.5 ± 37.8, (5.14)	24.7 ± 21.7 (3.98)	Cherries (42.2), red wine (24.1), olives (10.5), strawberries (10.1), grape (9.30), other foods (3.8)
▪ Chalcones	0.009 ± 0.18, (<0.01)	0.006 ± 0.01 (<0.01)	Beer (100)
▪ Dihydrochalcones	1.72 ± 1.59, (0.20)	0.98 ± 0.91 (0.16)	Apples (93.2), fruit juices from concentrate (6.77)
▪ Dihydroflavonols	2.62 ± 4.92, (0.31)	1.81 ± 3.43 (0.29)	Red wine (97.6), white wine (1.80), rosé wine (0.59)
▪ Catechines	28.1 ± 22.4, (3.32)	27.1 ± 20.7 (4.36)	Tea (23.0), red wine (19.2), apples (18.6), chocolate (11.6), peaches (6.0), cocoa powder (3.18), fruit juices from concentrate (2.83), other foods (15.6)
▪ Proanthocyanidins	204± 185, (24.1)	200.7 ± 189.4 (32.32)	Chocolate (42.7), apples (20.4), plums (9.53), red wine (7.09), cocoa powder (5.68), strawberries (4.20), other foods (10.4)
▪ Theaflavin	0.70 ± 1.81, (0.08)	0.57 ± 1.46 (0.09)	Tea (100)
▪ Flavanones	83.2 ± 76.6, (9.83)	58.1 ± 55.0 (9.35)	Oranges (71.3), natural orange juice (23.0), fruit juices from concentrate (3.22), other foods (2.09)
▪ Flavones	73.2 ± 47.4, (8.65)	54.7 ± 32.9 (8.81)	Whole-grain bread (30.0), bread (23.6), oranges (21.6), natural orange juice (8.53), artichoke (3.80), other foods (12.5).
▪ Flavonols	54.0 ± 22.3, (6.40)	35.6 ± 15.3 (5.73)	Onions (27.8), spinach (26.7), lettuce (11.9), red wine (6.02), olives (5.10), asparagus (4.93), other foods (17.55)
▪ Isoflavonoids	0.002 ± 0.004, (<0.01)	0.002 ± 0.003 (<0.01)	Beer (100)
Phenolic acids	280 ± 131, (33.1)	164.2 ± 70.8 (26.44)	
▪ Hydroxybenzoic acids	15.5 ± 10.3, (1.83)	20.5 ± 12.4 (3.30)	Red wine (21.2), olives (19.9), walnuts (18.1), tea (9.46), swiss chard leaves (6.15), white wine (1.34), other foods (23.8)
▪ Hydroxycinnamic acids	264 ± 129, (30.9)	141.6 ± 66.8 (22.80)	Decaffeinated coffee (37.7), coffee (26.1), plums (5.66), potatoes (5.50), olives (4.21), red wine (1.79), other foods (19.0)
▪ Hydroxyphenylacetic acids	0.90 ± 1.04, (0.10)	1.16 ± 1.40 (0.19)	Olives (87.2), red wine (6.57), beer (3.86), extra virgin olive oil (1.52), white wine (0.65)
▪ Hydroxyphenylpropanoic acids	0.48 ± 0.65, (0.06)	0.91 ± 1.23 (0.14)	Olives (100)
Stilbenes	2.13 ± 3.92, (0.25)	1.78 ± 3.19 (0.29)	Red wine (91.9), white wine (3.94), grapes (1.60), rosé wine (1.21), other foods (0.07)
Lignans	1.53 ± 0.56, (0.18)	1.33 ± 0.55 (0.21)	Extra virgin olive oil (16.7), seeds (9.84), oranges (9.73), green bean (5.42), pepper (5.32), peaches (4.97), broccoli (4.71), bread (4.48), red wine (4.16), cabbage (2.77), other foods (31.9)
Other polyphenols	70.8 ± 41.5, (8.37)	45.6 ± 27.8 (7.34)	
▪ Alkylmethoxyphenols	0.93 ± 0.87, (0.11)	0.93 ± 0.87 (0.15)	Decaffeinated coffee (74.1), coffee (16.2), beers (9.77)
▪ Alkylphenols	13.7 ± 17.8, (1.62)	13.8 ± 18.5 (2.23)	Whole-grain bread (69.1), whole-grain pastries (14.8), breakfast cereals (8.40), pasta (3.29), other foods (4.41)
▪ Furanocoumarins	0.37 ± 0.38, (0.04)	0.37 ± 0.39 (0.06)	Celery stalks (98.3), grapefruit juice (1.7)
▪ Hydroxybenzaldehydes	0.42 ± 0.65, (0.05)	0.42 ± 0.66 (<0.01)	Red wine (78.9), walnuts (14.5), beer (2.61), white wine (1.95), other foods (2.04)
▪ Hydroxybenzoketones	0.002 ± 0.004, (<0.01)	0.002 ± 0.003 (<0.01)	Beer (100)
▪ Hydroxycoumarins	0.10 ± 0.19, (0.01)	0.09 ±0.18 (<0.01)	Beer (73.6), white wine (26.3), cocoa powder (0.10)
▪ Methoxyphenols	0.13 ± 0.12, (0.01)	0.11 ± 0.12 (0.01)	Decaffeinated coffee (81.3), coffee (18.7)
▪ Naphtoquinones	0.82 ± 1.12, (0.09)	0.84 ± 1.14 (0.14)	Walnuts (100)
▪ Tyrosols	52.4 ± 37.8, (6.19)	30.0 ± 21.2 (4.83)	Olives (50.0), extra virgin olive oil (34.8), refined olive oil (5.17), red wine (3.29), other foods (6.74)
▪ Other	1.96 ± 2.30, (0.23)	0.66 ± 0.54 (0.11)	Orange juice (45.4), pears (18.2), coffee (16.0), other fruit juices (9.98), olives (5.86), other foods (4.56)

Table 3. Energy-adjusted intake of total polyphenol and their main subclasses according to sociodemographic and lifestyle characteristics.

	n	Total Polypenols (mg/d)	*p*	Flavonoids (mg/d)	*p*	Phenolic Acids (mg/d)	*p*	Stilbenes (mg/d)	*p*	Lignans (mg/d)	*p*	Other Polyphenols (mg/d)	*p*
Total population	6633	846 ± 275 [1]		491 ± 229		290 ± 127		2.13 ± 3.81		1.53 ± 0.54		70.8 ± 38.5	
Men	3424	830 ± 288	<0.001	469 ± 234	<0.001	285 ± 134	0.003	3.00 ± 4.74	<0.001	1.53 ± 0.54	0.933	72.1 ± 42.5	0.006
Women	3209	863 ± 259		515 ± 220		276 ± 118		1.21 ± 2.12		1.53 ± 0.53		69.5 ± 33.7	
Age (years)													
<65	3530	835 ± 275	0.002	476 ± 230	<0.001	285 ± 128	0.014	2.15 ± 4.03	0.605	1.51 ± 0.54	0.006	70.7 ± 39.2	0.967
65-70	2122	854 ± 271		503 ± 225		276 ± 123		2.07 ± 3.62		1.55 ± 0.52		71.0 ± 38.3	
>70	981	866 ± 281		517 ± 228		275 ± 127		2.21 ± 3.40		1.55 ± 0.54		70.8 ± 36.4	
BMI (Kg/m^2)													
<29.9	1762	847 ± 268	0.042	501 ± 225	0.004	272 ± 124	0.006	2.26 ± 3.85	<0.001	1.52 ± 0.49	0.679	69.9 ± 36.8	0.353
30-34.9	3258	852 ± 280		493 ± 232		284 ± 129		2.24 ± 3.90		1.53 ± 0.54		71.5 ± 39.7	
>35	1613	831 ± 270		475 ± 226		282 ± 124		1.77 ± 3.57		1.54 ± 0.57		70.5 ± 37.9	
Physical activity level													
Low	3953	833 ± 278	<0.001	480 ± 231	<0.001	280 ± 129	0.884	1.85 ± 3.48	<0.001	1.51 ± 0.54	<0.001	70.0 ± 38.5	0.034
Moderate	1253	861 ± 267		503 ± 217		282 ± 123		2.30 ± 3.79		1.55 ± 0.54		71.7 ± 36.6	
Active	1408	867 ± 271		510 ± 230		280 ± 123		2.76 ± 4.55		1.58 ± 0.53		72.8 ± 40.0	
Educational level													
Primary school	3266	834 ± 259	<0.001	482 ± 213	<0.001	278 ± 121	0.070	1.80 ± 3.38	<0.001	1.54 ± 0.55	0.093	70.9 ± 40.2	0.290
Secondary school	1913	840 ± 270		487 ± 227		279 ± 125		2.27 ± 3.98		1.51 ± 0.53		69.9 ± 38.1	
University	1450	880 ± 311		517 ± 260		287 ± 139		2.70 ± 4.40		1.55 ± 0.52		72.0 ± 35.0	
Smoking status													
Current smokers	821	841 ± 296	0.581	455 ± 243	<0.001	311 ± 143	<0.001	2.33 ± 4.43	0.114	1.47±0.53	<0.001	70.5 ± 46.3	0.768
Non-smokers	5812	847 ± 272		496 ± 226		276 ± 123		2.10 ± 3.72		1.54±0.54		70.9 ± 37.3	

[1] Mean ± Standard deviation. BMI: body mass index. Total and polyphenol subclasses were adjusted for total energy intake using the residual method. Comparison between subcategories was performed using ANOVA.

3. Results

The present study was conducted on 6633 participants from the PREDIMED-Plus study. The mean age was 65.0 ± 4.9 years, and mean BMI was 32.5 ± 3.44 kg/m^2. Table 1 shows the main characteristics of the participants according to quartiles of dietary total polyphenol intake. We observed that participants included in the highest quartile of polyphenol intake (>1019.3 mg/day) were mainly men and former smokers with a higher educational level (all three $p < 0.001$). We observed an inverse trend in the relationship between polyphenol intake and BMI ($p = 0.02$), whereas this trend was direct for waist circumference ($p = 0.01$) and body weight ($p < 0.001$). Moreover, fewer participants with insulin and nonsteroidal anti-inflammatory drug treatment were observed in the highest quartile of polyphenol intake (both $p = 0.01$).

Total polyphenol intake was 846 ± 318 mg/day, of which 58.0% were flavonoids (491 ± 253 mg/day), 33.1% phenolic acids (280 ± 131 mg/day), and the rest other polyphenols, stilbenes, and lignans (70.8 ± 41.5, 2.13 ± 3.92, and 1.53 ± 0.56 mg/day, respectively). The mean of the total polyphenol aglycone intake was 620.9 ± 273.5 mg/day. Table 2 shows the contribution (%) of each polyphenol subclass and polyphenol aglycones. The highest contributor to total polyphenol intake was hydroxycinnamic acids (30.9%). Regarding flavonoids, flavanols were the main contributors (24.1% from proanthocyanidins, 3.32% catechins, and 0.08% of theaflavins), followed by flavanones (9.83%), flavones (8.65%), flavonols (6.40%), and anthocyanins (5.14%). Additionally, tyrosols represented 6.19% of the total polyphenol intake, being the most abundant polyphenol classified within the group of other polyphenols.

The main food sources for each polyphenol subclass are also shown in Table 2. In the case of flavonoids, the most important contributors to the intake of proanthocyanidins were fruits and chocolate and its derivatives. Fruits (mainly oranges and orange juice) were the greatest contributors of flavanones, while vegetables (mainly onion, spinach, and lettuce) were the greatest contributors of flavones. Red wine, olives, tea, and wholegrain cereals were also important contributors to the remaining subclasses. Coffee was the most significant contributor of phenolic acids, especially of hydroxycinnamic acids, followed by olives and red wine. Stilbenes were mainly provided by red wine (91.9%). Lignans were widely distributed among foods, with extra virgin olive oil, fruits, and vegetables the main contributors. The main contributors of other polyphenols were olives, olive oil, cereals, coffee, and alcoholic beverages (mainly beer and red wine).

Table 3 shows the energy-adjusted intake of total polyphenols and the main subclasses by sex, age, BMI, level of physical activity, educational level, and smoking status. Total polyphenol intake was significantly higher in women due to their high intake of flavonoids ($p < 0.001$), whereas men consumed more phenolic acids ($p = 0.003$), stilbenes, and other polyphenols. The intake of total polyphenols, flavonoids, and lignans increased with age ($p = 0.002$, $p < 0.001$, and $p = 0.006$, respectively). Interestingly, participants with the highest BMI (>35 kg/m^2) showed the lowest total polyphenol ($p = 0.042$), flavonoid ($p = 0.004$), and stilbene intake ($p < 0.001$), whereas phenolic acid intake was significantly higher in this group ($p = 0.006$). The level of physical activity was directly associated with total polyphenol intake ($p < 0.001$) and with all polyphenol classes except for phenolic acids ($p < 0.001$ in all cases except $p = 0.03$ for other polyphenols). Participants with a higher educational level (high school) showed higher total polyphenol, flavonoid, and stilbene intake ($p < 0.001$ in all cases). Current smokers reported a significantly higher intake of coffee than non-smokers ($p < 0.001$) and, consequently, showed a significantly higher intake of phenolic acids ($p < 0.001$). Otherwise, the smokers group showed significantly lower intake of flavonoids and lignans than their counterparts ($p < 0.001$, both).

The associations between dietary polyphenol intake and MetS components after full adjustment are shown in Figure 2. High flavonoid and low phenolic acid intake were associated with lower waist circumference ($p = 0.02$ and $p < 0.001$, respectively). The highest intake of other polyphenols was significantly and inversely associated with systolic ($p = 0.001$) and diastolic blood pressure levels ($p = 0.002$). An inverse association was found between fasting plasma glucose levels and lignans ($p = 0.04$). Positive associations were found between HDL-c levels and all polyphenol classes except for phenolic acid and lignan intake. Lastly, triglyceride concentration was inversely associated with

lignans and stilbenes ($p = 0.006$ and $p = 0.004$, respectively). Changes in the linear regression models after adjustment are shown in the Supplementary Table (Supplementary Table S1).

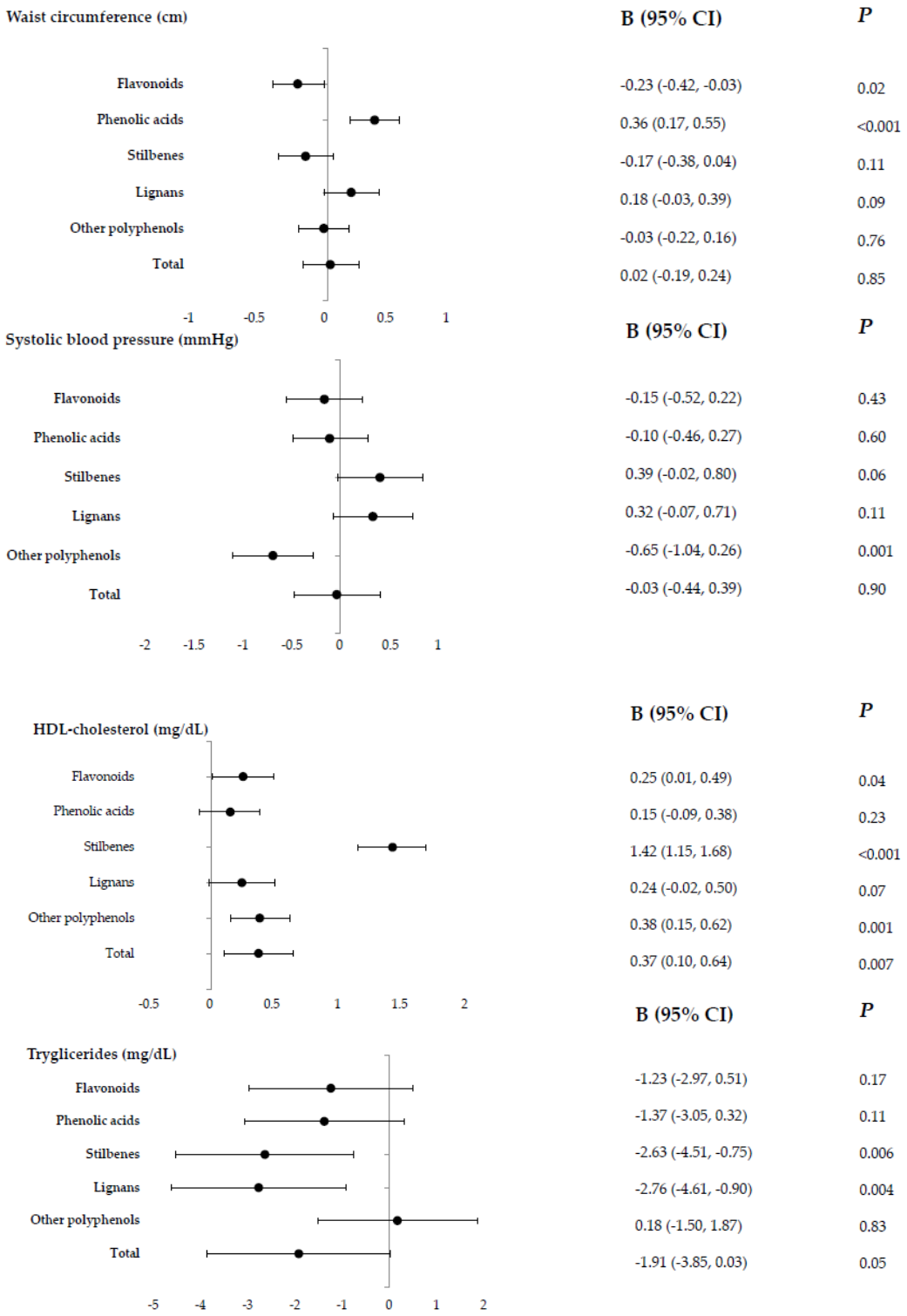

Figure 2. Energy-adjusted subclasses of dietary polyphenol intake by metabolic syndrome components (standardized β-coefficients [95% Confidence Intervals]).

4. Discussion

In this cross-sectional study of the PREDIMED-Plus study, we showed that high intake of some polyphenol subclasses was inversely associated with levels of the MetS components. These associations were especially observed for the subclasses whose contribution to total polyphenol intake was lower, such as other polyphenols, lignans, and stilbenes. Previous epidemiological studies have investigated the association between dietary polyphenol intake and MetS components in healthy populations or those at high risk of CVD, but to our knowledge there are no previous studies on these associations in subjects previously diagnosed with MetS.

In our study, the polyphenol intake was 846 ± 318 mg/day, and the intake was highest for flavonoids (58% of total), followed by phenolic acids (33.1%), similar to results of other Spanish cohorts [14,18]. By contrast, the total polyphenol intake was considerably lower than the intake observed in Mediterranean countries of the EPIC Study (1011 mg/day) [19], the SU.VI.MAX cohort study (1193 mg/day) [20], and the data from other studies conducted in non-Mediterranean countries, such as the UK National Diet and Nutrition Survey Rolling Programme for participants of similar age (1053 mg/day) [21]. The main noteworthy difference between our results and those of other countries was the relevant contribution of seeds, olives and olive oil, and red wine [14,20], while coffee, tea, and cocoa products are the foods most commonly observed in non-Mediterranean countries [22–24].

In addition to the differences observed according to geographical location and dietary habits, sociodemographic and lifestyle habits significantly influence the quantity and profile of intake of polyphenol subclasses. The intake of total polyphenols, particularly flavonoids and lignans, increased with age compared to younger participants (<65 years), although Grosso et al. reported the opposite observation [23]. In addition, BMI was inversely associated with total polyphenol intake, mainly with lower flavonoid and stilbene intake. This finding was also reported in the TOSCA.IT and EPIC studies [19,25].

The intake of polyphenol subclasses has been reported to have an impact on MetS components [26,27]. Even though flavonoids were the principal contributors of total polyphenol intake in our study, no associations were found with any of the MetS components, except for an inverse association with waist circumference. Similar findings were described in the HELENA study [28], where flavonoid intake was associated with lower BMI. Research on the mechanisms of action involved in the anti-obesogenic properties of flavonoids suggests that the improvements in glucose homeostasis are promoted by reducing insulin resistance and decreasing oxidative stress levels [29]. Phenolic acid intake was associated with higher fasting plasma glucose levels and waist circumference. These results are opposite from those observed in the HAPIEE cohort study, which described the beneficial effects of phenolic acid on the overall risk of developing MetS and lowering blood pressure [30]. Nevertheless, it must be taken into account that the dietary intake of phenolic acids and total polyphenol in the mentioned study doubled the amount estimated in our results, probably because of the higher intake of tea and its contribution to phenolic acid intake compared to our study population [23]. In Mediterranean countries, dietary intake of stilbenes is relatively high compared to other countries [19], with red wine being their main source (>90%). In this setting, higher stilbene intake was associated with higher HDL-c levels, but since HDL-c is the best-established cardiovascular protective factor by alcohol consumption, we cannot exclude that the alcohol content of red wine may interfere with this result [31]. In the PREDIMED study, the intake of red wine was associated with improvements in four out of five MetS criteria (i.e., elevated abdominal obesity, low HDL-c levels, high blood pressure, and high fasting plasma glucose levels) [32]. Other studies also found an inverse association between abdominal adiposity and stilbene intake, BMI, and waist circumference [30,33]. As a mechanistic pathway for stilbenes, resveratrol has shown potential anti-obesogenic effects decreasing adipocyte proliferation while activating lipolysis and β-oxidation [34]. However, the association with lower body weight and waist circumference observed in the present study and the promising effects against obesity associated with polyphenol intake observed in other studies were not clinically relevant [35]. Our results showed an inverse association between fasting glucose and lignans, and

an increase in HDL-c levels and lower levels of systolic and diastolic blood pressure measurements for other polyphenols. The same finding was described in a Brazilian cohort for hypertension and other polyphenols [36]. In contrast with our results, flavonoids, mainly anthocyanins, showed greater antihypertensive effects in another study [37]. Finally, the association between lignan intake and fasting glucose levels was not demonstrated to be linked with the diagnosis of type 2 diabetes (T2D) in the EPIC study [38], but this inverse association aligned with the results observed in the PREDIMED cohort and PREDIMED-Plus study [39,40]. The potential mechanism of action underlying this association might be explained by the improvements observed in gut microbiota. This assumption was also observed in a study of US women [41], showing an inverse association between levels of gut microbiota metabolites from dietary lignan intake and T2D incidence.

Interestingly, in our study we found an association between intake of all polyphenol subclasses except phenolic acids and lignans and higher HDL-c levels. These associations were also found with total polyphenol intake in the TOSCA.IT study in T2D subjects [25] and in a similar cohort of participants at high cardiovascular risk [42]. We also observed that triglyceride levels were inversely associated with stilbene and lignan intake. Despite the fact that the antioxidant properties of polyphenols for the prevention of LDL-c oxidation are well described, the effects of dietary polyphenols on the reduction of total cholesterol levels or triglycerides are controverted [43].

The major strengths of the present study are its large sample size, the multicenter design, and the use of the Phenol-Explorer as the most comprehensive food composition database on dietary polyphenols [15]. In prior studies, the FFQ was validated to evaluate total polyphenol intake in both clinical and cross-sectional studies [44]. Our study has also some limitations. First, it used a cross-sectional design which does not allow attributing conclusions to plausible causes. In order to establish causality, a randomized controlled trial based on the intake of different polyphenol subclasses should be performed. Second, potential residual confounding and the lack of generalizability of the results to other populations than middle-aged to elderly people with higher BMI and MetS are limitations. Third, the use of the FFQ may have led to a misclassification of the exposure due to self-reported information of food intake and to the fact that some polyphenol-rich foods are grouped in the same item (e.g., spices). Nevertheless, the FFQ used has been validated in the adult Spanish population and showed good reproducibility and validity [45]. Fourth, other factors that affect food polyphenol content, such as bioavailability, variety, ripeness, culinary technique, storage, region, and environmental conditions, were not collected.

Even though recent research postulates that polyphenols are effective in improving MetS, no single phenolic compound or food has an impact on all the MetS components, suggesting that healthy and polyphenol-rich dietary patterns such as the MedDiet may be an adequate strategy for MetS management. This research might be useful for setting dietary and health counseling for MetS patients, especially those with low HDL-c levels. The use of a consensus methodology and polyphenol database might facilitate this in future studies. Future large-scale clinical trials are needed to clarify the underlying mechanisms of action and establish safe doses for the potential health effects described.

5. Conclusions

This study provides detailed information about the relationship between polyphenol intake and the components of MetS in a population of overweight or obese adults. Higher intake of all the subclasses of polyphenols was associated with a better profile of the components of MetS, especially with HDL-c levels.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/12/3/689/s1, Table S1: Energy-adjusted sub-classes of dietary polyphenol intake by metabolic syndrome criterias.

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Manuscript II.

Title: "Associations between Dietary Polyphenols and Type 2 Diabetes in a Cross-Sectional Analysis of the PREDIMED-Plus Trial: Role of Body Mass Index and Sex"

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Associations between Dietary Polyphenols and Type 2 Diabetes in a Cross-Sectional Analysis of the PREDIMED-Plus Trial: Role of Body Mass Index and Sex

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Abstract: Overweight and obesity are important risk factors for type 2 diabetes (T2D). Moving towards healthier diets, namely, diets rich in bioactive compounds, could decrease the odds of suffering T2D. However, those individuals with high body mass index (BMI) may have altered absorption or metabolism of some nutrients and dietary components, including polyphenols. Therefore, we aimed to assess whether high intakes of some classes of polyphenols are associated with T2D in a population with metabolic syndrome and how these associations depend on BMI and sex. This baseline cross-sectional analysis includes 6633 participants from the PREDIMED-Plus trial. Polyphenol intakes were calculated from food frequency questionnaires (FFQ). Cox regression models with constant time at risk and robust variance estimators were used to estimate the prevalence ratios (PRs) for

polyphenol intake and T2D prevalence using the lowest quartile as the reference group. Analyses were stratified by sex and BMI groups (overweight and obese) to evaluate potential effect modification. Catechins, proanthocyanidins, hydroxybenzoic acids, and lignans were inversely associated with T2D. Hydroxycinnamic acids were directly related in men. These associations were different depending on sex and BMI, that is, women and overweight obtained stronger inverse associations.

Keywords: diet; obesity; flavonoids; catechins; proanthocyanidins; hydroxybenzoic acids; hydroxycinnamic acids; lignans; phenolic acids

1. Introduction

The number of people with diabetes has been increasing over the past few decades and the estimations for the near future are not encouraging. Currently, 8.5% of adults have diabetes worldwide, type 2 diabetes (T2D) being the most prevalent type. People with T2D have increased odds of premature death and are more likely to have myocardial infarction or angina, stroke, kidney failure, peripheral artery disease, vision loss, and neuropathy. Therefore, T2D may be considered a global public health problem with vast economic consequences [1].

Unlike type 1 diabetes, T2D can be prevented through different approaches, namely, exercising regularly, avoiding smoking, keeping weight under control, and adhering to a healthy diet. There is compelling evidence from prospective observational studies and clinical trials that individual nutrients, foods, and dietary patterns are crucial in the prevention and management of T2D. It is well known that plant-based diets including whole grains, fruits, vegetables, legumes, and nuts as well as moderate alcohol consumption have been associated with lowering the risk of T2D, whereas diets rich in refined grains, red or processed meats, and sugar-sweetened beverages increase the risk [2]. Plant foods are usually rich in polyphenols, a large and heterogeneous group of bioactive compounds that constitute the first source of antioxidants in the diet. Polyphenols are usually classified according to their structure into two main groups—flavonoids and non-flavonoids. The flavonoid group, with C6–C3–C6 structured compounds, includes flavones, flavonols, theaflavins, catechins, proanthocyanidins (polymeric forms), flavanones, anthocyanidins, and isoflavones. Phenolic acids, lignans, and stilbenes belong to the non-flavonoid group. High polyphenol intake has been associated with a reduced incidence of T2D in epidemiological studies [3], and better metabolic control in those participants with T2D. Some of the mechanisms by which dietary polyphenols and their metabolites exert their effects have been elucidated but others are still unknown [4].

Overweight and obese individuals are at higher risk of T2D than lean ones, therefore it seems that they would particularly benefit from polyphenol intake. However, some literature suggest that those with higher body mass index (BMI) may have altered absorption or metabolism of some nutrients and dietary components, including polyphenols [5,6].

Due to the heterogeneity of polyphenols, and to understand their antidiabetic properties, it is necessary to study them considering the different groups separately, because they have different absorption and metabolism [7]. Epidemiological and clinical studies have usually been focused on some polyphenolic groups, but comprehensive studies are still scarce. Moreover, to our knowledge, no previous studies have examined the associations between the intake of all polyphenolic groups and subgroups and the risk of T2D stratifying by sex and BMI. Therefore, our aim was to examine the hypothesis that higher intakes of some classes of polyphenols are associated with T2D in a population with metabolic syndrome and these associations depend on sex and BMI.

2. Materials and Methods

2.1. Study Design and Population

The present study was designed as a baseline cross-sectional analysis in the PREDIMED-Plus trial, a six-year parallel-group, multicenter, randomized, lifestyle intervention study for the primary prevention of cardiovascular disease involving 6874 participants recruited in 23 Spanish recruiting centers from October 2013 to December 2016. Eligible participants were men (aged 55–75 years) and women (aged 60–75 years) with a BMI between 27 and 40 kg/m^2 who met, at least, three components of the metabolic syndrome (updated harmonized criteria of the International Diabetes Federation and the American Heart Association and National Heart, Lung, and Blood Institute).

Details on the PREDIMED-Plus protocol can be found at http://predimedplus.com. The selection and the description of the studied sample have been previously reported [8]. The PREDIMED-Plus trial was registered at the International Standard Randomized Controlled Trial (ISRCTN89898870; registration date, 24 July 2014). All participants provided written informed consent, and the study protocol and procedures were approved according to the ethical standards of the Declaration of Helsinki by all the participating institutions: CEI Provincial de Málaga, CEI de los Hospitales Universitarios Virgen Macarena y Virgen del Rocío, CEI de la Universidad de Navarra, CEI de las Illes Balears, CEIC del Hospital Clínic de Barcelona, CEIC del Parc de Salut Mar, CEIC del Hospital Universitari Sant Joan de Reus, CEI del Hospital Universitario San Cecilio, CEIC de la Fundación Jiménez Díaz, CEIC Euskadi, CEI en Humanos de la Universidad de Valencia, CEIC del Hospital Universitario de Gran Canaria Doctor Negrín, CEIC del Hospital Universitario de Bellvitge, CEI de Córdoba, CEI de Instituto Madrileño De Estudios Avanzados, CEIC del Hospital Clínico San Carlos, CEI Provincial de Málaga, CEI de las Illes Balears, CCEI de la Investigación Biomédica de Andalucía and CEIC de León. The code of the Etical Committe aproval of the Coordinated Center (CEIC del Hospital Universitari Sant Joan de Reus) is 13-07-25/7proj2 (aproval date: 30/07/2013).

After the exclusion of 53 participants with missing baseline dietary data and 188 with extreme energy intakes (<500 or >3500 for women and <800 and >4000 for men) [9], 6633 participants were available for the present analysis.

2.2. Dietary Assessment and Polyphenol Intake

At baseline, participants filled out a validated 143-item semi-quantitative food frequency questionnaire (FFQ) [10] under the supervision of registered dietitians, from which total energy and nutrient intake were calculated on the basis of Spanish food composition tables [11,12]. Additionally, they were asked to fill out a 17-point score questionnaire measuring adherence to an energy-restricted traditional Mediterranean diet (MedDiet), an updated version of the 14-point score questionnaire used in the PREDIMED trial [13].

The 143-item FFQ was also used to calculate polyphenol intake together with the Phenol-Explorer database (www.phenol-explorer.eu). The validity of this particular FFQ to assess total polyphenol intake was tested in both clinical and cross-sectional studies [14,15]. Individual polyphenol intakes were obtained by multiplying the content of each polyphenol in each food item with polyphenols (mg/g) by the daily consumption of this food item (g/day) and then summing the product across all food items. Then, polyphenol subclasses were adjusted for total energy intake using the residual method [9]. After stratification by sex and BMI groups, namely, overweight (BMI < 30 kg/m^2) and obese (BMI ≥ 30 kg/m^2), polyphenol intakes were divided in quartiles.

2.3. Ascertainment of T2D

For the present analysis, the main endpoint was the prevalence of T2D, defined as previous clinical diagnosis of T2D, or glycated hemoglobin (HbA1c) ≥ 6.5%, or use of antidiabetic medication at baseline, or fasting plasma glucose > 126 mg/dl in both the screening visit and baseline visit.

2.4. Assessment of Covariates

Participants filled out a general questionnaire to provide data on lifestyle habits, education, concurrent diseases, and medication use. Physical activity was measured by a short Minnesota Leisure Time Physical Activity Questionnaire validated for the Spanish population [16]. Height and weight were measured by trained staff. BMI was calculated as weight in kilograms by the square of height in meters. After an overnight fasting, blood samples were collected. Serum glucose, triglyceride, as well as total and high-density lipoprotein (HDL) cholesterol levels were measured by routine laboratory tests using standard enzymatic methods.

2.5. Statistical Methods

Baseline characteristics according to quartiles of total polyphenol intake are presented as means (±SD) for quantitative variables and frequencies for categorical variables. One-factor ANOVA tests were used to assess the differences between quartiles of polyphenol consumption and chi-squared tests for categorical variables.

We used Cox regression models with constant time at risk and robust variance estimators to obtain the prevalence ratios (PRs) for polyphenol intake and T2D prevalence using the lowest quartile as the reference group [17]. We ran stratified analyses for sex and BMI groups (overweight and obese) to evaluate potential effect modification.

In multivariate models, we adjusted for age (continuous), smoking status (never, current, or former), physical activity (quintiles), and education (primary education, secondary education, academic/graduate) in model 1. In the fully adjusted model, we additionally adjusted for energy intake (continuous) as well as consumption of refined cereals and animal products (derived from the 17-point questionnaire) and of distilled beverages and liquors, sugar, soft drinks, cookies, and pastries (in g/day derived from the FFQ). All models were stratified by the recruiting center.

Interaction effect was calculated comparing the models, with and without the interaction term, with likelihood-ratio tests after estimation. We tested for linear trends across categories of polyphenol intake by assigning each participant the median value for each category and modelling this value as a continuous variable. Statistical analyses were conducted using STATA software (version 15.1; StataCorp, College Station, TX, USA). All *t*-tests were two-sided and *p*-values below 0.05 were considered significant.

3. Results

The present study was conducted on 6633 participants from the PREDIMED-Plus cohort, 51.6% men, with a mean age of 65.0 ± 4.9 years. Of these, 2042 had T2D at baseline (30.8%). Mean total polyphenol intake was 846 ± 275 mg/day. Approximately 58% corresponded to flavonoids, 33% were phenolic acids, and the rest were stilbenes, lignans, and other polyphenols. The flavonoid group was mainly composed of proanthocyanidins (205 ± 175 mg/day), flavanones (86 ± 75 mg/day), flavones (72 ± 45 mg/day), flavonols (54 ± 22 mg/day), anthocyanidins (44 ± 36 mg/day), and catechins (28 ± 22 mg/day). Other flavonoid subclasses, namely, chalcones, dihydrochalcones and dihydroflavonols, theaflavins, and isoflavonoids, contributed less than 1% and were not considered in the analyses. Within phenolic acids, hydroxycinnamic acids were the main subclass (>90%) and hydroxybenzoic acids followed them with 6%. Other minor phenolic acids were also not considered. Intake of energy-adjusted polyphenols by sex and BMI group is shown in Table S1, Supplementary Materials. Men tended to have higher intakes of total polyphenols and total phenolic acids, as well as anthocyanidins, catechins, hydroxycinnamic and hydroxybenzoic acids, stilbenes, and lignans, whereas women had higher intakes of flavones. Considering BMI groups, overweight men had higher intakes of total flavonoids and proanthocyanidins, but lower intakes of total phenolic acids and hydroxycinnamic acids compared to obese men. On the other hand, women within the overweight group had higher intakes of hydroxybenzoic acids and stilbenes but lower intakes of hydroxycinnamic acids.

Table 1 shows baseline participant characteristics by quartiles of energy-adjusted total polyphenol intake. At baseline, participants in the highest quartile were more likely to be men, physically active, smokers and former smokers, and with a higher education level. The same characteristics are summarized according to sex and BMI groups in Table S2, Supplementary Materials. In this cohort, men were younger (because of the inclusion criteria), had higher BMI, were more physically active, and were more highly educated than women. Within the group of men, there were more smokers, the prevalence of T2D was higher, as well of T2D treatment users. Their fasting glucose levels were more elevated, but not HbA1c. Overweight men and women had lower prevalence of T2D (although not significant in the case of men), lower fasting glucose levels, were more physically active, and were more educated. Moreover, overweight men were older, and overweight women used less metformin, had lower levels of HbA1c, and smoked more.

Table 1. Baseline characteristics by quartiles of energy-adjusted polyphenol intake (n = 6633).

Characteristics	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-Value
n	1659	1658	1658	1658	
Total polyphenols (mg/day), median (min–max)	561 (73–662)	736 (663–812)	896 (813–994)	1146 (995–3377)	
Sex, males, n (%)	775 (46.7)	871 (47.5)	775 (53.3)	679 (59.1)	<0.001
Age (years)	64.9 ± 4.9	65.0 ± 4.8	65.0 ± 4.9	65.1 ± 5.0	0.65
Body mass index (kg/m^2)	32.6 ± 3.5	32.6 ± 3.5	32.6 ± 3.5	32.4 ± 3.3	0.07
Diabetic, n (%)	489 (29.5)	523 (31.5)	519 (31.3)	511 (30.8)	0.57
Insulin, n (%)	82 (4.9)	88 (5.3)	76 (4.6)	68 (4.1)	0.40
Metformin, n (%)	356 (21.5)	405 (24.4)	394 (23.8)	389 (23.5)	0.21
Other glucose lowering medication, n (%)	306 (18.4)	342 (20.6)	341 (20.6)	336 (20.3)	0.35
Fasting glucose (mg/dL)	113 ± 29	113 ± 29	114 ± 31	113 ± 28	0.59
Glycated hemoglobin (mmol/L)	6.1 ± 0.9	6.1 ± 0.8	6.1 ± 1.0	6.1 ± 0.8	0.54
Physical activity, (METS·min/week)	2252 ± 2216	2434 ± 2249	2437 ± 2275	2733 ± 2435	<0.001
Smoking status, n (%)					
Smoker	231 (13.9)	235 (14.2)	242 (14.6)	258 (15.6)	<0.001
Former, >1 year	623 (37.6)	642 (38.7)	709 (42.8)	762 (46.0)	
Never	804 (48.5)	780 (47.0)	706 (42.6)	638 (38.5)	
Education level, n (%)					
High	308 (18.6)	319 (19.2)	363 (21.9)	464 (28.0)	<0.001
Medium	484 (29.2)	467 (28.2)	485 (29.3)	477 (28.8)	
Low	867 (52.3)	872 (52.6)	810 (48.8)	717 (43.2)	

Values are frequencies and percentages for categorical variables or means ± SD for continuous variables, except for polyphenol intake which is represented by medians (min–max). p-values were calculated by chi-squared tests for categorical variables and ANOVA for continuous variables.

In Table 2, we summarize nutrient and main food group consumption according to energy-adjusted quartiles of polyphenol intake. Those in the fourth quartile tended to have a healthier diet in general, with higher consumption of fruits, vegetables, and nuts, and lower consumption of cereals, dairy products, meat, and sugar. They also presented higher intake of total carbohydrates and fiber, and lower intake of fatty acids compared to those in the reference quartile. However, they also had greater intake of alcohol, soft drinks, and cookies, pastries, and sweets, and lower consumption of olive oil. Nutrient composition of the diet and main food items according to sex and BMI groups are presented in Table S3, Supplementary Materials. There were no significant differences in total caloric intake between overweight and obese, men or women. Generally, women had healthier diets (higher score in the 17-point MedDiet questionnaire), with greater consumption of fiber, vegetables, dairies, and fish, and less alcohol. No differences were observed for legumes and nuts between sex. Obese men also reported to consume more meat, but less sugar than overweight men. On the other hand, obese

women had poorer Mediterranean diet adherence, with less consumption of polyunsaturated fatty acids (PUFAs) and nuts, and less alcohol.

Table 2. Main dietary nutrient and food consumption according to quartiles of energy-adjusted polyphenol intake at baseline (n = 6633).

Nutrients and Foods	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*p*-Value
n	1659	1658	1658	1658	
Total energy (kJ/day) (kcal/day)	10272 ± 2293 (2454 ± 548)	9481 ± 2256 (2265 ± 539)	9670 ± 2293 (2310 ± 548)	10180 ± 2294 (2432 ± 548)	<0.001
Carbohydrates (g/day)	246 ± 75	229 ± 70	237 ± 71	251 ± 73	<0.001
Fiber (g/day)	23 ± 7	25 ± 8	27 ± 8	30 ± 10	<0.001
Proteins (g/day)	100 ± 22	95 ± 22	96 ± 22	99 ± 22	<0.001
MUFAs (g/day)	57 ± 16	52 ± 16	52 ± 16	54 ± 16	<0.001
PUFAs (g/day)	18 ± 7	16 ± 6	16 ± 6	17 ± 6	<0.001
SFAs (g/day)	28 ± 9	25 ± 8	25 ± 8	26 ± 9	<0.001
Alcohol (g/day)	10 ± 14	10 ± 14	11 ± 14	14 ± 17	<0.001
17-point MedDiet score	7.7 ± 2.5	8.5 ± 2.6	8.7 ± 2.6	9.2 ± 2.7	<0.001
Food items (g/day)					
Vegetables	299 ± 125	319 ± 129	336 ± 134	357 ± 160	<0.001
Fruits	262 ± 146	324 ± 160	381 ± 181	470 ± 261	<0.001
Legumes	21 ± 11	21 ± 11	20 ± 11	21 ± 12	0.23
Cereals	164 ± 83	144 ± 74	147 ± 77	146 ± 77	<0.001
Dairy	358 ± 205	338 ± 193	341 ± 196	347 ± 211	0.02
Meat	160 ± 62	145 ± 58	143 ± 56	143 ± 56	<0.001
Olive oil	43 ± 18	39 ± 17	39 ± 16	39 ± 16	<0.001
Fish	101 ± 50	101 ± 48	102 ± 45	105 ± 47	0.05
Nuts	13 ± 16	14 ± 17	15 ± 17	17 ± 19	<0.001
Cookies, pastries, and sweets	27 ± 31	23 ± 28	25 ± 28	32 ± 32	<0.001
Sugar	8 ± 13	7 ± 11	6 ± 11	6 ± 12	<0.001
Soft drinks	31 ± 82	20 ± 56	20 ± 62	16 ± 51	<0.001

Values are means ± SD. *p*-values were calculated by ANOVA tests. Monounsaturated fatty acids, MUFAs; polyunsaturated fatty acids, PUFAs; saturated fatty acids, SFAs; Mediterranean diet, MedDiet.

We performed Cox proportional models with constant time to study the association between T2D prevalence and quartiles of the following main polyphenol groups: flavonoids (proanthocyanidins, flavanones, flavones, flavonols, anthocyanidins, and catechins), phenolic acids (hydroxycinnamic and hydroxybenzoic acids), stilbenes, and lignans. The results of the fully adjusted model comparing the fourth versus the first quartiles for men and women are shown in Figure 1. Significant inverse and linear associations were found for catechins, hydroxybenzoic acids, and lignans for both men and women. Proanthocyanidin intake was also inversely associated with T2D, but in men the linearity was not significant. These associations were always stronger for women. On the other hand, hydroxycinnamic acids showed a strong direct association with T2D prevalence in men, but marginally significant in the case of women.

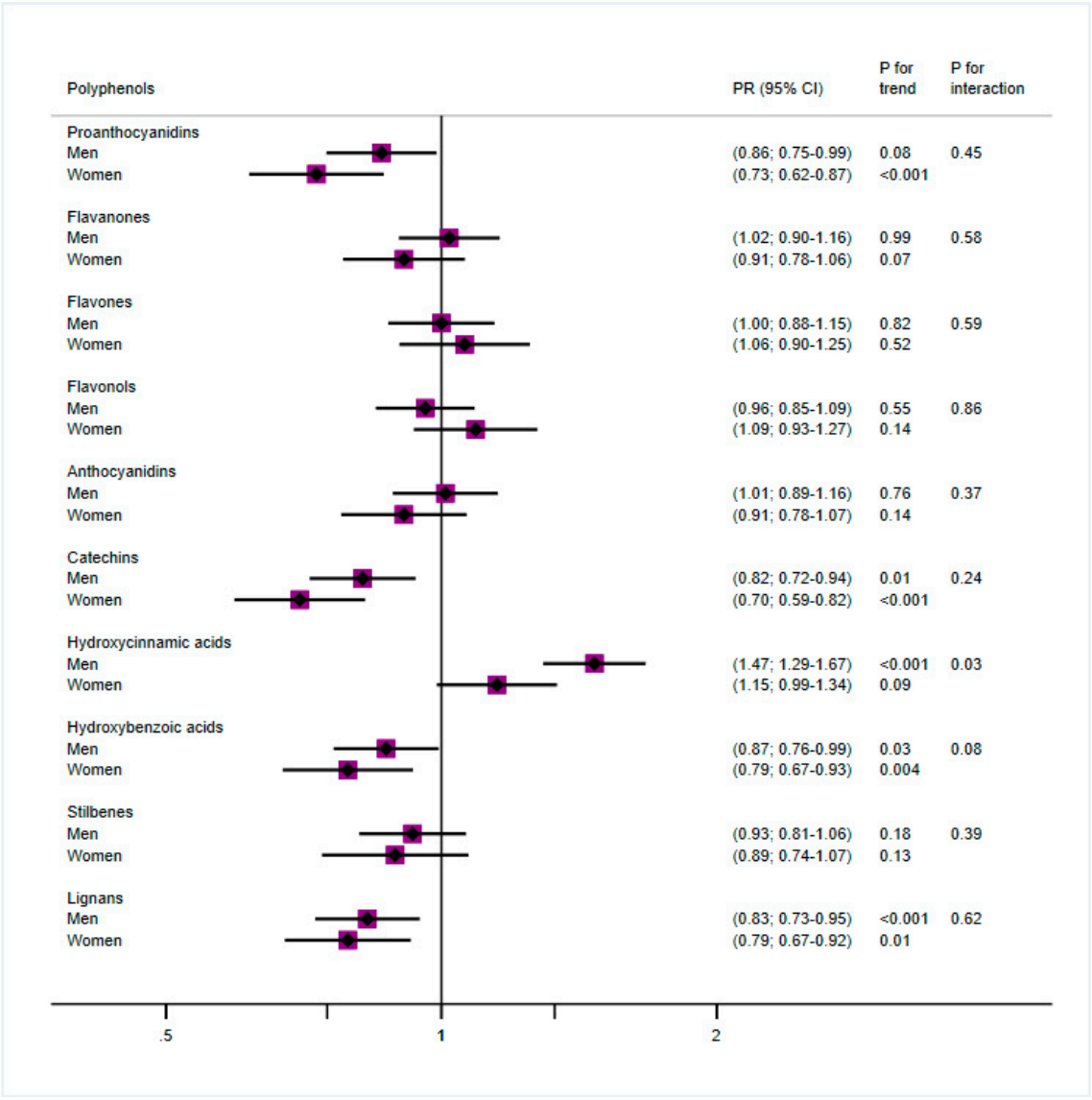

Figure 1. Cox proportional models for polyphenol groups and type 2 diabetes (T2D) by sex, comparing quartile 4 (Q4) vs. quartile 1 (Q1) and adjusted for age, education (basic studies, medium, high), body mass index (BMI) (overweight, obese), smoking (never, former, smoker), physical activity (quintiles), energy, consumption of refined cereals and animal products (from 17-point questionnaire) and of distilled beverages and liquors, sugar, soft drinks, cookies, and pastries (g/day), and stratified by recruitment center.

Because weight is an important risk factor for T2D, we also performed the same analysis but dividing the population in two groups according to the BMI—overweight (BMI < 30 kg/m^2), and obese (BMI ≥ 30 kg/m^2). In our cohort, three out of four participants were obese. The prevalence of T2D in the groups with obesity compared to the overweight groups was higher in both men (34% vs. 32%) and women (29% vs. 24%).

Figure 2 shows the results after adjustment for anthropometric, sociodemographic, lifestyle, and dietary variables (fully adjusted model) and stratifying by recruitment center, comparing the fourth versus the first quartiles separated by BMI groups. Significant inverse and linear associations were found for proanthocyanidins, anthocyanidins (only in overweight), catechins, hydroxybenzoic acids, stilbenes (only in obese), and lignans. In all of them, the PR was lower in overweight than in obese. Hydroxycinnamic acid intake was directly associated with T2D in both groups.

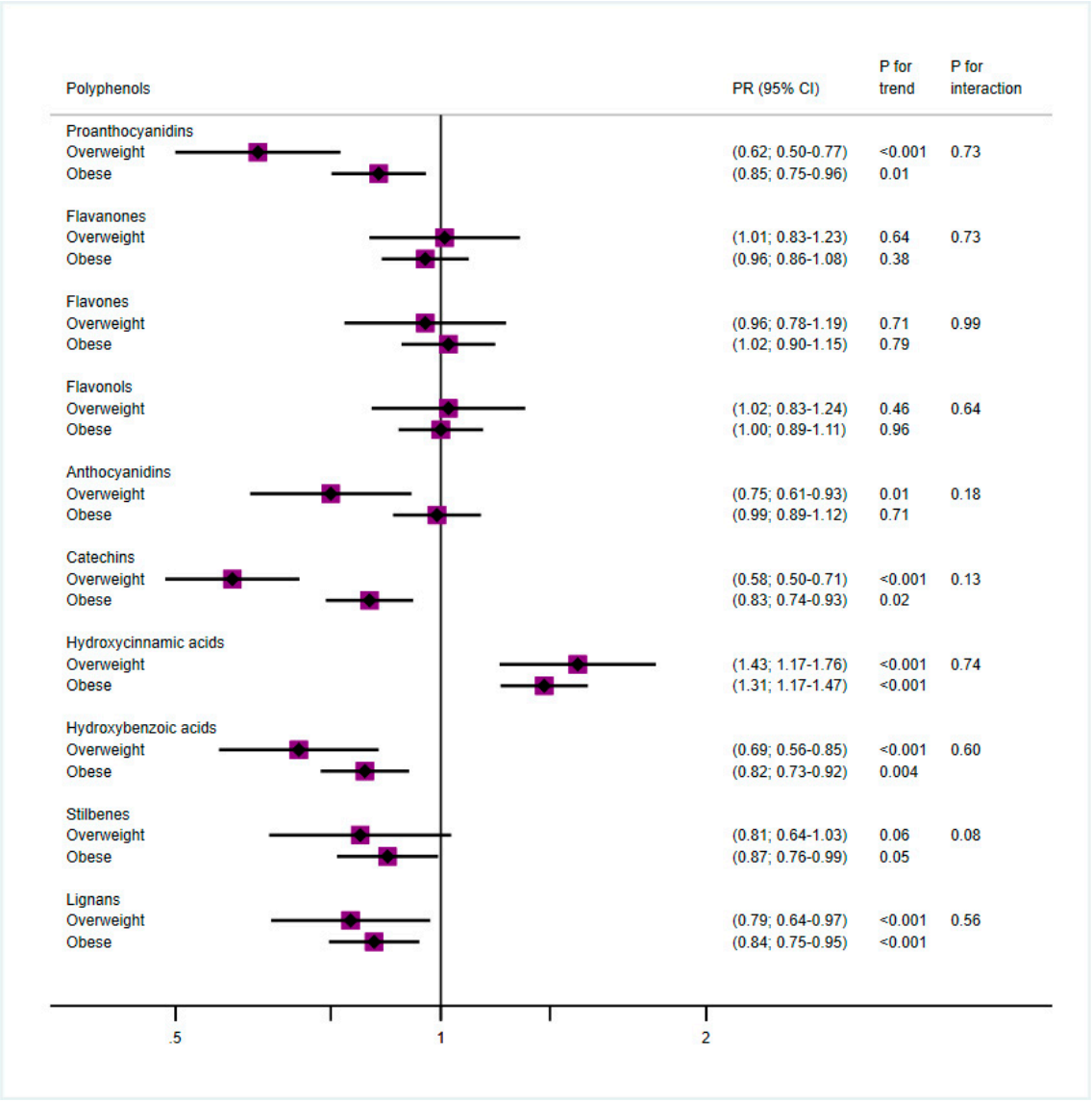

Figure 2. Cox proportional models for polyphenol groups and T2D by BMI groups, comparing Q4 vs. Q1 and adjusted for sex, age, education (basic studies, medium, high), smoking (never, former, smoker), physical activity (quintiles), energy, consumption of refined cereals and animal products (from 17-point questionnaire) and of distilled beverages and liquors, sugar, soft drinks, cookies, and pastries (g/day), and stratified by recruitment center.

Results of the fully adjusted model according to sex and BMI groups are shown in Table 3. We found similar patterns for proanthocyanidins, catechins, and hydroxybenzoic acids. In all cases, there were significant and linear inverse associations comparing extreme quartiles for overweight men and women, and for obese women, but not for obese men. The prevalence ratios were lower for the overweight than the obese. Regarding lignans, inverse associations were significant for all groups except for overweight women. Additionally, we found a significant and inverse linear trend for anthocyanidins and stilbenes in overweight men, although the PR did not reach significance.

Table 3. Prevalence ratio (PR) and confidence interval (CI) for prevalence of T2D and groups of energy-adjusted polyphenol intake by sex and BMI.

		Men						**Women**					
		Overweight (BMI < 30) *n* = 971 (314 cases, 32%)			Obese (BMI ≥ 30) *n* = 2453 (828 cases, 34%)			Overweight (BMI < 30) *n* = 802 (194 cases, 24%)			Obese (BMI ≥ 30) *n* = 2407 (706 cases, 29%)		
		Q4 vs. Q1	*p*-value	*p*-trend	Q4 vs. Q1	*p*-value	*p*-trend	Q4 vs. Q1	*p*-value	*p*-trend	Q4 vs. Q1	*p*-value	*p*-trend
Proanthocyanidins	Mean intake, mg/day	458.9 vs. 63.5			434.2 vs. 53.1			435.3 vs. 57.9			415.3 vs. 51.4		
	Cases	69 vs. 87			179 vs. 188			33 vs. 54			153 vs. 190		
	PR (CI)—unadjusted	0.81 (0.62–1.05)	0.11	0.18	0.96 (0.67–1.14)	0.67	0.79	0.62 (0.42–0.91)	0.02	0.01	0.81 (0.67–0.97)	0.02	0.02
	PR (CI)—model 1	0.81 (0.63–1.05)	0.12	0.20	0.98 (0.83–1.17)	0.83	0.95	0.64 (0.43–0.94)	0.02	0.01	0.82 (0.68–0.98)	0.03	0.03
	PR (CI)—model 2	0.75 (0.59–0.95)	0.02	0.04	0.93 (0.79–1.11)	0.43	0.48	0.51 (0.34–0.76)	0.001	<0.001	0.79 (0.66–0.95)	0.01	0.01
Flavanones	Mean intake, mg/day	185.3 vs. 16.6			180.4 vs. 16.8			179.8 vs. 17.6			183.6 vs. 15.3		
	Cases	90 vs. 74			211 vs. 203			48 vs. 53			179 vs. 184		
	PR (CI)—unadjusted	1.22 (0.95–1.57)	0.11	0.37	1.04 (0.89–1.22)	0.59	0.47	0.92 (0.66–1.30)	0.65	0.67	0.98 (0.82–1.16)	0.80	0.47
	PR (CI)—model 1	1.26 (0.98–1.62)	0.07	0.26	1.05 (0.90–1.23)	0.54	0.43	0.94 (0.67–1.33)	0.74	0.72	0.96 (0.81–1.14)	0.67	0.35
	PR (CI)—model 2	1.05 (0.84–1.32)	0.66	0.86	0.98 (0.85–1.15)	0.84	0.93	0.82 (0.58–1.16)	0.26	0.24	0.92 (0.78–1.09)	0.35	0.12
Flavones	Mean intake, mg/day	126.4 vs. 29.7			131.2 vs. 30.3			131.8 vs. 32.0			139.6 vs. 31.4		
	Cases	92 vs. 65			219 vs. 183			49 vs. 45			181 vs. 157		
	PR (CI)—unadjusted	1.41 (1.08–1.83)	0.01	0.004	1.21 (1.03–1.42)	0.02	0.01	1.11 (0.78–1.59)	0.54	0.87	1.16 (0.96–1.39)	0.12	0.13
	PR (CI)—model 1	1.42 (1.09–1.86)	0.01	0.003	1.22 (1.03–1.43)	0.02	0.009	1.16 (0.81–1.65)	0.43	0.69	1.18 (0.98–1.41)	0.08	0.08
	PR (CI)—model 2	1.00 (0.78–1.29)	0.99	0.65	0.99 (0.84–1.16)	0.90	0.90	0.94 (0.66–1.34)	0.73	0.50	1.08 (0.90–1.31)	0.39	0.35
Flavonols	Mean intake, mg/day	84.1 vs. 29.4			83.2 vs. 29.1			81.6 vs. 32.0			83.6 vs. 29.9		
	Cases	87 vs. 73			228 vs. 201			53 vs. 44			184 vs. 179		
	PR (CI)—unadjusted	1.24 (0.96–1.60)	0.11	0.06	1.13 (0.97–1.32)	0.11	0.18	1.22 (0.86–1.73)	0.26	0.14	1.03 (0.87–1.23)	0.72	0.54
	PR (CI)—model 1	1.25 (0.96–1.62)	0.10	0.05	1.13 (0.97–1.32)	0.12	0.21	1.27 (0.89–1.81)	0.11	0.09	1.09 (0.91–1.29)	0.34	0.25
	PR (CI)—model 2	0.97 (0.76–1.23)	0.79	0.95	0.96 (0.83–1.12)	0.62	0.45	1.16 (0.82–1.64)	0.40	0.19	1.05 (0.89–1.25)	0.57	0.50
Anthocyanidins	Mean intake, mg/day	94.9 vs. 13.9			97.6 vs. 13.3			82.0 vs. 11.8			87.5 vs. 10.1		
	Cases	67 vs. 73			224 vs. 174			47 vs. 47			172 vs. 185		
	PR (CI)—unadjusted	0.93 (0.70–1.23)	0.60	0.29	1.29 (1.10–1.52)	0.002	0.005	1.02 (0.72–1.45)	0.90	0.93	0.93 (0.79–1.11)	0.44	0.28
	PR (CI)—model 1	0.93 (0.70–1.23)	0.61	0.29	1.28 (1.09–1.51)	0.003	0.007	1.07 (0.75–1.54)	0.62	0.91	0.93 (0.79–1.11)	0.45	0.27
	PR (CI)—model 2	0.80 (0.62–1.05)	0.14	0.02	1.10 (0.94–1.29)	0.22	0.30	0.89 (0.63–1.26)	0.50	0.32	0.93 (0.78–1.10)	0.38	0.24
Catechins	Mean intake, mg /day	57.9 vs. 10.3			58.7 vs. 9.7			55.3 vs. 8.9			53.4 vs. 8.2		
	Cases	68 vs. 92			194 vs. 198			34 vs. 67			144 vs. 200		
	PR (CI)—unadjusted	0.75 (0.58–0.97)	0.03	0.03	0.99 (0.84–1.17)	0.92	0.69	0.51 (0.36–0.73)	0.001	<0.001	0.72 (0.60–0.86)	<0.001	<0.001
	PR (CI)—model 1	0.76 (0.58–0.98)	0.03	0.04	1.01 (0.86–1.19)	0.88	0.52	0.53 (0.37–0.77)	0.001	0.002	0.75 (0.62–0.90)	0.002	0.002
	PR (CI)—model 2	0.64 (0.51–0.81)	<0.001	<0.001	0.91 (0.77–1.06)	0.23	0.50	0.48 (0.33–0.69)	<0.001	<0.001	0.77 (0.64–0.92)	0.004	0.003

Table 3. *Cont.*

		Men						Women					
Hydroxycinnamic acids	Mean intake, mg/day	435.3 vs. 122.3			457.0 vs. 127.2			407.2 vs. 123.6			426.1 vs. 125.7		
	Cases	97 vs. 63			244 vs. 180			45 vs. 50			195 vs. 174		
	PR (CI)—unadjusted	1.54 (1.18–2.00)	0.001	0.003	1.36 (1.16–1.59)	<0.001	<0.001	1.13 (0.80–1.61)	0.49	0.46	1.13 (0.95–1.33)	0.17	0.19
	PR (CI)—model 1	1.52 (1.17–1.98)	0.002	0.005	1.35 (1.16–1.58)	<0.001	<0.001	1.14 (0.81–1.62)	0.45	0.44	1.12 (0.94–1.33)	0.20	0.20
	PR (CI)—model 2	1.62 (1.26–2.08)	<0.001	<0.001	1.43 (1.23–1.66)	<0.001	<0.001	1.15 (0.82–1.62)	0.41	0.40	1.16 (0.98–1.37)	0.08	0.09
Hydroxybenzoic acids	Mean intake, mg/day	30.7 vs. 6.5			31.7 vs. 5.8			24.2 vs. 5.2			22.3 vs. 4.2		
	Cases	72 vs. 78			208 vs. 197			38 vs. 59			149 vs. 191		
	PR (CI)—unadjusted	0.93 (0.71–1.22)	0.61	0.32	1.06 (0.90–1.24)	0.48	0.36	0.65 (0.45–0.93)	0.02	0.02	0.78 (0.65–0.94)	0.008	0.005
	PR (CI)—model 1	0.93 (0.71–1.22)	0.61	0.32	1.05 (0.89–1.23)	0.56	0.44	0.68 (0.47–0.97)	0.04	0.04	0.82 (0.68–0.99)	0.04	0.03
	PR (CI)—model 2	0.76 (0.60–0.97)	0.03	0.01	0.92 (0.79–1.07)	0.28	0.34	0.62 (0.43–0.89)	0.01	0.01	0.83 (0.69–1.00)	0.05	0.04
Stilbenes	Mean intake, mg/day	9.9 vs. 0.0			9.9 vs. 0.0			3.86 vs. 0.00			3.04 vs. 0.00		
	Cases	68 vs. 71			221 vs. 191			44 vs. 42			148 vs. 185		
	PR (CI)—unadjusted	0.99 (0.74–1.31)	0.93	0.50	1.16 (0.99–1.36)	0.07	0.09	1.04 (0.72–1.52)	0.74	0.83	0.81 (0.67–0.97)	0.03	0.02
	PR (CI)—model 1	0.99 (0.75–1.32)	0.97	0.54	1.16 (0.99–1.36)	0.07	0.09	1.10 (0.75–1.59)	0.55	0.63	0.83 (0.69–1.00)	0.05	0.04
	PR (CI)—model 2	0.80 (0.61–1.05)	0.12	0.03	0.99 (0.84–1.16)	0.78	0.97	0.92 (0.61–1.37)	0.68	0.63	0.86 (0.70–1.06)	0.15	0.12
Lignans	Mean intake, mg/day	2.20 vs. 1.00			2.22 vs. 1.00			2.12 vs. 1.33			2.15 vs. 1.00		
	Cases	71 vs. 75			221 vs. 207			44 vs. 47			156 vs. 197		
	PR (CI)—unadjusted	0.93 (0.71–1.22)	0.61	0.52	1.06 (0.91–1.24)	0.44	0.71	0.95 (0.66–1.36)	0.77	0.61	0.80 (0.67–0.95)	0.012	0.005
	PR (CI)—model 1	0.94 (0.66–1.24)	0.66	0.59	1.07 (0.91–1.25)	0.95	0.65	1.00 (0.69–1.45)	0.99	0.81	0.81 (0.68–0.97)	0.02	0.01
	PR (CI)—model 2	0.70 (0.54–0.90)	0.005	0.004	0.88 (0.76–1.03)	0.06	0.04	0.88 (0.61–1.26)	0.47	0.34	0.77 (0.64–0.92)	0.004	0.002

Model 1—adjusted by age, education (basic studies, medium, high), smoking (never, former, smoker), physical activity (quintiles). Model 2—adjusted by the variables used in model 1 plus total energy intake, consumption of refined cereals and animal products (from 17-point questionnaire) and of distilled beverages and liquors, sugar, soft drinks, cookies, and pastries (g/day).

Hydroxycinnamic acids, however, were directly associated with T2D in both overweight and obese men, showing a significant linear trend. Non-significant results were found for other polyphenol groups, namely, flavanones, flavones, and flavonols, after adjusting for all potential confounders. Nevertheless, it is worth mentioning that the intake of flavones was directly associated with T2D in men in model 2, prior to adjustment for energy and dietary variables.

4. Discussion

In this baseline cross-sectional study within the PREDIMED-Plus trial, we observed that higher intakes of some polyphenolic classes were inversely and lineally associated with the prevalence of T2D in a population at high cardiovascular risk, and these associations depended on sex and BMI. We also found that hydroxycinnamic acids were directly associated with T2D.

Previous epidemiological studies have investigated the association between intake of polyphenols and T2D, but this is the first study that quantifies the association between the intake of different polyphenol subgroups and T2D accounting for differences by sex and BMI.

In our study, catechins were the main source of flavan-3-ols because theaflavin intake was very low. Together with proanthocyanidins, these compounds are classified as flavanols. The main sources of flavanols in the PREDIMED-Plus cohort were cocoa and chocolate, apples, plums, red wine, and tea. Proanthocyanidins and catechins were strongly and inversely associated with T2D in overweight men and women, and obese women. In a previous longitudinal study conducted in a similar cohort (PREDIMED study), proanthocyanidins and catechins showed the same association with new-onset T2D when baseline glucose levels were taken out of the model but not in the fully adjusted model. In a large, prospective, case-cohort study, Zamora-Ros et al. concluded that all flavan-3-ol monomers, including catechins, as well as proanthocyanidins of low polymerization degree were associated with a lower risk of developing T2D. As in our case, they did not find associations for flavonols, except for myricetin intake [18].

Many studies have focused on other monomers of flavan-3-ols, especially epigallocatechin gallate, usually found in tea, grapes, and some seeds, and chocolate catechins. The antidiabetic effects of flavan-3-ols have been reported in animal and cell-culture studies, as well as several clinical studies [4,19,20]. Data from a recent meta-analysis on cocoa intake and cardio-metabolic risk suggested significant improvements on insulin-related outcomes [21].

Anthocyanidins are the blue, red, and purple pigments of most fruits and vegetables, such as berries and wine. Although we only found an inverse linear trend with T2D in overweight men, results from in vitro, animal, epidemiological, and human studies have suggested that anthocyanidins might play an important role modulating T2D [20], even though they have low bioavailability. In the frame of the EPIC-InterAct study, Zamora-Ros et al. did not find a significant association between dietary anthocyanidins and incident T2D, which is in agreement with our findings [22]. However, Jennings et al. found that higher anthocyanin and flavone intake, as well as anthocyanin-rich food consumption, were associated with improvements of insulin resistance in a cross-sectional study with women [23], and higher intakes of these colored compounds were also inversely associated with a lower risk of T2D in the Nurses' Health Study [24].

Hydroxybenzoic acids followed the same pattern as that of proanthocyanidins and catechins, showing inverse associations in overweight men and women but not in obese men. Red wine, olives, and walnuts were their principal sources in this cohort. Perhaps because they are the minor components of the phenolic acid group, they have not received much attention in previous studies. Hydroxycinnamic acids, on the other hand, accounted for more than 90% of the phenolic acid group, coffee being the main food source. In our cohort, hydroxycinnamic acid intake was associated with higher prevalence of T2D in men and almost in women. This result contradicts other studies regarding coffee and their polyphenols, such as caffeic acid, or chlorogenic acid. Large observational studies have pointed towards a significant inverse association between coffee consumption and T2D, especially with decaffeinated coffee [25,26]. In a double-blind, placebo-controlled, cross-over design, Lane

et al. concluded that caffeine impaired postprandial glucose metabolism, especially when caffeine was ingested with carbohydrates. These results differ from those obtained with healthy subjects, suggesting that caffeine, but not other compounds of coffee, could have negative consequences in glucose homeostasis only in diabetics [27]. Our results support the notion that high caffeine intake could be directly associated with T2D.

Stilbenes are non-flavonoid polyphenols, usually known due to its main representative—resveratrol. Red wine is by far the main source of this group of compounds, accounting for more than 90% of the intake. Stilbenes were strongly associated with lower odds of developing T2D in the PREDIMED study [28]; however, we only found a mild association and a linear trend for overweight man in the present cohort. It is worth mentioning that the intake of stilbenes was three times higher in men than in women. More than 50% of women had less than 0.1 mg/day of stilbenes. Clinical and animal models have described the antidiabetic effects of stilbenes [29,30]. Nevertheless, whether stilbenes are feasible for the prevention and/or management of T2D is still controversial [31].

Lignans are phytoestrogens usually found in fiber-rich foods since they are constituents of plant cell walls. In western populations, the consumption of lignans are usually greater than isoflavones. The urinary excretion of lignan metabolites produced by the microbiota (mainly enterodiol and enterolactone) is a marker for fiber intake and whole-grain products [32]. In our population, the main source of lignans was virgin olive oil. Those in the highest quartile of lignan intake had lower odds of having T2D except for overweight women where no association was found. These results agree with the inverse association found a few years ago in the PREDIMED cohort [28]. Also in the same line, lower T2D incidence was found in U.S. women with higher enterodiol and enterolactone in urine, which are gut microbiota metabolites of dietary lignans [33]. However, Zamora-Ros et al. found no significant associations between lignans and T2D incidence in the EPIC study [22].

We did not find significant associations with T2D prevalence for any of the following groups: flavanones, flavones, and flavonols, although in a similar population, flavanones were inversely associated with T2D incidence [28]. In a prospective study conducted in two female large cohorts, urinary excretion of hesperetin (a flavanone) was related to a decreased risk of T2D after a long follow-up. Other polyphenol metabolites, including naringenin, quercetin, isorhamnetin, and caffeic acid, were only inversely associated in the short term [34]. Two large observational studies also support the antidiabetic effects of flavonols [22,35].

We noticed that, in our population, prevalence ratios in overweight participants were generally lower than in obese ones. These results agree with the fact that obesity is associated with lower nutrient bioavailability. Novotny et al. conducted a pilot study to investigate the pharmacokinetic response of grape polyphenols in overweight and obese vs. lean volunteers. They found higher concentrations for catechin, epicatechin, quercetin, and resveratrol in individuals with normal BMI, suggesting that obesity could compromise polyphenol absorption [6]. A previous randomized crossover study with blackberries also reported that lean volunteers had better anthocyanin metabolism than overweight/obese ones [5].

The exact mechanisms by which polyphenols affect T2D are still unknown. Several in vitro and animal studies have pointed out that polyphenols decrease glucose absorption in the small intestine due to the inhibition of α-glucosidase, α-amylase, and sucrase in the gut mucosa, and they also limit their reabsorption in the kidney. Other mechanisms of action are related to peripheral tissues. That includes inhibition of gluconeogenesis, adrenergic stimulation of glucose uptake, and stimulation of insulin release by pancreatic β-cells [36].

The main limitation of the present study is the cross-sectional design, which does not allow us to assess causality, but also the estimation of polyphenol intake through FFQs, so bioavailability could not be considered. Furthermore, as in all epidemiological studies, residual confounding cannot be excluded. Finally, these results might not be generalizable to other populations. Our study has also several strengths, including the big sample size, the blinded assessment of the endpoint, the

multicenter design, and the comprehensive data on dietary intake and risk factors of T2D and other potential confounders.

Many research studies endorse the role of polyphenols in modulating the risk of diseases. However, there are many issues to deal with when assessing these effects such as their wide variability of structures, the difficulty of analyzing polyphenol content in foods, the interactions between them, other food components and food matrices, and the complexity of the human body [37].

Authors should discuss the results and how they can be interpreted in the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

5. Conclusions

We can conclude that high intakes of some polyphenols are inversely associated with the prevalence of T2D in older adults with metabolic syndrome, these relationships being more especially observed in overweight subjects than in obese ones. However, hydroxycinnamic acids are directly associated with T2D. In that sense, randomized controlled trials would be useful to confirm the promising benefits of polyphenols for the prevention and management of T2D.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3921/8/11/537/s1, List of the PREDIMED-Plus study investigators, Table S1: Energy-adjusted polyphenol intake (mg/day) according to sex and BMI groups (n = 6633), Table S2: Baseline characteristics by sex and BMI groups (n = 6633), Table S3: Main dietary nutrient and food composition according to sex and BMI groups (n = 6633).

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Article

Adopting a High-Polyphenolic Diet Is Associated with an Improved Glucose Profile: Prospective Analysis within the PREDIMED-Plus Trial

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Abstract: Previous studies suggested that dietary polyphenols could reduce the incidence and complications of type-2 diabetes (T2D); although the evidence is still limited and inconsistent. This work analyzes whether changing to a diet with a higher polyphenolic content is associated with an improved glucose profile. At baseline, and at 1 year of follow-up visits, 5921 participants (mean age 65.0 $\pm$ 4.9, 48.2% women) who had overweight/obesity and metabolic syndrome filled out a validated 143-item semi-quantitative food frequency questionnaire (FFQ), from which polyphenol intakes were calculated. Energy-adjusted total polyphenols and subclasses were categorized in tertiles of changes. Linear mixed-effect models with random intercepts (the recruitment centers) were used to assess associations between changes in polyphenol subclasses intake and 1-year plasma glucose or glycosylated hemoglobin (HbA1c) levels. Increments in total polyphenol intake and some classes were inversely associated with better glucose levels and HbA1c after one year of follow-up. These associations were modified when the analyses were run considering diabetes status separately. To

our knowledge, this is the first study to assess the relationship between changes in the intake of all polyphenolic groups and T2D-related parameters in a senior population with T2D or at high-risk of developing T2D.

Keywords: antioxidants; Mediterranean diet; flavonoids; phenolic acids; obesity; glucose; HbA1c; glycosylated hemoglobin; metabolic syndrome

1. Introduction

The prevalence of diabetes is experiencing an increasing trend, and in 2019 it was the ninth leading cause of death in the world. Additionally, individuals with diabetes are more likely to suffer from other noncommunicable diseases such as heart attacks, strokes, or kidney disease. The expectations for the forthcoming years are not encouraging since the prevalence of diabetes has been increasing over the past decades. Nevertheless, type-2 diabetes (T2D), the most prevalent type, can be prevented by modifying harmful behavioral risk factors such as smoking, an unhealthy diet, sedentarism, and alcohol abuse [1]. In the search for the best dietary pattern to prevent or stop the progression of T2D, plant-based diets such as Mediterranean-style, vegetarian or vegan diets have been studied in several prospective observational studies and clinical trials [2].

Healthy plant-based diets are based on the consumption of large amounts of whole grains, fruits, vegetables, legumes, and nuts, as well as healthy fats such as extra virgin olive oil, which are associated with a lower risk of developing cardiovascular disease and T2D [3]. A trait all these foods have in common is a richness in polyphenols, bioactive plant secondary metabolites with a vast structural diversity. According to their structure, polyphenols are classified into two main groups: flavonoids and non-flavonoids. Polyphenols in the flavonoid group share the C6-C3-C6 structure and can be divided into the following subgroups: flavones, flavonols, theaflavins, catechins, proanthocyanidins (polymeric forms), flavanones, anthocyanidins, and isoflavones, whereas the non-flavanoids are classified as phenolic acids, lignans, and stilbenes [4].

Protective effects of polyphenols against the incidence and complications of T2D are supported by mechanistic studies conducted in animals [5] as well as clinical and epidemiological studies [6], although the available evidence is still limited and inconsistent. Furthermore, no previous study has examined the association between changes in the intake of all polyphenolic groups and subgroups and T2D-related parameters in a population with or at high-risk of T2D. The aim of the present work was to determine whether changing to a high polyphenol diet is associated with an improved glucose profile. Due to the heterogeneity of polyphenols in terms of bioavailability and metabolism, they were studied in separate groups.

2. Materials and Methods

2.1. Study Design and Participants

The present study is a prospective cohort analysis conducted in the context of the PREDIMED-Plus trial [7,8], an ongoing six-year multicenter, parallel group, randomized, lifestyle intervention study involving 6874 participants enrolled in 23 recruitment centers in Spain from October 2013 to December 2016. Eligible participants were men (aged 55–75 years) and women (aged 60–75 years) with a body mass index (BMI) between 27 and 40 kg/m^2 and the presence of three or more components of metabolic syndrome (updated harmonized criteria of the International Diabetes Federation and the American Heart Association and National Heart, Lung and Blood Institute) [9].

Participants were randomly assigned, in a 1:1 ratio, to one of two groups: an intensive weight-loss intervention group (based on an energy-restricted Mediterranean diet, individualized physical activity plan, and behavioral support) or a control group (based on the traditional Mediterranean diet and usual health care). The detailed study

protocol and eligible and exclusion criteria can be found elsewhere [8,10], including at http://predimedplus.com (accessed on 10 January 2022).

For the present analysis, 777 participants with missing dietary data and 176 with extreme energy intakes (<500 or >3500 for women and <800 and >4000 for men) [9] either at baseline or at the annual visit were excluded. Consequently, a total of 5921 participants were available for the analysis (Figure 1).

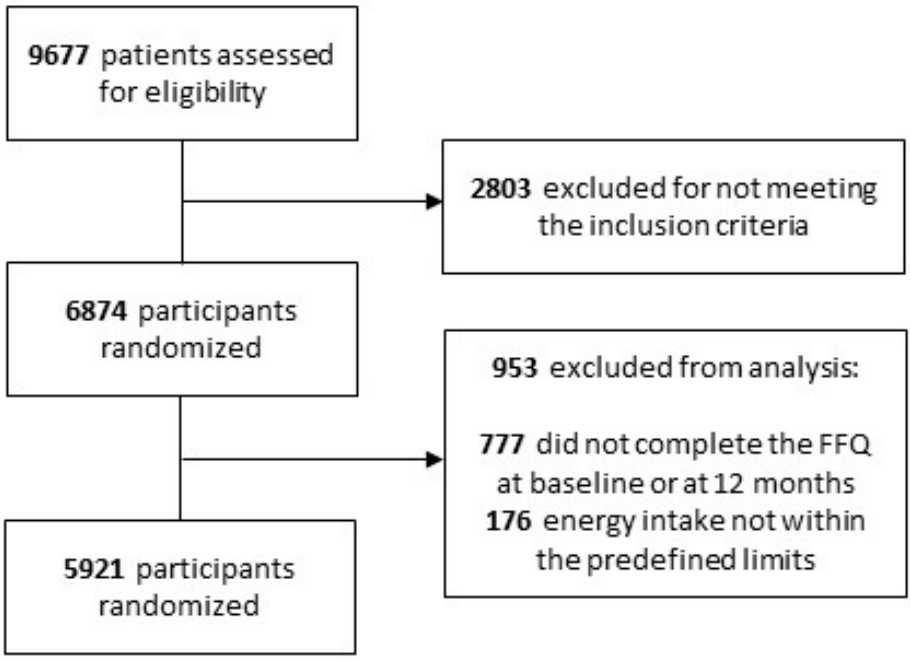

Figure 1. Flow chart of the participants.

2.2. Dietary Assessment and Polyphenol Intake

At baseline, and at one year of follow-up visits, registered dietitians collected data on dietary intake using a validated 143-item semi-quantitative food-frequency questionnaire (FFQ) [11], from which the total energy and nutrient intake were calculated based on Spanish food composition tables [12]. Additionally, a validated 17-point score questionnaire on adherence to an energy-restricted traditional Mediterranean diet was filled out [13].

The 143-item FFQ was also used to calculate polyphenol intake together with the Phenol-Explorer database (www.phenol-explorer.eu (accessed on 15 September 2021)). Individual polyphenol intakes were obtained by multiplying the content of each polyphenol in each food item with polyphenols (mg/g) by the daily consumption of this food item (g/day) and then summing the product across all food items. Total polyphenols and polyphenol subclasses were then adjusted for total energy intake using the residual method [14], and variables were transformed into tertiles of changes (one year vs. baseline).

2.3. Ascertainment of the Endpoints

The main endpoints were one-year changes of fasting plasma glucose (mg/dL) and glycosylated hemoglobin (HbA1c) (%) levels. Both parameters were measured in overnight fasting blood samples by routine laboratory tests.

2.4. Assessment of Covariates

Participants filled out a general questionnaire to provide data on lifestyle habits, education, concurrent diseases, and medication use. Physical activity was measured by a Regicor Short Physical Activity Questionnaire validated for the Spanish population [15].

Anthropometric parameters were measured at baseline and every follow-up visit by trained dietitians according to the PREDIMED-Plus protocol. Height, weight, waist, and

hip circumference were measured in duplicate by trained staff. BMI was calculated as weight in kilograms divided by height in meters squared.

Blood samples were collected after overnight fasting and stored frozen (−80 °C). Serum triglyceride and total and high-density lipoprotein (HDL) cholesterol levels were measured by routine laboratory tests using standard enzymatic methods.

Sociodemographic and lifestyle variables were categorized in four categories as follows: education (primary, secondary, or high school), physical activity (sedentary, moderately active, and active), smoking status (never, former, or current smoker), and BMI (27.0–29.9, 30.0–34.9, or ≥35 kg/m^2).

Previous diagnosis of T2D was also registered, as well as glucose-lowering treatment. T2D was diagnosed according to American Diabetes Association guidelines: fasting plasma glucose levels ≥ 7.0 mmol/L (≥126 mg/dL), HbA1c levels ≥ 6.5% or 2 h plasma glucose levels ≥ 11.1 mmol/L (≥200.0 mg/dL) after an oral dose of 75 g glucose [13]. Prediabetes was defined according to the criteria of the American Diabetes Association as impaired fasting glucose (5.6–6.9 mmol/L, or 100–125 mg/dL) and/or raised HbA1c of 39–47 mmol/mol (5.7–6.4%) [16].

2.5. Statistical Analyses

Baseline characteristics according to tertiles of changes in total polyphenol intake are presented as means (±SD) for quantitative variables and frequencies for categorical variables. One-factor ANOVA tests were used to assess the differences between tertiles and chi square tests for categorical variables.

Linear mixed-effect models with random intercepts at the recruitment center and cluster family level were used to assess associations between changes in polyphenol subclasses intake and glucose and HbA1c levels over the first year of follow-up. The intake of total polyphenols and the main polyphenol subclasses were distributed into tertiles of changes in consumption after one year of follow-up. To assess the linear trend (p for trend) across tertiles of polyphenol intake, the mean value was assigned to each tertile. Model 1 was minimally adjusted for age, sex, and study arm. Model 2 was additionally adjusted for smoking status and levels of education and physical activity at baseline (all categorical). Model 3 was further adjusted for baseline variables such as BMI, energy intake, and intakes of carbohydrates, protein, saturated fatty acids, and alcohol (continuous), and glucose-lowering treatment (Yes/No).

To account for multiple comparisons, we applied the Bonferroni correction to interpret the results. Considering the 12 polyphenols analyzed, significance was established at a p value threshold of 0.004 (p value < 0.05/12 = 0.004), although all p values below 0.05 have been mentioned. Statistical analyses were performed using STATA software (version 16; StataCorp, College Station, TX, USA), and statistical significance was set at $p < 0.05$. We used the PREDIMED-Plus longitudinal database generated on 26 June 2020 (202006290731_PREDIMEDplus).

3. Results

This work involved 5921 participants from the PREDIMED-plus cohort that completed the first year of the study. The mean age of the population was 65.0 ± 4.9 years, and 48.2% were women; 30.7% had been diagnosed with diabetes at baseline, and 48.5% were prediabetic. The mean total polyphenol intake was 854 ± 318 mg/day at baseline and 855.0 ± 293 mg/day after one year, indicating no overall change. Breaking down the polyphenols by type, 58% corresponded to flavonoids, 33% were phenolic acids, and the rest were stilbenes, lignans and others, which remained the same after one year. Hydroxycinnamic acids were the most consumed polyphenol class (30%), followed by flavanols (27%), proanthocyanidins (24%), flavanones (10.6%), flavones (9%), flavonols (6%), anthocyanidins (5%), catechins (3%) and hydroxybenzoic acids (2%).

Table 1 summarizes the baseline characteristics of participants classified in tertiles according to changes in total polyphenol intake adjusted for energy using the residual

method. During the first year, participants in the lowest tertile (T1) reduced their polyphenol intake by a mean of 249 mg/day, whereas those in the highest tertile (T3) increased their intake by a mean of 256 mg/day. The intake in the middle tertile (T2) remained quite stable. T3 included the highest percentage of men and participants from the intervention group (energy-restricted Mediterranean diet plus physical activity). No significant differences across tertiles were observed regarding age, educational level, smoking habit, physical activity, diabetes status, glucose and HbA1c levels at baseline, and all groups had lower levels of glucose and HbA1c after one year. This is due to the interventions that all participants received, which were (1) an intensive weight-loss intervention based on an energy-restricted Mediterranean diet, individualized physical activity plan, and behavioral support or (2) an intervention based on the traditional Mediterranean diet and usual health care (control group). According to the Mediterranean Diet score, participants from all groups had healthier diets after one year. Although, the greatest reduction in glucose was observed among the participants in T3, that is, those who adopted a high polyphenol diet. It is worth mentioning that fasting glucose and HbA1c levels were also significantly lower in T1. This could be explained because participants were divided in tertiles of change of polyphenol intake, but not all variables across the groups were equally distributed. For instance, the ones who decreased polyphenol intake after one year also had the highest consumption of olive oil. Therefore, the real associations appeared after the statistical models were adjusted for confounders.

Table 2 shows dietary changes after one year corresponding to each tertile of changes in total polyphenol intake. Although all the participants adopted healthier dietary patterns, there were differences between groups. For instance, those in T3 reduced their total calory intake per day by almost 200 kcal, compared to 129 kcal in T1. This difference can be explained by the higher reduction in dietary protein and saturated fatty acids in T3. Nevertheless, the most notable reduction in alcohol intake was in T1. We observed that participants who reduced their total polyphenol consumption also had lower carbohydrate and higher MUFA and PUFA intakes. The improvements in these parameters seem to be correlated with changes in diet, as these participants reduced their consumption of cookies, pastries, and fruit. Overall, participants in T3 obtained the highest score in the Mediterranean-diet adherence test after one year, and they had consumed more vegetables, fruits, and fiber and fewer cereals, dairy, meat, and sugary items (cookies, pastries and sweets, sugar, and soft drinks). No differences were observed for fish and nuts.

Table 1. Characteristics of the study participants, according to tertiles of changes in total polyphenol intake.

	Tertiles of Δ Polyphenol Intake after 1 Year			
Change of polyphenol intake (mg/day), median (min to max)	T1 −249 (−2400 to −106)	T2 1.52 (−106 to 107)	T3 256 (107 to 1400)	*p*
No. of subjects	1974	1974	1973	
Allocated in the intervention group	901 (45.6)	955 (48.4)	1044 (52.9)	<0.001
Age (years), mean ± SD	65.2 ± 4.9	65.0 ± 4.8	64.9 ± 4.9	0.09
Women, n (%)	987 (50.0)	971 (49.2)	898 (45.5)	0.01
Education, *n* (%)				
Primary school	981 (49.7)	971 (49.2)	990 (50.2)	0.11
High school	545 (27.6)	606 (30.7)	550 (27.9)	
University	448 (22.7)	397 (20.1)	433 (21.9)	
Current smoker, *n* (%)				
Baseline	243 (12.3)	242 (12.2)	250 (12.7)	0.06
After 1 year	205 (11.9)	202 (11.9)	214 (13.3)	0.45

Table 1. *Cont.*

	Tertiles of Δ Polyphenol Intake after 1 Year			
Physical activity (METS.min/week), mean ± SD				
Baseline	2543 ± 2283	2454 ± 2329	2527 ± 2353	0.34
1-year change	518 ± 2480 *	506 ± 2363 *	567 ± 2433 *	0.70
Diabetes status				
Nondiabetic participants	384 (19.5)	432 (21.9)	415 (21.0)	0.36
Pre-diabetic participants	981 (49.7)	950 (48.1)	941 (47.7)	
Diabetic participants	609 (30.9)	592 (30.0)	617 (31.3)	
Glucose (mg/dL), mean ± SD				
Baseline	113.67 ± 28.70	112.18 ± 29.08	114.29 ± 28.97	0.06
1-year change	−2.41 ± 20.92 *	−1.59 ± 23.13	−3.4 ± 23.04 *	0.04
HbA1c (%), mean ± SD				
Baseline	6.12 ± 0.87	6.08 ± 0.87	6.11 ± 0.83	0.25
1-year change	−0.09 ± 0.53 *	−0.05 ± 0.53	−0.09 ± 0.57 *	0.07

Values are frequencies and percentages for categorical variables or means ± SDs for continuous variables, except for polyphenol intake, which are median (min-max). Analysis of variance one factor (ANOVA) was used for continuous variables and the χ^2 test for categorical variables. BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); SD, standard deviation. * Significant differences between baseline and one-year data.

Table 2. Changes in daily intake of nutrients, food items, and Mediterranean diet score after one year, according to tertiles of changes in total polyphenol intake.

	Tertiles of Δ Polyphenol Intake after 1 Year				
	T1 −249 (−2400 to −106)	T2 1.52 (−106 to 107)	T3 256 (107 to 1400)	*p*	Adjusted *p*
No. of subjects	1974	1974	1973		
Total energy (Kcal/d)					
Baseline	2382 ± 547	2289 ± 541	2430 ± 552	<0.001	<0.001
1-year change	−128.7 ± 524.8	−135.1 ± 483.5	−194.4 ± 541.7	0.002	<0.001
Carbohydrates (g/d)					
Baseline	246 ± 73	231 ± 71	245 ± 73	<0.001	<0.001
1-year change	−34.7 ± 70.0	−29.2 ± 66.0	−31.6 ± 75.6	0.05	<0.001
Fiber (g/d)					
Baseline	29 ± 10	25 ± 8	25 ± 8	<0.001	<0.001
1-year change	0.5 ± 9.7	3.6 ± 8.3	6.8 ± 9.1	<0.001	<0.001
Proteins (g/d)					
Baseline	98 ± 22	96 ± 22	99 ± 22	<0.001	<0.001
1-year change	−1.2 ± 21.8	−2.1 ± 20.8	−4.0 ± 22.1	<0.001	<0.001

Table 2. *Cont.*

	Tertiles of Δ Polyphenol Intake after 1 Year				
	T1 −249 (−2400 to −106)	T2 1.52 (−106 to 107)	T3 256 (107 to 1400)	*p*	Adjusted *p*
MUFA (g/d)					
Baseline	53 ± 16	53 ± 16	56 ± 16	<0.001	<0.001
1-year change	7.0 ± 18.4	4.6 ± 16.8	1.9 ± 18.2	<0.001	<0.001
PUFA (g/d)					
Baseline	18 ± 6	17 ± 6	19 ± 7	<0.001	<0.001
1-year change	1.7 ± 7.2	1.0 ± 6.8	0.1 ± 7.3	<0.001	<0.001
SFA (Kcal/d)					
Baseline	26 ± 9	25 ± 8	27 ± 9	<0.001	<0.001
1-year change	−2.5 ± 8.1	−3.3 ± 7.3	−4.8 ± 8.2	<0.001	<0.001
Alcohol (g/d)					
Baseline	11 ± 14	11 ± 15	12 ± 16	0.05	0.31
1-year change	−2.0 ± 11.3	−1.2 ± 10	−0.5 ± 11.8	<0.001	<0.001
17-points MedDiet score					
Baseline	8.86 ± 2.7	8.45 ± 2.63	8.22 ± 2.65	<0.001	<0.001
1-year change	2.6 ± 3.1	3.3 ± 3.2	3.9 ± 3.3	<0.001	<0.001
Food items, g/day					
Vegetables					
Baseline	348 ± 146	326 ± 136	313 ± 127	<0.001	<0.001
1-year change	6.4 ± 153.5	32.0 ± 143.7	60.7 ± 146.8	<0.001	<0.001
Fruits					
Baseline	430 ± 240	341 ± 179	308 ± 167	<0.001	<0.001
1-year change	−58.5 ± 236.0	42.2 ± 177.9	146.9 ± 204.4	<0.001	<0.001
Legumes					
Baseline	21 ± 11	20 ± 11	20 ± 11	0.001	<0.001
1-year change	3.8 ± 13.6	4.6 ± 13.1	4.1 ± 13.3	0.15	0.04
Cereals					
Baseline	146 ± 77	145 ± 74	161 ± 82	<0.001	<0.001
1-year change	−15.4 ± 82.0	−23.1 ± 76.6	−35.2 ± 88.1	<0.001	<0.001
Dairy					
Baseline	348 ± 206	336 ± 193	349 ± 203	0.07	0.05
1-year change	−11.8 ± 191.7	−19.9 ± 177.2	−27.7 ± 193.4	0.03	<0.001
Meat					
Baseline	144 ± 57	146 ± 57	153 ± 61	<0.001	<0.001
1-year change	−8.6 ± 57.1	−16.1 ± 55.3	−24.1 ± 57.0	<0.001	<0.001
Olive oil					
Baseline	39 ± 16	40 ± 17	42 ± 17	<0.001	<0.001
1-year change	6.8 ± 18.9	4.7 ± 17.7	1.4 ± 19.1	<0.001	<0.001
Fish					
Baseline	105 ± 47	101 ± 47	101 ± 47	0.02	0.02
1-year change	8.1 ± 53.0	10.1 ± 50.7	9.7 ± 50.3	0.43	0.30
Nuts					
Baseline	16 ± 17	14 ± 17	15 ± 17	0.04	0.04
1-year change	13.8 ± 23.1	13.3 ± 21.0	14.3 ± 22.1	0.37	0.29

Table 2. *Cont.*

	Tertiles of Δ Polyphenol Intake after 1 Year				
	T1 −249 (−2400 to −106)	T2 1.52 (−106 to 107)	T3 256 (107 to 1400)	*p*	Adjusted *p*
Cookies, pastries, and sweets					
Baseline	30 ± 31	23 ± 27	27 ± 31	<0.001	<0.001
1-year change	−14.7 ± 29.7	−10.4 ± 26.4	−11.6 ± 31.2	<0.001	<0.001
Sugar					
Baseline	6 ± 12	7 ± 12	7 ± 12	0.32	0.62
1-year change	−2.5 ± 9.8	−3.1 ± 10.0	−3.6 ± 10.8	0.003	<0.001
Soft drinks					
Baseline	20 ± 59	21 ± 64	23 ± 66	0.16	0.27
1-year change	−7.0 ± 67.8	−10.4 ± 64.1	−12.9 ± 77.9	0.03	0.004

Values are means ± SD. *p*-values were calculated by ANCOVA tests adjusted for sex, age, intervention group, education level, and recruitment center. MUFA, Monounsaturated Fatty Acids; PUFA, Polyunsaturated Fatty Acids; SFA, Saturated Fatty Acids; MedDiet, Mediterranean Diet.

We generated linear mixed models to study the association between changes in glucose and HbA1c levels and tertiles of change in polyphenol intake after one year (Table 3). Analyses were performed for total polyphenols, total flavonoids (including anthocyanidins, catechins, proanthocyanidins, flavanones, flavones, and flavonols), total phenolic acids (including hydroxycinnamic acids and hydroxybenzoic acids), lignans, and stilbenes. We compared the participants in T1 and T3 using T2 as a reference, as the polyphenol intake in this group did not change. The extreme groups were also compared with each other (T1 vs. T3).

In multivariable-adjusted models, considering anthropometric, sociodemographic, lifestyle, and dietary variables after one year of follow-up, an increment in total polyphenol intake was inversely associated with glucose levels (β = −1.76; 95% CI −3.18, −0.34) when comparing T3 with T2. Moreover, HbA1c values were lower in T1 than in T2 (β = −0.039; 95% CI −0.076, −0.002), although further analyses revealed that this result was correlated with the hydroxycinnamic acid intake (β = −0.04; 95% CI −0.077, −0.004; T1 vs. T2).

Due to the heterogeneity of polyphenols, they were studied separately. The increase in total flavonoids was also correlated with a decrease in glucose levels (β = −1.56; 95% CI −2.99, −0.13; T3 vs. T1). Among the flavonoids, flavones and flavonols were both inversely associated with glucose and HbA1c, the last with a lineal relationship. Anthocyanidins were also inversely associated with HbA1c (β = −0.037; 95% CI −0.075, 0.000; T3 vs. T2), but, after adjusting for all the potential confounding variables, the association was not significant ($p = 0.05$). Some correlations with glucose and HbA1c were also found for the non-flavonoids: lignans and stilbenes. In the case of lignans, the association was also linear.

We wanted to study if diabetes status was an important factor when analyzing the impact of polyphenol intake on glucose and HbA1c, so the analyses were repeated after dividing the population in three groups: those without diabetes, and prediabetic and diabetic participants (Table 4 and Figure 2). Interestingly, the role of polyphenols was found to differ considerably depending on the diabetes status. No significant associations were found within the non-diabetic group, whereas the participants who most benefited from a higher polyphenol intake were prediabetic. In this group, several polyphenol classes were inversely associated with levels of glucose (total polyphenols, total flavonoids, proanthocyanidins, flavanones, and flavones) or HbA1c (flavones and lignans). Once again, hydroxycinnamic acid intake was directly associated with HbA1c. Fewer polyphenol groups were associated with glucose-related parameters in diabetic participants (flavonols, lignans, and stilbenes).

Table 3. Changes in glucose (mg/dL) and glycosylated hemoglobin (HbA1c) (%) according to tertiles of change of polyphenol intake (mg/dL) after one year. Results from linear mixed models.

		T1 (vs. T2)	*p*	T2	T3 (vs. T2)	*p*	T3 (vs. T1)	*p*	*p*-Trend
	Total polyphenols	−249 (−2400, −106) [a]		2 (−106, 107)	257 (107, 1400)				
Glucose	Model 1	−2.56 (−3.46, −1.66) [b]	<0.001	ref.	−2.34 (−3.24, −1.44)	<0.001	0.22 (−0.74, 1.18)	0.66	0.12
	Model 2	−2.78 (−3.72, −1.84)	<0.001	ref.	−2.56 (−3.51, −1.61)	<0.001	0.23 (−0.78, 1.23)	0.62	0.33
	Model 3	−0.94 (−2.36, 0.48)	0.19	ref.	−1.76 (−3.18, −0.34)	0.015	−0.82 (−2.24, 0.61)	0.23	0.43
HbA1c	Model 1	0.015 (−0.008, 0.037)	0.19	ref.	0.008 (−0.015, 0.031)	0.50	−0.007 (−0.031, 0.018)	0.55	0.76
	Model 2	0.011 (−0.012, 0.034)	0.35	ref.	0.001 (−0.023, 0.025)	0.92	−0.01 (−0.035, 0.016)	0.44	0.80
	Model 3	−0.039 (−0.076, −0.002)	0.04	ref.	−0.032 (−0.069, 0.005)	0.09	0.007 (−0.031, 0.044)	0.36	0.46
	Total flavonoids	−195 (−2405, −78)		3 (−78, 84)	193 (84, 1383)				
Glucose	Model 1	0.46 (−0.45, 1.37)	0.32	ref.	−0.6 (−1.51, 0.31)	0.20	−1.06 (−2.04, −0.07)	0.04	0.16
	Model 2	0.01 (−0.95, 0.96)	0.99	ref.	−0.79 (−1.75, 0.18)	0.11	−0.79 (−1.83, 0.24)	0.14	0.16
	Model 3	0.17 (−1.25, 1.59)	0.81	ref.	−1.39 (−2.82, 0.04)	0.06	−1.56 (−2.99, −0.13)	0.03	0.16
HbA1c	Model 1	−0.029 (−0.052, −0–006)	0.014	ref.	−0.033 (−0.057, −0.01)	0.006	−0.004 (−0.029, 0.021)	0.79	0.42
	Model 2	−0.019 (−0.043, 0.005)	0.12	ref.	−0.021 (−0.045, 0.004)	0.09	−0.002 (−0.027, 0.024)	0.95	0.23
	Model 3	−0.024 (−0.061, 0.013)	0.21	ref.	−0.024 (−0.061, 0.013)	0.20	−0.001 (−0.038, 0.037)	0.99	0.72
	Anthocyanidins	−25 (−526, −10)		0 (−10, 10)	25 (10, 209)				
Glucose	Model 1	1.87 (0.96, 2.78)	<0.001	ref.	1.72 (0.81, 2.63)	<0.001	−0.15 (−1.1, 0.8)	0.76	0.05
	Model 2	1.42 (0.46, 2.37)	0.004	ref.	1.74 (0.77, 2.70)	<0.001	0.32 (−0.68, 1.32)	0.56	0.03
	Model 3	0.06 (−1.36, 1.48)	0.93	ref.	−0.43 (−1.86, 1.00)	0.56	−0.49 (−1.92, 0.94)	0.50	0.89
HbA1c	Model 1	0.024 (0.001, 0.047)	0.04	ref.	−0.026 (−0.05, −0.003)	0.03	−0.05 (−0.075, −0.025)	<0.001	0.83
	Model 2	0.019 (−0.004, 0.043)	0.11	ref.	−0.019 (−0.044, 0.005)	0.12	−0.039 (−0.064, −0.013)	0.003	0.35
	Model 3	−0.028 (−0.065, 0.009)	0.14	ref.	−0.037 (−0.075, 0.000)	0.05	−0.009 (−0.047, 0.028)	0.66	0.62
	Catechins	−14 (−162, −5)		0 (−5, 6)	14 (6, 176)				
Glucose	Model 1	−0.46 (−1.35, 0.42)	0.31	ref.	0.25 (−0.65, 1.14)	0.59	0.71 (−0.2, 1.61)	0.12	0.77
	Model 2	0.00 (−0.93, 0.93)	0.99	ref.	0.29 (−0.64, 1.23)	0.54	0.29 (−0.66, 1.24)	0.55	0.51
	Model 3	0.76 (−0.66, 2.17)	0.30	ref.	−0.11 (−1.53, 1.31)	0.90	−0.87 (−2.29, 0.55)	0.23	0.27
HbA1c	Model 1	0.038 (0.016, 0.06)	0.001	ref.	0.029 (0.006, 0.052)	0.01	−0.009 (−0.032, 0.014)	0.36	0.89
	Model 2	0.044 (0.021, 0.067)	<0.001	ref.	0.028 (0.004, 0.051)	0.02	−0.017 (−0.04, 0.007)	0.15	0.90
	Model 3	0.008 (−0.028, 0.045)	0.65	ref.	−0.019 (−0.057, 0.018)	0.30	−0.028 (−0.065, 0.009)	0.14	0.35
	Proanthocyanidins	−122 (−2169, −48)		−4 (−48, 40)	106 (40, 1207)				
Glucose	Model 1	1.97 (1.07, 2.87)	<0.001	ref.	0.27 (−0.63, 1.18)	0.55	−1.7 (−2.64, −0.75)	<0.001	0.10
	Model 2	1.15 (0.19, 2.11)	0.02	ref.	−0.35 (−1.31, 0.61)	0.47	−1.51 (−2.5, −0.51)	0.003	0.01
	Model 3	1.13 (−0.29, 2.55)	0.12	ref.	−0.15 (−1.58, 1.29)	0.84	−1.28 (−2.71, 0.15)	0.08	0.61
HbA1c	Model 1	0.031 (0.008, 0.054)	0.009	ref.	−0.015 (−0.039, 0.008)	0.20	−0.046 (−0.071, −0.022)	<0.001	0.001
	Model 2	0.031 (0.007, 0.055)	0.01	ref.	−0.014 (−0.038, 0.011)	0.28	−0.044 (−0.07, −0.019)	0.001	<0.001
	Model 3	−0.012 (−0.049, 0.025)	0.51	ref.	−0.02 (−0.058, 0.018)	0.30	−0.008 (−0.045, 0.030)	0.70	0.56

Table 3. *Cont.*

		T1 (vs. T2)	*p*	T2	T3 (vs. T2)	*p*	T3 (vs. T1)	*p*	*p*-Trend
	Flavanones	−42 (−554, −5)		13 (−5, 36)	77 (36, 860)				
Glucose	Model 1	−1.48 (−2.38, −0.58)	0.001	ref.	−2.27 (−3.19, −1.35)	<0.001	−0.79 (−1.76, 0.18)	0.11	0.75
	Model 2	−1.37 (−2.31, −0.43)	0.004	ref.	−2.52 (−3.49, −1.55)	<0.001	−1.15 (−2.16, −0.15)	0.04	0.30
	Model 3	0.53 (−0.88, 1.95)	0.46	ref.	−0.33 (−1.75, 1.09)	0.65	−0.86 (−2.28, 0.56)	0.24	0.38
HbA1c	Model 1	−0.026 (−0.048, −0.003)	0.02	ref.	0.019 (−0.004, 0.043)	0.11	0.045 (0.021, 0.07)	<0.001	<0.001
	Model 2	−0.013 (−0.036, 0.010)	0.28	ref.	0.011 (−0.013, 0.036)	0.36	0.024 (−0.001, 0.05)	0.06	0.02
	Model 3	−0.015 (−0.052, 0.022)	0.43	ref.	0.007 (−0.030, 0.044)	0.71	0.022 (−0.015, 0.059)	0.25	0.10
	Flavones	−21 (−305, 1)		13 (1, 30)	55 (30, 344)				
Glucose	Model 1	0.71 (−0.2, 1.63)	0.12	ref.	−0.46 (−1.44, 0.52)	0.36	−1.17 (−2.19, −0.16)	0.02	0.007
	Model 2	1.05 (0.08, 2.02)	0.03	ref.	−0.09 (−1.12, 0.94)	0.86	−1.14 (−2.2, −0.08)	0.03	0.008
	Model 3	−1.2 (−2.64, 0.23)	0.10	ref.	−1.56 (−3.02, −0.11)	0.62	−1.56 (−3.02, −0.11)	0.04	0.20
HbA1c	Model 1	−0.053 (−0.076, −0.029)	<0.001	ref.	−0.026 (−0.051, 0.000)	0.05	0.027 (0.001, 0.053)	0.019	<0.001
	Model 2	−0.045 (−0.07, −0.021)	<0.001	ref.	−0.024 (−0.05, 0.003)	0.08	0.022 (−0.005, 0.049)	0.06	<0.001
	Model 3	−0.015 (−0.053, 0.022)	0.41	ref.	−0.049 (−0.087, −0.012)	0.01	−0.034 (−0.072, 0.004)	0.08	0.12
	Flavonols	−15 (−103, −4)		4 (−4, 12)	26 (12, 177)				
Glucose	Model 1	−1.59 (−2.5, −0.67)	0.001	ref.	1.4 (0.44, 2.36)	0.004	2.99 (1.96, 4.01)	<0.001	0.51
	Model 2	−1.4 (−2.37, −0.44)	0.004	ref.	1.3 (0.3, 2.3)	0.01	2.7 (1.63, 3.78)	<0.001	0.85
	Model 3	0.13 (−1.29, 1.55)	0.86	ref.	−1.36 (−2.78, 0.06)	0.06	−1.49 (−2.93, −0.05)	0.04	0.03
HbA1c	Model 1	0.019 (−0.004, 0.042)	0.11	ref.	−0.053 (−0.077, −0.029)	<0.001	−0.072 (−0.098, −0.045)	<0.001	<0.001
	Model 2	0.017 (−0.007, 0.04)	0.17	ref.	−0.036 (−0.061, −0.011)	0.004	−0.053 (−0.08, −0.026)	<0.001	0.05
	Model 3	−0.005 (−0.042, 0.033)	0.81	ref.	−0.073 (−0.11, −0.036)	<0.001	−0.069 (−0.106, −0.031)	<0.001	0.003
	Hydroxycinnamic acids	−103 (−725, −32)		−3 (−32, 26)	93 (26, 739)				
Glucose	Model 1	−3.07 (−3.96, −2.19)	<0.001	ref.	−1.28 (−2.17, −0.38)	0.005	1.8 (0.86, 2.73)	<0.001	<0.001
	Model 2	−3.24 (−4.17, −2.3)	<0.001	ref.	−1.28 (−2.22, −0.34)	0.007	1.96 (0.97, 2.94)	<0.001	<0.001
	Model 3	−0.94 (−2.36, 0.47)	0.19	ref.	−0.26 (−1.68, 1.16)	0.72	0.69 (−0.74, 2.11)	0.34	0.47
HbA1c	Model 1	−0.015 (−0.037, 0.007)	0.18	ref.	0.02 (−0.003, 0.042)	0.09	0.035 (0.011, 0.059)	0.004	0.41
	Model 2	−0.018 (−0.041, 0.006)	0.14	ref.	0.016 (−0.007, 0.039)	0.18	0.034 (0.009, 0.058)	0.008	0.85
	Model 3	−0.04 (−0.077, −0.004)	0.03	ref.	−0.021 (−0.058, 0.016)	0.26	0.019 (−0.018, 0.057)	0.31	0.40
	Hydroxybenzoic acids	−13 (−55, −7)		−4.4 (−7, −1)	3.3 (−1, 64)				
Glucose	Model 1	0.13 (−0.73, 0.99)	0.77	ref.	−0.06 (−0.94, 0.82)	0.89	−0.19 (−1.14, 0.76)	0.70	0.79
	Model 2	0.27 (−0.63, 1.18)	0.55	ref.	0.04 (−0.88, 0.96)	0.93	−0.23 (−1.22, 0.76)	0.64	0.93
	Model 3	−0.1 (−1.53, 1.33)	0.89	ref.	0.33 (−1.09, 1.76)	0.65	0.44 (−1.02, 1.89)	0.56	0.80
HbA1c	Model 1	−0.038 (−0.06, −0.016)	0.001	ref.	−0.027 (−0.049, −0.004)	0.02	0.011 (−0.013, 0.036)	0.32	0.77
	Model 2	−0.035 (−0.058, −0.013)	0.002	ref.	−0.028 (−0.051, −0.005)	0.016	0.007 (−0.018, 0.032)	0.53	0.70
	Model 3	0.001 (−0.036, 0.038)	0.96	ref.	0.007 (−0.03, 0.044)	0.70	0.006 (−0.032, 0.044)	0.75	0.77
	Lignans	−0.4 (−7,2, −0.1)		0.1 (−0.1, 0.3)	0.5 (0.3, 5.8)				
Glucose	Model 1	0.27 (−0.67, 1.2)	0.58	ref.	0.63 (−0.31, 1.58)	0.19	0.37 (−0.66, 1.4)	0.50	0.05
	Model 2	0.33 (−0.65, 1.3)	0.52	ref.	0.49 (−0.52, 1.49)	0.34	0.16 (−0.91, 1.23)	0.59	0.006
	Model 3	0.54 (−0.89, 1.96)	0.46	ref.	−1.08 (−2.51, 0.35)	0.14	−1.62 (−3.07, −0.17)	0.03	0.08

Table 3. *Cont.*

		T1 (vs. T2)	*p*	T2	T3 (vs. T2)	*p*	T3 (vs. T1)	*p*	*p*-Trend
HbA1c	Model 1	0.025 (0.002, 0.049)	0.03	ref.	0.001 (−0.024, 0.026)	0.96	−0.025 (−0.052, 0.002)	0.06	0.09
	Model 2	0.03 (0.006, 0.054)	0.01	ref.	0.003 (−0.023, 0.029)	0.82	−0.027 (−0.055, 0)	0.04	0.21
	Model 3	0.031 (−0.006, 0.068)	0.10	ref.	−0.041 (−0.078, −0.003)	0.03	−0.072 (−0.11, −0.034)	<0.001	0.003
	Stilbenes	−1.4 (−30.3, −0.6)		0.0 (−0.6, 0.6)	1.7 (0.6, 27.1)				
Glucose	Model 1	−0.54 (−1.43, 0.35)	0.24	ref.	−1.17 (−2.04, −0.3)	0.08	−0.64 (−1.54, 0.27)	0.15	0.004
	Model 2	−0.28 (−1.21, 0.66)	0.46	ref.	−1.09 (−2, −0.18)	0.02	−0.81 (−1.76, 0.14)	0.08	0.008
	Model 3	1.1 (−0.35, 2.55)	0.14	ref.	−0.6 (−2.08, 0.87)	0.42	−1.7 (−3.25, −0.16)	0.03	0.81
HbA1c	Model 1	−0.057 (−0.08, −0.035)	<0.001	ref.	−0.024 (−0.046, −0.002)	0.03	0.033 (0.011, 0.056)	0.004	0.60
	Model 2	−0.051 (−0.074, −0.028)	<0.001	ref.	−0.02 (−0.042, 0.003)	0.09	0.032 (0.008, 0.055)	0.009	0.41
	Model 3	−0.005 (−0.042, 0.033)	0.81	ref.	−0.038 (−0.076, 0.001)	0.06	−0.033 (−0.073, 0.007)	0.11	0.15

[a] Median intake (min and max) in mg/day for each tertile. [b] (β; 95% CI). We used generalized linear mixed models with the following levels: recruitment center and household. Model 1 is adjusted for sex, age (continuous), and intervention group. Model 2 is as model 1 plus education, smoking status (never, former and smokers), and physical activity in leisure time (sedentary, moderately active, active). Model 3 is as model 2 plus BMI (<30, 30–35, <35), energy intake, intake of carbohydrates, saturated fatty acids, and proteins, alcohol, and glucose-lowering treatment.

Table 4. Changes in glucose (mg/dL) and glycosylated hemoglobin (HbA1c) (%) according to tertiles of change in polyphenol intake after one year and stratified by diabetes status at baseline. Results from linear mixed models.

		Non Diabetic Participants (N = 1231, 21%)		**Prediabetic Participants (N = 2872, 48%)**		**Diabetic Participants (N = 1818, 30%)**	
		T3 vs. T1	***p***	**T3 vs. T1**	***p***	**T3 vs. T1**	***p***
Total polyphenols (mg/d)	Glucose	−0.21 (−1.95, 1.52) [a]	0.81	−1.16 (−2.25, −0.06)	0.04	−0.83 (−4.95, 3.30)	0.69
	HbA1c	0.011 (−0.040, 0.062)	0.68	0.011 (−0.017, 0.04)	0.44	−0.011 (−0.118, 0.097)	0.85
Total flavonoids (mg/d)	Glucose	−0.99 (−2.72, 0.75)	0.27	−1.66 (−2.75, −0.56)	0.001	−2.24 (−6.39, 1.90)	0.29
	HbA1c	0.025 (−0.028, 0.077)	0.36	−0.009 (−0.037, 0.02)	0.55	−0.014 (−0.122, 0.095)	0.81
Anthocyanidins (mg/d)	Glucose	0.44 (−1.31, 2.19)	0.62	−0.4 (−1.5, 0.7)	0.48	−0.80 (−4.95, 3.35)	0.71
	HbA1c	−0.004 (−0.057, 0.049)	0.87	0.003 (−0.026, 0.031)	0.85	−0.025 (−0.134, 0.083)	0.65
Catechins (mg/d)	Glucose	−0.19 (−1.91, 1.53)	0.83	−0.67 (−1.76, 0.42)	0.23	−2.08 (−6.18, 2.02)	0.32
	HbA1c	0.007 (−0.044, 0.059)	0.79	−0.003 (−0.031, 0.025)	0.84	−0.101 (−0.208, 0.006)	0.06
Proanthocyanidins (mg/d)	Glucose	−0.58 (−2.31, 1.15)	0.51	−1.2 (−2.3, −0.1)	0.03	−2.46 (−6.59, 1.68)	0.24
	HbA1c	0.033 (−0.019, 0.085)	0.21	−0.008 (−0.036, 0.021)	0.58	−0.033 (−0.141, 0.076)	0.56
Flavanones (mg/d)	Glucose	−0.08 (−1.81, 1.66)	0.93	−1.02 (−2.11, 0.08)	0.07	−1.22 (−5.34, 2.89)	0.56
	HbA1c	0.016 (−0.035, 0.068)	0.54	0.007 (−0.022, 0.035)	0.64	0.048 (−0.060, 0.155)	0.39
Flavones (mg/d)	Glucose	−1.03 (−2.78, 0.72)	0.25	−1.27 (−2.39, −0.15)	0.03	−3.02 (−7.24, 1.20)	0.16
	HbA1c	−0.000 (−0.052, 0.051)	0.99	−0.03 (−0.059, 0.000)	0.05	−0.084 (−0.194, 0.025)	0.13
Flavonols (mg/d)	Glucose	−1.39 (−3.13, 0.34)	0.12	−0.95 (−2.05, 0.15)	0.09	−2.46 (−6.58, 1.66)	0.24
	HbA1c	0.009 (−0.042, 0.061)	0.72	−0.036 (−0.065, −0.007)	0.01	−0.148 (−0.256, −0.040)	0.01
Total phenolics acids (mg/d)	Glucose	0.26 (−1.46, 1.97)	0.77	0.48 (−0.62, 1.57)	0.39	0.01 (−4.09, 4.11)	1.00
	HbA1c	0.018 (−0.032, 0.069)	0.48	0.022 (−0.007, 0.05)	0.13	0.006 (−0.101, 0.113)	0.91
Hydroxycinnamic acids (mg/d)	Glucose	0.53 (−1.19, 2.25)	0.54	0.2 (−0.89, 1.3)	0.72	0.14 (−3.97, 4.24)	0.95
	HbA1c	0.02 (−0.031, 0.071)	0.44	0.03 (0.001, 0.058)	0.04	0.003 (−0.104, 0.110)	0.95
Hydroxybenzoic acids (mg/d)	Glucose	−0.13 (−1.90, 1.65)	0.89	0.34 (−0.77, 1.46)	0.55	1.58 (−2.62, 5.78)	0.46
	HbA1c	0.009 (−0.042, 0.061)	0.72	−0.007 (−0.036, 0.022)	0.63	0.034 (−0.077, 0.144)	0.55
Lignans (mg/d)	Glucose	0.1 (−1.64, 1.85)	0.91	−0.62 (−1.73, 0.49)	0.27	−4.83 (−8.99, −0.66)	0.02
	HbA1c	−0.029 (−0.08, 0.023)	0.28	−0.039 (−0.067, −0.01)	0.01	−0.169 (−0.277, −0.062)	0.002
Stilbenes (mg/d)	Glucose	0.61 (−1.26, 2.48)	0.52	−0.21 (−1.39, 0.96)	0.73	−5.51 (−9.87, −1.14)	0.01
	HbA1c	0.022 (−0.034, 0.077)	0.45	−0.021 (−0.051, 0.01)	0.18	−0.079 (−0.192, 0.034)	0.17

[a] (β; 95% CI). We used generalized linear mixed models with the following levels: recruitment center and household. Regression models are adjusted for sex, age (continuous), and intervention group, education, smoking status (never, former and smokers), physical activity at leisure time (sedentary, moderately active, active), BMI (<30, 30–35, <35), energy intake, and intake of carbohydrates and saturated fatty acids.

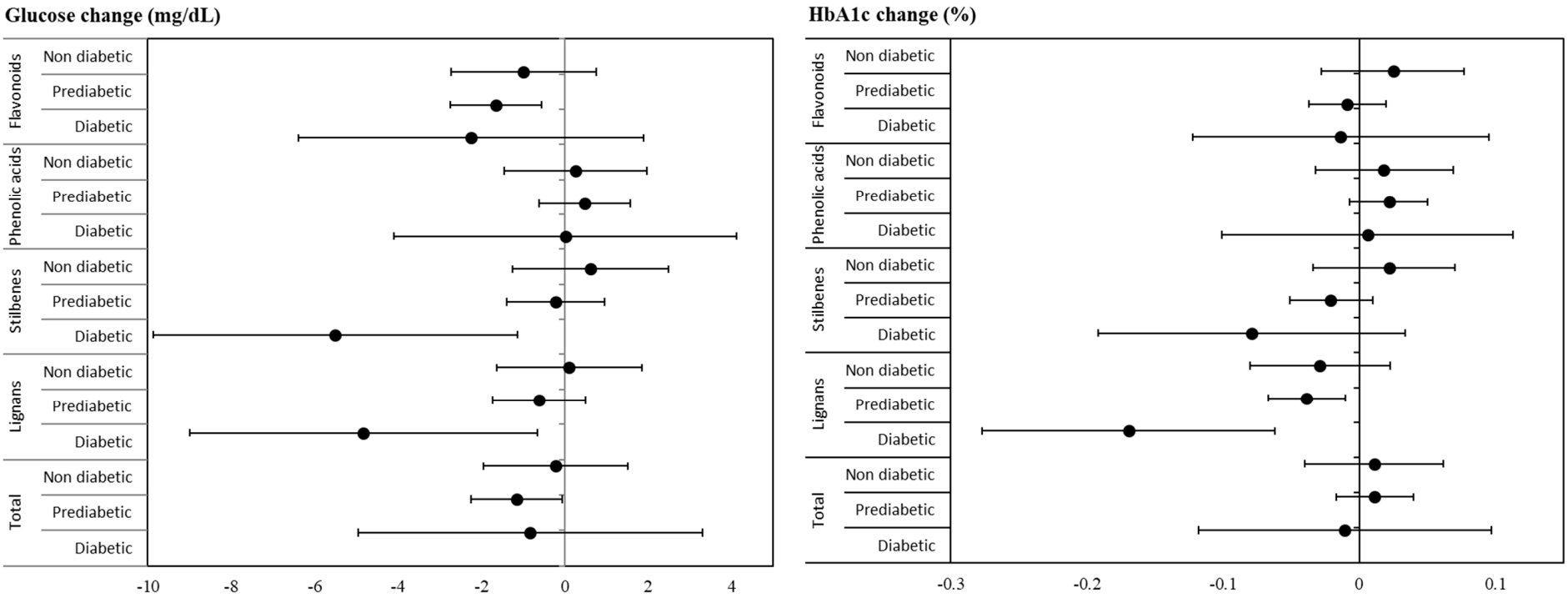

Figure 2. Glucose (mg/dL) and glycosylated hemoglobin (%) changes after one year (β; 95% CI) comparing extreme tertiles of energy-adjusted polyphenol intake by diabetes status at baseline. Results from fully adjusted linear mixed models.

4. Discussion

This work shows a longitudinal inverse association between certain classes of polyphenols and levels of glucose and HbA1c in the PREDIMED-Plus cohort after one year of follow-up. To our knowledge, this is the first study to assess the relationship between changes in the intake of all polyphenol groups and T2D-related parameters in a senior population with or at high risk of T2D.

Although evidence is still limited, it has been suggested that the benefits of dietary polyphenols regarding T2D may include anti-inflammatory, antioxidant, and glucose metabolism regulatory effects, such as the inhibition of α-amylases and α-glucosidases, protection against glucose toxicity in pancreatic β-cells [17], and modulation of glucose transporter type-4 (GLUT4) receptors.

4.1. Anthocyanidins

Anthocyanidins are a subtype of flavonoids responsible for many of the red to violet colors in fruits and vegetables. The main food sources of anthocyanins are berries, including grapes and derivatives such as wine [4]. We found a significant inverse association between changes in anthocyanin intake and HbA1c levels (T3 vs. T2), although it was insignificant for glucose levels. In line with this result, a 12-week randomized double blind placebo-controlled trial showed that daily supplementation of 320 mg of anthocyanidins in 160 prediabetic participants significantly reduced HbA1c, while no significant changes were observed in glucose levels [18]. In the same study population, a higher anthocyanin intake was correlated with a lower prevalence of T2D in overweight men [19]. Moreover, in a meta-analysis of cohort studies, Guo et al. showed a 5% decrease in T2D risk with each 7.5 mg/day increment in anthocyanin intake [20].

4.2. Proanthocyanidins

Proanthocyanidins are classified as flavanols, together with catechins and theaflavins [4]. An increase in proanthocyanidin intake was associated with a decrease in fasting glucose and HbA1c levels, although not in the fully adjusted model. In the stratified analyses no significant results were observed, except for fasting glucose levels in prediabetic participants. This was in line with the null effects observed in clinical trials administering proanthocyanidin supplements in T2D patients [21,22], whereas some evidence suggests this flavanol can improve insulin resistance [23]. Although previous studies with the same population found inverse associations between proanthocyanidin and catechin intake and T2D risk, in the present work only proanthocyanidins had an effect on T2D indicators [19].

4.3. Flavones

Flavones were inversely associated with fasting glucose levels and HbA1c in prediabetic participants. The main food sources of flavones in the study population were whole grain products, bread, and oranges. No associations between flavones and T2D risk were previously found in the same study population or a similar cohort at high risk of cardiovascular disease [19,24].

4.4. Flavonols

In the case of flavonols, the main dietary sources were red wine and vegetables such as onion, spinach, and lettuce. After one year of follow-up, a significant increase in vegetable consumption was observed, especially in participants with a higher intake of dietary polyphenols. Changes in flavonol intake were inversely associated with changes in HbA1c levels in both prediabetic and diabetic participants, which is in line with the antidiabetic effects of flavonols postulated by two large observational studies [25,26]. However, in the present study, an increase in flavonol intake was significantly correlated with higher fasting glucose levels, although the correlation was not significant in the stratified analysis. These findings agree with previous observations for T2D risk in the same cohort [19].

4.5. Hydroxybenzoic and Hydroxycinnamic Acids

Total phenolic acid intake was not associated with T2D parameters, yet interestingly, the intake of hydroxycinnamic acids was directly associated with an increase in fasting glucose and HbA1c levels, except in the fully adjusted model, and the highest increase in HbA1c levels was observed in prediabetic participants. Hydroxycinnamic acids accounted for more than 90% of the phenolic acid intake, coffee being the main food source. In contrast, in a prospective analysis of 4923 T2D participants, drinking two or more cups of coffee per day was associated with a 41% reduction in all-cause mortality risk [27]. Similar findings were reported in a meta-analysis of ten prospective cohort studies, where the risk for all-cause mortality was reduced in a coffee-consuming T2D population [28]. It should be stressed that the antidiabetic properties of coffee are likely to be mediated by the polyphenol content rather than caffeine [29,30]. No significant association was observed for hydroxybenzoic acids; among them, ellagic acid has been correlated with lower HbA1c and fasting glucose levels and is reported to promote insulin secretion [31].

4.6. Lignans

Even though the ingestion of lignans is low compared to other polyphenol subclasses, its intake has been associated with several health benefits. The main food sources of lignans in this cohort were fiber-rich foods, such as whole grain cereals and olive oil. In the present analysis, a higher intake of fiber was observed in participants who increased their dietary polyphenol intake after one year of follow-up. Those with the highest increase in lignan intake, especially prediabetic and diabetic participants, had lower levels of fasting glucose and HbA1c in the fully adjusted model. These results agree with previous findings in the PREDIMED cohort and in two U.S. women cohorts [32]. Dietary lignan intake has been linked with improved glycemic control, mainly HbA1c and fasting plasma glucose levels, but the evidence from observational studies assessing its effect on T2D risk is limited [33–35]. The antidiabetic effects exerted by lignans may be mediated by improvements in central obesity [36]. Other potential mechanisms of action include an inhibition of α-amylase and α-glucosidase, improvements in insulin sensitivity, activation of AMPk and GLUT4 receptors, and acting as antagonists of adiponectin receptors [37]. Associated improvements in fasting plasma glucose levels in non-diabetic patients have also been observed [34].

4.7. Stilbenes

Stilbenes, mainly resveratrol, have been previously associated with a lower risk of T2D in the PREDIMED cohort [24]. However, in the present study changes in stilbene intake showed only a mild inverse association with alterations in fasting plasma and HbA1c levels, which was stronger in diabetic participants. Lui et al. performed a meta-analysis of 11 controlled trials administering trans resveratrol in overweight or obese individuals to assess whether its consumption affected glycemic status or insulin sensitivity [38]. Notably, in alignment with our findings, non-significant effects on glycemic measurements were observed in non-diabetic participants. The main food source of stilbenes is red wine, and its moderate intake has been associated with a lower risk of T2D [39].

4.8. Effect Modification by Diabetes Status

The improvements in HbA1c levels observed in the present study are similar to those arising from other dietary interventions in T2D patients, such as high-fiber diets or health education programs [10,40]. According to the United States Food and Drug Administration, even a modest reduction in HbA1c levels (0.3 to 0.4%) reduces the risk of developing diabetes [41].

Polyphenol intake has been shown to have a modulatory effect on the gut microbiota profile [42]. It is also recognized that the gut microbiota plays a key role in the development of T2D, due to its implication in carbohydrate metabolism. Moreover, intestinal dysbiosis has been described in both T2D and prediabetic patients, indicating that certain compo-

sitional changes in the microbiota participate in the development of the disease [43,44]. Another potential mechanism underlying the antidiabetic effect of polyphenols is the induction of GLP-1 secretion [45]. The GLP-1 signaling pathway has been extensively explored to develop effective therapies for T2D, and emerging evidence shows that some phenolic compounds can stimulate GLP-1 secretion from intestinal L-cells and may, therefore, be helpful in improving glucose homeostasis [45].

4.9. Strengths and Limitations

The limitations of this study are mostly related to the estimation of polyphenol intake and the confounding variables, as residual confounding factors may still be present. Regarding polyphenols, although we used the most updated and comprehensive database available (Phenol-explorer), not all foods from the FFQ were included in the database (e.g., honey), and the questionnaire does not cover all polyphenol-rich foods (e.g., spices) or the polymeric, non-extractable polyphenols associated with cell wall macromolecules. Furthermore, phenolic intake can be affected by the variable polyphenol content in foods, which depends on ripeness, environmental factors, processing and storage, and variety [4]. Finally, bioavailability was not considered, and the results might not be generalizable to different populations.

The main strengths of the study are the large sample size, the multicenter design, and longitudinal approach. Regarding sociodemographic and lifestyle variables (confounders), a standardized protocol was used to reduce the information bias.

5. Conclusions

Evidence suggests that a regular consumption of dietary polyphenols is associated with improvements in essential biological outcomes for T2D prevention and management. However, assessing the health benefits of polyphenol intake is complex due to their diverse chemical structure and variable bioavailability, the complexity of estimating their content in foods and therefore their intake, potential interactions with other nutrients or polyphenols, and biological aspects that may modify metabolization [46]. Even though the protective role of dietary polyphenols in health has been widely demonstrated, more randomized clinical trials are needed to clarify how their consumption affects biomarkers related to T2D. Moreover, more information is needed to determine which polyphenol subclasses are the most beneficial and which food sources produce the best results in terms of T2D prevention and management.

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Informed Consent Statement: All participants gave written informed consent.

Data Availability Statement: Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval of the PREDIMED-Plus Steering Committee. There are restrictions on the availability of data for the PREDIMED-Plus trial, due to the signed consent agreements around data sharing, which only allow access to external researchers for studies following the project purposes. Requestors wishing to access the PREDIMED-Plus trial data used in this study can make a request to the PREDIMED-Plus trial Steering Committee chair: jordi.salas@urv.cat. The request will then be passed to members of the PREDIMED-Plus Steering Committee for deliberation.

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Manuscript IV

Title: "Association between changes in dietary polyphenol intake and body adiposity after one-year of follow-up: PREDIMED-Plus sub-study."

Authors: Sara Castro-Barquero, Anna Tresserra-Rimbau, Eric B Rimm, Dora Romaguera, J Alfredo Martínez, Jordi Salas-Salvadó, Miguel Ángel Martínez, Josep Vidal, Miguel Ruiz-Canela, Jadwiga Konieczna, Rosa Casas, Montse Fitó, Rosa Lamuela-Raventós, Ramon Estruch.

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Tittle: Association between changes in dietary polyphenol intake and body adiposity after one-year of follow-up: PREDIMED-Plus sub-study.

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Abstract (162 words):

Recent evidence suggests that dietary intake of different polyphenols has been associated with obesity and obesity-related inflammation. However, the essential step towards the understanding of the protective effects of polyphenols on overweight/obesity and body composition depends on the adequate estimation of their consumption by dietary recalls. A prospective analysis of 1,195 participants included in the PREDIMED-Plus study was performed. Total polyphenols and polyphenol subclasses intake were measured by food-frequency questionnaires (FFQ) by matching food consumption data with Phenol-Explorer database. Dual-energy X-ray Absorptiometry (DXA) measurement was performed to obtain body weight, android, gynoid and visceral fat mass. Participants were distributed into tertiles of changes in consumption after 1 year, with tertil 1 (decreased intake) as the reference category. Lineal mixed-effect models were used to assess associations between changes in polyphenol subclasses intake and adiposity indicators over the first year of follow-up. We found an inverse association between some classes of polyphenols and body adiposity markers, mainly visceral adipose tissue and total fat mass.

Keywords: Polyphenols, adiposity, Mediterranean diet, flavonoids, body composition.

Abbreviatures:

DXA: Dual-energy X-ray absorptiometry

VAT: Visceral adipose tissue

SAT: Subcutaneous adipose tissue

MD: Mediterranean diet

MetS; Metabolic syndrome

CVD: Cardiovascular diseases

BMI: Body mass index

PA: Physical activity

FFQ: Food frequency questionnaire

EVOO: Extra virgin olive oil

1. Introduction

Global overweight and obesity are increasing in an alarming rate and the health consequences associated have been widely recognized: as a risk factor of overall mortality, cardiovascular diseases (CVD), metabolic disturbances such as high blood pressure, dyslipidemia, alteration of glucose metabolism, cancer, osteoarthritis, chronic kidney disease, gynecological alteration, among others [1]. Obesity is a complex multifactorial disease defined as an excess of fat mass, which occurs through adipocyte hypertrophy and hyperplasia. Nevertheless, fat distribution, particularly excess of abdominal fat and visceral adipose tissue (VAT), has been associated with higher cardiovascular (CV) risk than general obesity or peripheral fat mass [2,3]. Adipose tissue is an endocrine organ with the capacity to secrete hormones, such as leptin, adiponectin and resistin, and wide range of inflammatory adipocytokines, such as TNF-α and interleukins. However, excess of VAT is associated with higher secretion of pro-inflammatory adipocytokines, leading to systemic inflammation and the obesity-related metabolic disturbances mentioned [4,5]. This excess of adipose tissue and inflammatory response can be reversed by life-style modification, including physical activity promotion and following a healthy dietary pattern such as Mediterranean Diet (MD) [6,7]. Following a MD prevents the development of CVD, as reported by observational and intervention studies such as the PREDIMED (Prevención con DIeta MEDiterránea) [8,9]. MD is rich in polyphenols, plant-derived bioactive compounds characterized by the presence of aromatic ring and attached hydroxyl groups, which are present in the main key-foods of

this dietary pattern (extra virgin olive oli (EVOO), nuts, vegetables, wine, vegetables, fruits and whole-grain cereals). Phenolic compounds are potentially responsible for the beneficial effects of the MD as its prolonged intake has been associated to improvements in all the classical CVD risk factors and anti-inflammatory effects [10,11]. Therefore, other polyphenol effects involved in body weight management have been described as inducing satiety, prebiotic effect for gut microbiota, inducing β-oxidation, among others [12]. Among phenolic compounds, the intake of resveratrol showed significant reduction in body weight, body mass index (BMI), waist circumference and fat mass in obese subjects [13,14]. Its intake has been associated to anti-inflammatory and anti-oxidative properties, which may also improve obesity-related indices of metabolic disorders [15]. Besides, another interesting phenolic-rich food is green tea, which intake has been associated to anti-obesogenic effects [16]. Evidence for polyphenol's effects on obesity and weight control is inconsistent because of the heterogeneity among study designs, characteristics of study population, duration of the intervention and the formulation and dosage of polyphenol supplements. Our objective was to evaluate whether higher intake of dietary polyphenols in an energy-restricted MD diet and physical activity (PA) promotion or control diet based on the traditional MD on the 12-months effects of the intervention was associated with greater improvements on body composition parameters

Methods

1.1. Study design

The present study was a prospective cohort analysis based on data collected during the first year of the PREDIMED-Plus (PREvención don DIeta MEDiterránea Plus) study, an ongoing randomized, large-scale, parallel, multicenter intervention-controlled trial conducted in 23 centers of the National Spanish Health System designed to assess the effect of an energy restricted MD and physical activity promotion on cardiovascular morbidity and mortality in individuals with metabolic syndrome (MetS).

Volunteers were men (aged 55-75 years) and women (60-75 years) free of documented history of CVD with overweight/obesity (BMI ≥27 and <40 kg/m^2) who met at least three or more criteria of the MetS: abdominal obesity (waist circumference >102 cm in men or ≥88 cm in women), hypertension, hypertriglyceridemia, low HDL-cholesterol levels and hyperglycemia or diagnosed with type 2 diabetes mellitus [17]. Recruitment period lasted from 5 September 2013 to 31 October 2016. The study protocol, eligible and exclusion criteria can be found at http://predimedplus.com. Individuals were randomly assigned to an intervention or control group. Intervention arm promote an energy-restricted MD together with PA and behavioral support while the control group received usual health

care for CVD prevention and advice on following traditional MD without energy restriction or PA promotion.

The trial protocol was approved by local institutional ethic committees, registered under the International Standard Randomized Controlled Trial Number 89898870 (ISRCT:http://www.isrctn.com/ISRCTN89898870). All participants provided written informed consent before joining the study.

For the present analysis, of the total sample of 6,874 randomized participants, subsample of 1,569 participants from 7 recruiting centers underwent total body DXA scans at baseline. Of those, we excluded 37 participants reported energy intake values outside the predefined limits (<3,347kJ [800 kcal]/day or >17,573 kJ [4,000 kcal]/day for men; <2,510 kJ [500 kcal]/day or >14,644 kJ [3,500 kcal]/day for women) at baseline and during follow-up [18]. We also excluded participants having missing data on dietary information (n=159) and lack of data on DXA (n=178) at baseline and during the follow-up. Finally, a total of 1,195 participants were available for the present analysis. Of those, only 1,157 participants had available data on VAT at baseline.

1.2. Dietary assessment and polyphenol intake

At baseline, after six months and yearly visits participants completed a validated 143-item semi-quantitative FFQ [19], from which total energy, nutrients and polyphenol intake were estimated according to Spanish food composition tables and Phenol-Explorer database (version 3.6) [20]. The validity of this FFQ to assess total polyphenol intake was tested [21,22]. As described elsewhere [23], dietary polyphenol intake was estimated by multiplying polyphenol content in food (mg/100g of food) by the daily consumption of each food (g/day). Total polyphenol intake and polyphenol subclasses were calculated as the sum of all individual polyphenol intakes from the food sources reported from the FFQ. Afterward, polyphenol subclasses were adjusted for total energy intake using the residual method [18].

1.3. Anthropometric and body composition parameters

Anthropometric parameters were measured in every follow-up visit by trained dietitians according to the PREDIMED-Plus protocol. Body weight (kg) and height (cm) were measured in light clothing without shoes or accessories using a high-quality calibrated scale and wall-mounted stadiometer, respectively. Waist circumference (cm) was measured midway between the lowest rib and the iliac crest and hip circumference (cm) was determined at the widest part. BMI was calculated as weight (kg) divided by the square of height (m^2).

Direct measures of body composition were performed with DXA scanner (GE Healthcare/DXA Lunar Prodigy Primo and Lunar iDXA; Madison, WI) connected with enCore^{TM} software. For VAT measure, scans were reanalyzed using validated CoreScan software application [24]. The measures obtained are indicators of overall adiposity, as total fat mass (g) and total body fat (%), and regional adiposity, as trunk, leg, android and gynoid fat mass (g). As described elsewhere [25], to calculate subcutaneous adipose tissue (SAT) (g), VAT is subtracted from android fat mass. Body composition measures had to be performed preferably within two months from the follow-up visit (at baseline, six months and yearly). DXA scans were performed and calibrated daily by trained operators according to the standard protocols provided by the manufacturer. Participants were scanned wearing examination gown. Android-to-gynoid fat ratio was calculated by dividing total fat mass (g) from the android fat mass (g).

1.4. Covariables assessment

Self-reported questionnaire at baseline provided data on sex, age, smoking status, educational level, history of medical condition, lifestyle habits and medication use. A 17-point score was used to assess Mediterranean diet adherence [26] at baseline and one-year follow-up visits. The validated REGICOR short self-reported physical activity questionnaire was used to assess total leisure-time PA (METs min/week) [27] and the validated Spanish version of the Nurses' Health Study questionnaire to assess sedentary behaviors [28]. Sociodemographic and lifestyle variables were categorized as follows: educational level (three categories: primary, secondary, or high school), physical activity level (three categories: low, moderate, or high), BMI (three categories: 27.0–29.9, 30.0–34.9, or ≥35 kg/m2), and smoking status (three categories: never, former, or current smoker).

1.5. Statistical analysis

Baseline characteristics of study participants were expressed as means and standard deviations (SD) for continuous variables, and frequencies and percentages for categorical variables. Differences in baseline characteristics by tertiles of change in polyphenol consumption 1 year versus baseline were analyzed using one-way ANOVA or X^2 test, as appropriate.

Adiposity indicators became normalized into sex-specific z-scores (mean=0; SD=1), in order to ensure comparability considering sexual dimorphism in body fat distribution. Linear mixed-effect models with random intercepts at recruiting center, cluster family and patient level were used to assess associations between changes in polyphenol subclasses intake and adiposity indicators over the first year of follow-up. Total

polyphenol and the main polyphenol subclasses intake were distributed according into tertiles of changes in consumption after 1 year, with tertil 1 (decreased intake) as the reference category. To assess the linear trend (*p* for trend) across tertiles of polyphenol intake, the mean value was assigned to each tertil. Model 1 was minimally adjusted for age, sex, study arm and follow-up time. Model 2 was further adjusted for educational and physical activity levels and smoking status (all categorical). Model 3 was further adjusted for baseline variables such as height and repeatedly measured energy intake, carbohydrate, protein, saturated fatty acids and alcohol intake (all continues). Statistical analyses were performed using Stata v16.0 and statistical significance was set at $p < 0.05$ We used the PREDIMED-Plus longitudinal database generated on 26th of June 2020 (202006290731_PREDIMEDplus).

2. Results

Baseline characteristics of study participants according to tertiles of changes in polyphenol consumption are shown in **Table 1**. The mean age of study participants was 65.5 years, 46.2% of the participants were women, and with elevated BMI (mean 32.4 kg/m^2, SD 3.3 kg/m^2) and waist circumference (mean 107.1 cm, SD 9.2 cm). Participants with the highest total dietary polyphenol consumption (T3, mean total polyphenol consumption 1 year compared to baseline: 241.1 mg/day) showed significantly lower adherence to 17-points MD score (*p*-value 0.004). Interestingly, significantly higher rate of participants were located in intervention arm (*p*-value 0.024). Furthermore, significant differences among tertiles were observed for energy intake and antihypertensive treatment (*p*-value 0.042 and 0.033, respectively).

The associations between changes in the main polyphenol classes intake and sex-specific z-scores of VAT, android-to-gynoid ratio and total fat mass after controlling for potential confounders are shown in **Table 2.** No significant association were observed between the main polyphenol subclasses and VAT, except for flavonoids, which T3 showed a significant inverse association with VAT changes (β -0.10 z-score, 95% CI [-0.019 to -0.01]) and linear trend was significant (*p* for trend = 0.038). No significant associations were found for android-to-gynoid ratio. In the case of total fat mass, higher lignans intake were inversely associated with total fat mass (*p* for trend = 0.001; T2 -0.09 [-0.16 to -0.02] and T3 -0.12 [-0.20 to -0.05]). The same inverse associations were found between VAT and changes in flavonoids intake (*p* for trend = 0.041) (**Table 3**). When we analyzed the association with the sub-classes of polyphenols (**Table 3.1**), we found significant inverse associations between VAT and flavonoids subclasses, such as

catechins (full-adjusted model, T2: -51.3g [-118.0 to 15.3]; and T3: -85.2g [-152.0 to -18.3]; *p* for trend 0.013) and flavonols for all the tertiles (all *p* for trend <0.001). Moreover, significant inverse association between VAT and hydroxybenzoic acids were observed for all the adjusted levels (all *p* for trend <0.01). **Supplementary Table 1** showed the associations between polyphenol intake and fat distribution indicators using tertil 2 (no-changes in dietary polyphenol intake) as the reference category. Inverse associations were observed between T1 vs T2 and T2 vs T3 of lignans and total fat mass (full-adjusted model, T1 vs T2: 0.09 [0.02 to 0.16] and T2 vs T3: -0.03 [-0.10 to 0.04], *p* for trend 0.001). In this sense, similar associations were observed between tertiles of stilbens and total fat mas (full-adjusted model, T1 vs T2: -0.00 [-0.07 to 0.07] and T2 vs T3: -0.08 [-0.15 to -0.00], *p* for trend 0.041). We evaluated the associations between VAT and tertiles of change of the main polyphenol families and subclasses according to sex (**Supplementary Table 2**). No significant associations were observed in VAT changes and polyphenol intake for men. In the case of women, significant inverse association were found between catechin intake and VAT (full-adjusted model *p* for trend = 0.036). Moreover, significant associations were found between flavonoids and lignans with VAT changes in the minimally adjusted model (*p* for trend = 0.046, both).

3. Discussion

The present study shows an inverse association between some dietary polyphenol subclasses and body adiposity parameters, such as VAT and total fat mass after 1 year of follow-up in the PREDIMED-Plus trial. To our knowledge, this is the first study that assessed the relationship between changes in dietary polyphenol intake and body adiposity parameters measured by DXA scan in a middle-aged population with MetS.

In weight-loss oriented interventions, such as the energy-restricted MD and physical activity promotion designed in the PREDIMED-Plus study, body composition is a key role of the intervention effectiveness. Lean body mass/total body fat ratio, which is consider as a more favorable body composition, suggests that life-style interventions for weight-loss should include physical activity promotion in order to prevent or delay age and weight-loss related sarcopenia. Moreover, DXA-derived indicators of body composition, especially abdominal obesity, showed higher ability to predict abnormal cardiovascular and metabolic risk parameters than classical anthropometric indicators [29]. The magnitudes of the present associations were small per increased tertil or sex-specific z-score (SD). However, losing even small amounts of body weight are associated with health improvements [30,31]. The benefits of dietary polyphenol associated with body

composition include activation of lipolysis and β-oxidation, inhibit lipogenesis and adipocyte proliferation and exert a prebiotic effect for gut microbiota [12].

Flavonoid's intake has been associated with weight maintenance and several mechanisms underlying body weight regulation have been described for flavonoids, such as decrease fat absorption, inhibit adipogenesis and increase glucose uptake and energy expenditure [32–36].

Among flavonoids subclasses, anti-obesogenic effects of catechins have been previously reported, especially with green tea catechins enriched beverages or supplements. Hursel *et al.* observed in a meta-analysis of 11 trials, a significative weight loss (mean body weight changes -1.31kg, 95% IC: -2.05 to -0.57; *P*<0001) [37]. The inverse association observed between catechins intake and fat distribution has been already described by Hibi *et al.* [38]. A post hoc analysis from six human trials with 921 participants observed a significant reduction in total fat area (17.7cm^2,95%CI: -20.9 to -14.4) and visceral fat area (-7.5cm^2, 95%CI: -9.3 to -5.7) [38]. Nonetheless, the effects of a supplement of epigallactocatechin-3-gallate combined with resveratrol on total body weight were not demonstrated in the randomized doble-blind study with 38 overweight and obese participants, while VAT tended to decrease after the twelve-weeks intervention [39].

In the case of anthocyanidins, which contribution to total flavonoid intake is significant, Jenning *et al.* observed in a cross-sectional study of 618 participants aged 25 to 83 years a significant association between higher consumption of anthocyanins with lower VAT (T3-T1: -0.49 dm^3; p-value 0.03) [40].

Quercetin, a flavonoid abundant in onions, fruit, tea and wine, has been associated with anti-obesogenic properties due to its capacity to inhibit fat accumulation by the suppression of the expression of peroxisome proliferator-activated receptor γ, which increase the expression of cyclic adenosine monophosphate to promote lipolysis [41,42]. Nevertheless, inconclusive results are observed in quercetin-rich intervention in human trials [43].

In the present study, no significant associations were observed between total phenolic acids intake with body composition parameters. Coffee, which is the main food source of phenolic acids in diet, has been associated with improvements in several MetS components, including total fat mass [44,45]. Moreover, aligned with our results, the QUENCH trial showed non-significant effects on body composition after 4-weeks supplemented with dried purple carrot rich in phenolic acids (259,2 mg/day) [46].

Interestingly, despite non-significant results were observed for total phenolic acids intake, significant associations with hydroxybenzoic acids and VAT, but no association were found for hydroxycinnamic acids.

Even though lignan intake is low compared to flavonoids or phenolic acids intake, several cross-sectional data have described the association between lignans intake and lower fat mass and central obesity [47,48]. In this sense, a prospective study with 1,111 US women showed significant association between lower BMI at baseline and higher urinary excretion of lignan metabolization and slower weight gain [49]. Similar finding was observed in a middle-aged SU.VI.MAX cohort after 6-years of follow-up, showing an inverse association between higher lignans intake and lower BMI (-0.28 kg/m^2, 95%CI: -1.63; -0.09) and waist circumference (-1.16 cm, 95%CI: -1.80; -0.51) [50]. Considering body adiposity measurements, Morisset *et al.* reported significant associations between plasma enterolactone, the main biological active lignan metabolite, and body fat distribution measured by computed tomography [51]. Similar to our findings, higher intake of lignans, which was represented by plasma enterolactone levels, was associated with a trend for a lower total and VAT areas in 115 post-menopausal women.

Stilbene intake, mainly represented by resveratrol, has been associated with body composition improvements, but most of the meta-analysis and randomized clinical trials are conducted with resveratrol supplementation, not estimating dietary stilbenes intake. Mousavi *et al.* conducted a meta-analysis of 28 randomized controlled trials assessing the effect of resveratrol supplementation on body composition [13]. Significant effects were observed for body weight (mean differences -0.51 kg, 95%CI: -0.94 to -0.09), BMI (-0.17 kg/m^2, 95%CI: -0.32 to -0.03) and waist circumference (-0.79 cm, 95%CI: -1.39 to -0.2). Interestingly, non-significant results were observed for total fat mass, while our results showed significant association with total fat mass. Aligned with our findings, a recent meta-analysis of 36 randomized clinical trials showed significant effect of resveratrol supplementation on total fat mass (-0.32%, 95%CI: -0.62 to -0.03) [14] .

The major strengths of the present study are its large sample size, the multicenter design, and high-quality data as we have gathered clinical data on numerous health variables collected in repeated in-person study visits. Unlike previous studies, we examined wider spectrum of body composition using DXA-scan rather than anthropometry. Moreover, measurements of adiposity, food intake, physical activity and other covariables were based on physical and laboratory examinations performed by trained nutritionist and other stuff with use of standardized protocols, in order to minimize the measurement errors. VAT data was determined based on an automated algorithm

from the DXA manufactures, avoiding predictive estimation that may underestimate this fat depot. Additionally, the use of the Phenol-Explorer as the most comprehensive food composition database on dietary polyphenols and the FFQ used was validated to evaluate total polyphenol intake in both clinical and cross-sectional studies [22]. We acknowledge some limitations. First, we used a prospective cohort design, which does not allow attributing conclusions to plausible causes. Second, generalizability of our findings to other study populations than middle edge to elderly Caucasian population with MetS is hindered. Third, self-reported information on drug use, dietary intake, physical activity, and some other covariates may imply some residual confounding in our analyses. Fourth, regarding to polyphenol intake, other factors that may affect food polyphenol content were not collected, such as variety, ripeness, culinary technique, storage among others.

4. Conclusion

Regular consumption of dietary polyphenols is associated with improvements in body composition, especially flavonoids and stilbenes. However, assessing dietary polyphenol intake is complex due to its bioavailability, its content in foods and content variations due to food processing and the potential interaction with other nutrients and dietary components. Even though significant associations were observed between several polyphenol subclasses and body composition parameters, more randomized clinical trials are needed it clarify how its consumption and which polyphenol subclasses affects body composition.

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Figure 1: Study flow-chart

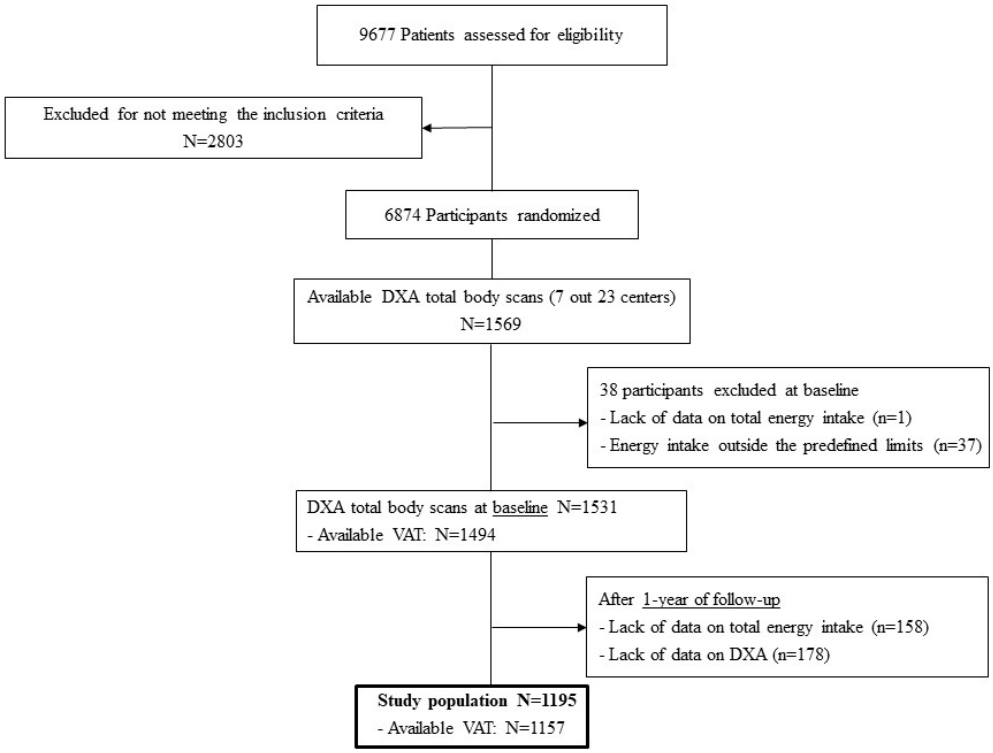

Table 1. Baseline characteristics according to tertiles of changes in energy-adjusted polyphenol intake (n=1195)

1 year – baseline intake of total polyphenol intake (*mg/day*), median (min to max)	**Total**	**T1** -214.3 (-2008; -91.5)	**T2** 6.5 (-91.4; 105.5)	**T3** 241.1 (105.5; 1408)	**P-value**
No. of subjects	1195	399	398	398	
Age, *years*	65.5±4.9	65.7±5.0	65.2±4.9	65.5±4.9	0.381
Women, *n (%)*	552 (46.2)	183 (45.9)	186 (46.7)	183 (46.0)	0.965
BMI, kg/m^2	32.4±3.3	32.2±3.1	32.5±3.3	32.5±3.4	0.255
Waist circumference, *cm*	107.1±9.2	106.6±8.8	107.3±9.3	107.4±9.5	0.406
Intervention arm, *n (%)*	583 (48.8)	175 (43.9)	195 (49.0)	213 (53.5)	0.024
Adherence to ER-MedDiet, 17p score	8.5±2.6	8.8±2.6	8.3±2.6	8.3±2.6	0.004
Total energy intake, *kcal/day*	2397±523	2404±522	2347±518	2440±525	0.042
Type 2 diabetes prevalence, *n (%)*	331 (27.7)	120 (30.1)	97 (24.4)	114 (28.6)	0.174
Current smokers, *n (%)*	155 (13.0)	50 (12.5)	55 (13.8)	50 (12.6)	0.974
Physical activity, (*MET.min/week), mean±SD*	2765±2343	2880±2503	2589±2149	2825±2360	0.177
Medication*, n (%)*					
Antihypertensive agents	961 (80.4)	336 (84.2)	306 (76.9)	319 (80.1)	0.033
Colesterol-lowering agents	601 (50.3)	211 (52.9)	188 (47.2)	202 (50.7)	0.274
Insulin	42 (3.51)	16 (4.01)	13 (3.27)	13 (3.27)	0.805
Metformina	251 (21.0)	87 (21.8)	86 (21.6)	78 (19.6)	0.699
Other hypoglycemic agents	204 (17.1)	72 (18.0)	62 (15.6)	70 (17.6)	0.616
Aspirin or antiplatelet agents	157 (13.1)	61 (15.3)	41 (10.3)	55 (13.8)	0.101
NSAIDS	284 (23.8)	84 (21.0)	94 (23.6)	106 (26.6)	0.180
Vitamin and minerals	116 (9.71)	34 (8.52)	47 (11.8)	35 (8.79)	0.220
Sedative or tranquillisers agents	269 (22.5)	79 (19.8)	88 (22.1)	102 (25.6)	0.140
Hormonal treatment (only women)	46 (3.85)	22 (5.51)	14 (3.52)	10 (2.51)	0.135
Educational level, *n (%)*					0.776

Primary school	579 (48.4)	197 (49.4)	189 (47.5)	193 (48.5)	0.888
Secondary school	362 (30.3)	114 (28.6)	123 (30.9)	125 (31.4)	
University and other studies	254 (21.2)	88 (22.1)	86 (21.6)	80 (20.1)	

Energy-restricted Mediterranean diet (ER-MedDiet), body mass index (BMI), high-density and nonsteroidal anti-inflammatory drugs (NSAIDs). Continue variables are expressed as mean (±SD). Categorical variables are expressed as number (n) and percentage (%). Comparisons between groups with Pearson's chi square test for categorical variables or one-way ANOVA for continuous variables.

Table 2: Association between changes in total polyphenol and main polyphenols subclasses intake and fat distribution (sex-specific z-scores) from baseline to 12-months follow-up visits.

	T1	T2	T3	
	B (95% CI)	B (95% CI)	B (95% CI)	*P* for trend
		Total polyphenols		
Visceral fat				
Model 1	Reference	-0.00 (-0.09; 0.08)	-0.08 (-0.17; 0.01)	0.081
Model 2	Reference	-0.01 (-0.09; 0.08)	-0.08 (-0.17; 0.01)	0.079
Model 3	Reference	-0.01 (-0.10; 0.08)	-0.08 (-0.16; 0.01)	0.099
Android-to-gynoid fat ratio				
Model 1	Reference	0.03 (-0.05; 0.12)	-0.01 (-0.10; 0.07)	0.744
Model 2	Reference	0.03 (-0.05, 0.12)	-0.01 (-0.10; 0.07)	0.748
Model 3	Reference	0.03 (-0.06; 0.11)	-0.02 (-0.10; 0.07)	0.718
Total fat mass				
Model 1	Reference	-0.02 (-0.09; 0.06)	-0.05 (-0.12; 0.03)	0.150
Model 2	Reference	-0.02 (-0.09; 0.05)	-0.05 (-0.13; 0.02)	0.146
Model 3	Reference	-0.02 (-0.09; 0.06)	-0.05 (-0.12; 0.03)	0.198
		Flavonoids		
Visceral fat				
Model 1	Reference	-0.00 (-0.09; 0.08)	-0.08 (-0.17; 0.01)	0.068
Model 2	Reference	-0.01 (-0.10; 0.08)	-0.09 (-0.18; 0.00)	0.057
Model 3	Reference	-0.02 (-0.11; 0.07)	-0.10 (-0.19; -0.01)*	**0.038**
Android-to-gynoid fat ratio				
Model 1	Reference	-0.01 (-0.10; 0.07)	-0.03 (-0.11; 0.06)	0.504
Model 2	Reference	-0.01 (-0.10; 0.07)	-0.03 (-0.12; 0.05)	0.451
Model 3	Reference	-0.02 (-0.10; 0.07)	-0.04 (-0.12; 0.05)	0.416
Total fat mass				
Model 1	Reference	0.03 (-0.05; 0.10)	-0.04 (-0.11; 0.03)	0.258

Model 2	Reference	0.02 (-0.05; 0.09)	-0.04 (-0.12; 0.03)	0.240
Model 3	Reference	0.02 (-0.05; 0.09)	-0.04 (-0.12; 0.03)	0.248
		Phenolic acids		
Visceral fat				
Model 1	Reference	0.02 (-0.07; 0.11)	-0.03 (-0.12; 0.05)	0.454
Model 2	Reference	0.02 (-0.07; 0.11)	-0.03 (-0.12; 0.05)	0.451
Model 3	Reference	0.02 (-0.06; 0.11)	-0.03 (-0.12; 0.06)	0.556
Android-to-gynoid fat ratio				
Model 1	Reference	0.04 (-0.04; 0.13)	-0.00 (-0.09; 0.08)	0.945
Model 2	Reference	0.04 (-0.04; 0.13)	-0.00 (-0.09; 0.08)	0.921
Model 3	Reference	0.04 (-0.04; 0.13)	-0.00 (-0.09; 0.08)	0.930
Total fat mass				
Model 1	Reference	0.02 (-0.05; 0.10)	-0.04 (-0.12; 0.03)	0.232
Model 2	Reference	0.02 (-0.05; 0.09)	-0.04 (-0.12; 0.03)	0.230
Model 3	Reference	0.02 (-0.05; 0.09)	-0.04 (-0.11; 0.03)	0.292
		Lignans		
Visceral fat				
Model 1	Reference	-0.01 (-0.10; 0.08)	-0.07 (-0.15; 0.02)	0.147
Model 2	Reference	-0.01 (-0.10; 0.08)	-0.07 (-0.16; 0.02)	0.146
Model 3	Reference	-0.02 (-0.10; 0.07)	-0.07 (-0.16; 0.02)	0.128
Android-to-gynoid fat ratio				
Model 1	Reference	-0.02 (-0.11; 0.06)	-0.04 (-0.12; 0.05)	0.405
Model 2	Reference	-0.03 (-0.11; 0.06)	-0.04 (-0.12; 0.05)	0.376
Model 3	Reference	-0.03 (-0.11; 0.06)	-0.04 (-0.13; 0.04)	0.325
Total fat mass (%)				
Model 1	Reference	-0.09 (-0.16; -0.02)	-0.12 (-0.19; -0.05)	**0.001**
Model 2	Reference	-0.09 (-0.16; -0.02)	-0.12 (-0.19; -0.05)	**0.001**
Model 3	Reference	-0.09 (-0.16; -0.02)	-0.12 (-0.20; -0.05)	**0.001**
		Stilbenes		
Visceral fat				

Model 1	Reference	0.03 (-0.06; 0.12)	-0.09 (-0.17; 0.00)	0.061
Model 2	Reference	0.03 (-0.06; 0.11)	-0.09 (-0.18; -0.00)	**0.048**
Model 3	Reference	0.02 (-0.07; 0.11)	-0.08 (-0.18; 0.01)	0.077
Android-to-gynoid fat ratio				
Model 1	Reference	0.07 (-0.01; 0.16)	0.01 (-0.07; 0.10)	0.787
Model 2	Reference	0.07 (-0.02; 0.15)	0.01 (-0.08; 0.09)	0.866
Model 3	Reference	0.07 (-0.02; 0.16)	0.01 (-0.08; 0.10)	0.793
Total fat mass				
Model 1	Reference	-0.00 (-0.07; 0.07)	-0.08 (-0.15; -0.01)	**0.029**
Model 2	Reference	-0.00 (-0.07; 0.07)	-0.09 (-0.16; -0.01)*	**0.021**
Model 3	Reference	-0.00 (-0.07; 0.07)	-0.08 (-0.15; -0.00)*	**0.044**

Analyses were performed using linear mixed-effects models with random intercepts at recruiting center, cluster family and patient level. Beta represents changes in adiposity indicators in each tertil of polyphenol consumption, compared to tertil 1, the reference category. Model 1 was controlled for age, sex, study arm and follow-up time. Model 2 was further adjusted for educational and physical activity levels and smoking status. Model 3 was further adjusted for baseline variables such as height and repeatedly measured energy intake, carbohydrate, protein, saturated fatty acids, and alcohol intake.

Table 3: Association between changes **visceral fat mass** and energy-adjusted tertils of main polyphenol subclasses intake changes after one-year of follow-up

	T1	T2	T3	
Total polyphenols	B (95% CI)	B (95% CI)	B (95% CI)	*P* for trend
Model 1	Reference	3.4 (-62.7; 69.5)	-57.7 (-124.1; 8.61)	0.088
Model 2	Reference	1.6 (-64.5; 67.8)	-58.4 (-124.7; 7.94)	0.084
Model 3	Reference	-0.9 (-67.5; 65.7)	-54.7 (-121.6; 12.3)	0.109
Flavonoids intake				
Model 1	Reference	10.5 (-55.4; 76.4)	-60.1 (-126.7; 6.53)	0.079
Model 2	Reference	4.9 (-61.2; 71.1)	-62.9 (-129.5; 3.79)	0.065
Model 3	Reference	-1.9 (-68.6; 64.7)	-70.3 (-137.9; -2.76)	**0.041**
Phenolic acids				
Model 1	Reference	11.2 (-55.1; 77.4)	-23.0 (-89.4; 43.4)	0.498

Model 2	Reference	8.7 (-57.9; 75.2)	-23.8 (-90.3; 42.7)	0.483
Model 3	Reference	14.0 (-52.6; 80.7)	-17.5 (-84.2; 49.1)	0.605
Lignans				
Model 1	Reference	-12.3 (-78.3; 53.7)	-48.7 (-115.3; 17.9)	0.153
Model 2	Reference	-14.7 (-81.0; 51.5)	-49.1 (-116.0; 17.7)	0.150
Model 3	Reference	-17.0 (-83.3; 49.3)	-51.7 (-118.9; 15.5)	0.132
Stilbenes				
Model 1	Reference	21.3 (-45.0; 87.7)	-54.9 (-121.7; 11.8)	0.108
Model 2	Reference	19.3 (-47.1; 85.7)	-59.5 (-126.5; 7.49)	0.084
Model 3	Reference	14.3 (-53.2; 81.7)	-51.8 (-121.5; 17.8)	0.148

Analyses were performed using linear mixed-effects models with random intercepts at recruiting center, cluster family and patient level. Beta represents changes in adiposity indicators in each tertil of polyphenol consumption, compared to tertil 1, the reference category. Model 1 was controlled for age, sex, study arm and follow-up time. Model 2 was further adjusted for educational and physical activity levels and smoking status. Model 3 was further adjusted for baseline variables such as height and repeatedly measured energy intake, carbohydrate, protein, saturated fatty acids and alcohol intake.

Table 3.1.: Association between **visceral fat mass** and energy-adjusted tertils of polyphenol sub-classes intake changes after one-year of follow-up

	T1	**T2**	**T3**	
Anthocyanins	B (95% CI)	B (95% CI)	B (95% CI)	*P* for trend
Model 1	Reference	-6.0 (-72.2; 60.2)	-39.6 (-106.3; 27.1)	0.245
Model 2	Reference	-7.6 (-74.0; 58.8)	-41.7 (-108.6; 25.2)	0.222
Model 3	Reference	-14.7 (-81.5; 52.2)	-37.1 (-104.6; 30.5)	0.282
Catechins				
Model 1	Reference	-43.3 (-109.4; 22.8)	-77.9 (-144.0; -11.9)*	**0.021**
Model 2	Reference	-45.4 (-111.5; 20.7)	-84.4 (-150.6; -18.2)*	**0.012**
Model 3	Reference	-51.3 (-118.0; 15.3)	-85.2 (-152.0; -18.3)*	**0.013**

Proanthocyanidins				
Model 1	Reference	-24.4 (-90.4; 41.7)	-49.4 (-115.5; 16.7)	0.143
Model 2	Reference	-21.2 (-87.5; 45.1)	-49.9 (-116.1; 16.2)	0.139
Model 3	Reference	-22.0 (-88.3; 44.3)	-45.5 (-112.0; 21.0)	0.180
Flavanones				
Model 1	Reference	-1.8 (-67.9; 64.4)	-18.1 (-84.5; 48.4)	0.594
Model 2	Reference	-3.5 (-69.8; 62.8)	-17.2 (-83.7; 49.4)	0.613
Model 3	Reference	-4.0 (-70.6; 62.7)	-16.6 (-83.4; 50.3)	0.627
Flavones				
Model 1	Reference	33.8 (-32.3; 99.9)	-16.6 (-83.3; 50.1)	0.638
Model 2	Reference	32.9 (-33.5; 99.2)	-15.9 (-82.7; 50.8)	0.651
Model 3	Reference	29.9 (-38.0; 97.8)	-14.0 (-81.7; 53.7)	0.673
Flavonols				
Model 1	Reference	-63.6 (-129.3; 2.01)	-134.3 (-200.3; -68.3)**	<0.001
Model 2	Reference	-62.1 (-127.8; 3.63)	-132.4 (-198.6; -66.2)**	**<0.001**
Model 3	Reference	-68.0 (-134.4; -1.59)*	-140.1 (-207.4; -72.7)**	**<0.001**
Hydroxycinnamic acid				
Model 1	Reference	-35.1 (-101.2; 31.0)	-21.3 (-87.8; 45.1)	0.522
Model 2	Reference	-36.4 (-102.6; 29.8)	-20.1 (-86.7; 46.5)	0.546
Model 3	Reference	-28.0 (-94.5; 38.6)	-6.9 (-74.1; 60.3)	0.833
Hydroxybenzoic acids				
Model 1	Reference	-78.1 (-144.3; -12.0)*	-89.9 (-156.2; -23.5)**	**0.008**
Model 2	Reference	-76.5 (-142.8; -10.2)*	-91.6 (-158.2; -25.0)**	**0.007**
Model 3	Reference	-84.2 (-150.8; -17.6)*	-95.6 (-163.7; -27.5)**	**0.006**

Analyses were performed using linear mixed-effects models with random intercepts at recruiting center, cluster family and patient level. Beta represents changes in adiposity indicators in each tertil of polyphenol consumption, compared to tertil 1, the reference category. Model 1 was controlled for age, sex, study arm and follow-up time. Model 2 was further adjusted for educational and physical activity levels and smoking status. Model 3 was further adjusted for baseline variables such as height and repeatedly measured energy intake, carbohydrate, protein, saturated fatty acids and alcohol intake.

Supplementary 1: Association between changes in total polyphenol and main polyphenols subclasses intake and fat distribution (sex-specific z-scores) from baseline to 12-months follow-up visits.

	T1	T2	T3 vs T2	T3 vs T1	P for trend
	B (95% CI)	B (95% CI)	B (95% CI)	B (95% CI)	
Total polyphenols (mean intake mg/day)					
Visceral fat					
Model 1	0.00 (-0.08; 0.09)	Reference	-0.07 (-0.16; 0.01)	-0.08 (-0.17; 0.01)	0.081
Model 2	0.01 (-0.08; 0.09)	Reference	-0.07 (-0.16; 0.02)	-0.08 (-0.17; 0.01)	0.079
Model 3	0.01 (-0.08; 0.10)	Reference	-0.07 (-0.15; 0.02)	-0.08 (-0.16; 0.01)	0.099
Android-to-gynoid fat ratio					
Model 1	-0.03 (-0.12; 0.05)	Reference	-0.05 (-0.13; 0.04)	-0.01 (-0.10; 0.07)	0.744
Model 2	-0.03 (-0.12; 0.05)	Reference	-0.05 (-0.13; 0.04)	-0.01 (-0.10; 0.07)	0.748
Model 3	-0.03 (-0.11; 0.06)	Reference	-0.04 (-0.13; 0.04)	-0.02 (-0.10; 0.07)	0.718
Total fat mass					
Model 1	0.02 (-0.06; 0.09)	Reference	-0.04 (-0.11; 0.03)	-0.05 (-0.12; 0.03)	0.150
Model 2	0.02 (-0.05; 0.09)	Reference	-0.04 (-0.11; 0.03)	-0.05 (-0.13; 0.02)	0.146
Model 3	0.02 (-0.06; 0.09)	Reference	-0.03 (-0.10; 0.04)	-0.05 (-0.12; 0.03)	0.198
Flavonoids (mean intake mg/day)					
Visceral fat					
Model 1	0.00 (-0.08; 0.09)	Reference	-0.08 (-0.17; 0.01)	-0.08 (-0.17; 0.01)	0.068
Model 2	0.01 (-0.08; 0.10)	Reference	-0.08 (-0.17; 0.01)	-0.09 (-0.18; 0.00)	0.057
Model 3	0.02 (-0.07; 0.11)	Reference	-0.08 (-0.17; 0.01)	-0.10 (-0.19; -0.01)*	**0.038**
Android-to-gynoid fat ratio					
Model 1	0.01 (-0.07; 0.10)	Reference	-0.02 (-0.10; 0.07)	-0.03 (-0.11; 0.06)	0.504
Model 2	0.01 (-0.07; 0.10)	Reference	-0.02 (-0.10; 0.07)	-0.03 (-0.12; 0.05)	0.451
Model 3	0.02 (-0.07; 0.10)	Reference	-0.02 (-0.10; 0.07)	-0.04 (-0.12; 0.05)	0.416
Total fat mass					

Model 1	-0.03 (-0.10; 0.05)	Reference	-0.07 (-0.14; 0.00)	-0.04 (-0.11; 0.03)	0.258
Model 2	-0.02 (-0.09; 0.05)	Reference	-0.07 (-0.14; 0.01)	-0.04 (-0.12; 0.03)	0.240
Model 3	-0.02 (-0.09; 0.05)	Reference	-0.07 (-0.14; 0.01)	-0.04 (-0.12; 0.03)	0.248
Lignans (mean intake mg/day)					
Visceral fat					
Model 1	0.01 (-0.08; 0.10)	Reference	-0.06 (-0.14; 0.03)	-0.07 (-0.15; 0.02)	0.147
Model 2	0.01 (-0.08; 0.10)	Reference	-0.05 (-0.14; 0.04)	-0.07 (-0.16; 0.02)	0.146
Model 3	0.02 (-0.07; 0.10)	Reference	-0.05 (-0.14; 0.03)	-0.07 (-0.16; 0.02)	0.128
Android-to-gynoid fat ratio					
Model 1	0.02 (-0.06; 0.11)	Reference	-0.01 (-0.10; 0.07)	-0.04 (-0.12; 0.05)	0.405
Model 2	0.03 (-0.06; 0.11)	Reference	-0.01 (-0.10; 0.07)	-0.04 (-0.12; 0.05)	0.376
Model 3	0.03 (-0.06; 0.11)	Reference	-0.02 (-0.10; 0.07)	-0.04 (-0.13; 0.04)	0.325
Total fat mass (%)					
Model 1	0.09 (0.02; 0.16)*	Reference	-0.03 (-0.11; 0.04)	-0.12 (-0.19; -0.05)	**0.001**
Model 2	0.09 (0.02; 0.16)*	Reference	-0.03 (-0.10; 0.04)	-0.12 (-0.19; -0.05)	**0.001**
Model 3	0.09 (0.02; 0.16)*	Reference	-0.03 (-0.10; 0.04)	-0.12 (-0.20; -0.05)*	**0.001**
Stilbenes (mean intake mg/day)					
Visceral fat					
Model 1	-0.03 (-0.12; 0.06)	Reference	-0.11 (-0.20; -0.02)*	-0.09 (-0.17; 0.00)	0.061
Model 2	-0.03 (-0.11; 0.06)	Reference	-0.12 (-0.21; -0.03)*	-0.09 (-0.18; -0.00)*	**0.048**
Model 3	-0.02 (-0.11; 0.07)	Reference	-0.10 (-0.19; -0.01)*	-0.08 (-0.18; 0.01)	0.077
Android-to-gynoid fat ratio					
Model 1	-0.07 (-0.16; 0.01)	Reference	-0.06 (-0.15; 0.02)	0.01 (-0.07; 0.10)	0.787
Model 2	-0.07 (-0.15; 0.02)	Reference	-0.06 (-0.15; 0.03)	0.01 (-0.08; 0.09)	0.866
Model 3	-0.07 (-0.16; 0.02)	Reference	-0.06 (-0.15; 0.03)	0.01 (-0.08; 0.10)	0.793
Total fat mass					
Model 1	0.00 (-0.07; 0.07)	Reference	-0.08 (-0.15; -0.01)*	-0.08 (-0.15; -0.01)	**0.029**
Model 2	0.00 (-0.07; 0.07)	Reference	-0.08 (-0.16;-0.01)*	-0.09 (-0.16; -0.01)*	**0.021**
Model 3	-0.00 (-0.07; 0.07)	Reference	-0.08 (-0.15; -0.00)*	-0.08 (-0.15; -0.00)*	**0.041**

Analyses were performed using linear mixed-effects models with random intercepts at recruiting center, cluster family and patient level. Beta represents changes in adiposity indicators in each tertil of polyphenol consumption, compared to tertil 1, the reference category. Model 1 was controlled for age, sex, study arm and follow-up time. Model 2 was further adjusted for educational and physical activity levels and smoking status. Model 3 was further adjusted for baseline variables such as height and repeatedly measured energy intake, carbohydrate, protein, saturated fatty acids, and alcohol intake

Supplementary 2: Association between changes **visceral fat mass** and energy-adjusted tertiles of main polyphenol subclasses intake changes after one-year of follow-up according to sex.

	Men				Women			
	T1	T2	T3	P for trend	T1	T2	T3	P for trend
Total polyphenols	B (95% CI)	B (95% CI)	B (95% CI)		B (95% CI)	B (95% CI)	B (95% CI)	
Model 1	Reference	16.1 (-85.2; 117.5)	-66.3 (-169.4; 36.8)	0.206	Reference	-41.7 (-121.3; 37.9)	-40.8 (-120.3; 38.6)	0.314
Model 2	Reference	11.0 (-90.5; 112.6)	-65.6 (-168.5; 37.3)	0.209	Reference	-38.2 (-118.6; 42.2)	-35.6 (-115.6; 44.4)	0.383
Model 3	Reference	2.2 (-99.4; 103.8)	-58.4 (-161.6; 44.8)	0.265	Reference	-41.1 (-123.2; 40.9)	-40.4 (-122.9; 42.1)	0.339
Flavonoids intake								
Model 1	Reference	49.0 (-52.8; 150.9)	-57.9 (-160.9; 45.1)	0.270	Reference	-76.7 (-155.7; 2.3)	-80.4 (-159.8; -1.0)*	**0.046**
Model 2	Reference	33.1 (-69.6; 135.8)	-64.0 (-167.5; 39.5)	0.224	Reference	-71.2 (-151.0; 8.6)	-74.9 (-155.1; 5.2)	0.067
Model 3	Reference	22.7 (-80.6; 126.0)	-71.6 (-175.8; 32.6)	0.173	Reference	-76.9 (-158.5; 4.8)	-80.4 (-162.5; 1.8)	0.057
Phenolic acids								
Model 1	Reference	38.8 (-63.1; 140.7)	-17.2 (-119.2; 84.8)	0.734	Reference	7.3 (-72.3; 86.9)	-34.5 (-113.9; 44.8)	0.396
Model 2	Reference	34.4 (-67.3; 136.2)	-19.4 (-121.2; 82.4)	0.702	Reference	8.9 (-71.4; 89.2)	-30.6 (-110.8; 49.5)	0.455
Model 3	Reference	44.6 (-56.7; 145.9)	-6.3 (-107.8; 95.3)	0.898	Reference	7.1 (-74.1; 88.4)	-35.1 (-116.9; 46.7)	0.401

Lignans								
Model 1	Reference	-9.6 (-111.3; 92.1)	-49.9 (-152.7; 53.0)	0.341	Reference	-3.1 (-82.3; 76.1)	-80.9 (-160.7; -1.2)*	**0.046**
Model 2	Reference	-7.6 (-109.9; 94.7)	-46.9 (-149.9; 56.1)	0.371	Reference	-1.9 (-81.6, 77.8)	-76.0 (-156.5; 4.6)	0.065
Model 3	Reference	-14.9 (-116.5; 86.7)	-56.2 (-159.3; 47.0)	0.286	Reference	-4.1 (-84.5; 76.3)	-79.6 (-161.5; 2.4)	0.058
Stilbenes								
Model 1	Reference	-10.6 (-112.9; 91.6)	-52.5 (-154.5; 49.5)	0.311	Reference	-15.0 (-94.5; 64.5)	-76.2 (-156.0; 3.6)	0.060
Model 2	Reference	-13.9 (-115.9; 88.1)	-57.6 (-159.8; 44.6)	0.268	Reference	-14.8 (-94.7; 65.2)	-72.3 (-153.0; 8.3)	0.078
Model 3	Reference	-6.6 (-111.1; 97.9)	-7.7 (-118.4; 103.0)	0.892	Reference	-22.6 (-106.3; 61.0)	-77.6 (-160.2; 5.1)	0.063
Catechins								
Model 1	Reference	-48.5 (-150.4; 53.4)	-69.4 (-171.6; 32.8)	0.183	Reference	-41.4 (-120.7; 38.0)	-88.4 (-167.9; -8.8)*	**0.029**
Model 2	Reference	-48.5 (-150.5; 53.5)	-81.9 (-184.3; 20.5)	0.117	Reference	-39.0 (-118.9; 40.9)	-82.9 (-163.1; -2.8)*	**0.042**
Model 3	Reference	-50.3 (-153.3; 52.7)	-77.3 (-180.9; 26.4)	0.145	Reference	-44.1 (-125.2; 37.1)	-87.5 (-169.2; -5.8)*	**0.036**
Anthocyanidins								
Model 1	Reference	11.4 (-90.6; 113.4)	-49.1 (-151.2; 53.0)	0.341	Reference	-27.6 (-107.4; 52.1)	-16.7 (-96.8; 63.3)	0.684
Model 2	Reference	11.6 (-90.4; 113.5)	-53.3 (-155.5; 48.9)	0.303	Reference	-32.1 (-112.5; 48.4)	-17.4 (-98.5; 63.6)	0.677
Model 3	Reference	-4.4 (-107.0; 98.1)	-45.6 (-148.2; 56.9)	0.380	Reference	-38.4 (-120.6; 43.7)	-21.4 (-103.8; 61.0)	0.621
Hydroxycinnamic acid								
Model 1	Reference	-15.1 (-116.9; 86.7)	-27.0 (-128.8; 74.7)	0.602	Reference	12.0 (-67.7; 91.7)	-2.1 (-81.6; 77.4)	0.961
Model 2	Reference	-18.5 (-120.1; 83.0)	-24.2 (-125.7; 77.4)	0.641	Reference	14.1 (-66.3; 94.5)	3.6 (-76.8; 84.1)	0.929

Model 3	Reference	-9.6 (-111.0; 91.7)	-6.7 (-108.5; 94.5)	0.893	Reference	13.1 (-68.7; 95.0)	0.5 (-82.1; 83.1)	0.993
Hydroxybenzoic acids								
Model 1	Reference	-37.3 (-139.0; 64.4)	-74.6 (-176.1; 26.9)	0.149	Reference	-90.3 (-169.9; -10.7)*	-80.5 (-159.8; -1.09)*	**0.049**
Model 2	Reference	-42.9 (-145.1; 59.3)	-85.6 (-187.6; 16.3)	0.099	Reference	-90.1 (-170.4; -9.9)	-78.5 (-158.2; 1.2)	0.055
Model 3	Reference	-47.4 (-150.2; 55.4)	-75.3 (-180.3; 29.7)	0.160	Reference	-93.3 (-174.5; -12.1)*	-82.7 (-163.9; -1.5)*	**0.045**

Analyses were performed using linear mixed-effects models with random intercepts at recruiting center. Beta represents changes in adiposity indicators in each tertil of polyphenol consumption, compared to tertil 1, the reference category. Model 1 was controlled for age, study arm and follow-up time. Model 2 was further adjusted for educational and physical activity levels and smoking status. Model 3 was further adjusted for baseline variables such as repeatedly measured energy intake, carbohydrate, protein, saturated fatty acids and alcohol intake.

Manuscript V

Title: "Loss of visceral fat is associated with a reduction in inflammatory status in patients with metabolic syndrome.**"**

Authors: Sara Castro-Barquero, Rosa Casas, Eric B Rimm, Anna Tresserra-Rimbau, Dora Romaguera, J Alfredo Martínez, Jordi Salas-Salvadó, Miguel Ángel Martínez, Josep Vidal, Miguel Ruiz-Canela, Jadwiga Konieczna, Emilio Sacanella, Montse Fitó, Ramon Estruch.

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Keywords: Adipokines, Inflammation, Lifestyle, Mediterranean diet, Visceral adipose tissue.

Abbreviatures:

DXA: Dual-energy X-ray absorptiometry

VAT: Visceral adipose tissue

SAT: Subcutaneous adipose tissue

MD: Mediterranean diet

MetS; Metabolic syndrome

CVD: Cardiovascular diseases

BMI: Body mass index

PA: Physical activity

FFQ: Food frequency questionnaire

EVOO: Extra virgin olive oil

TNF-α: Tumor necrosis factor alpha

IL: Interleukin

GLP-1: Glucagon-like peptide 1

MCP-1: Monocyte chemoattractant protein-1

PAI-1: Plasminogen activator inhibitor-1

SFA: Saturated fatty acids

MUFA: Monounsaturated fatty acids

PUFA: Polyunsaturated fatty acids

RCT: Randomized clinical trial

Abstract (180 words)

Excessive visceral adipose tissue (VAT) is associated with higher secretion of pro-inflammatory molecules, contributing to systemic inflammation and obesity-related metabolic disturbances. This prospective analysis includes 117 overweight/obese adults (55-75 years) from the PREDIMED-Plus study. Fourteen inflammatory markers and adipokines were measured using a Bio-Plex assay with multiplex technology: insulin, glucagon, IL-6, visfatin, ghrelin, GLP-1, TNF-α, MCP-1, PAI-1, resistin, C-peptide, leptin, adipsin and adiponectin. Participants were categorized into tertiles according to changes in VAT after 1-year of follow-up, determined by dual-energy X-Ray absorptiometry. Participants allocated in tertile 3, which represent an increase of VAT content after 1-year of follow-up compared to tertile 1, showed significant differences in insulin (T3 vs. T1, fully adjusted model: $p = 0.033$, p for trend 0.042), PAI-1 (fully adjusted model: $p = 0.042$, p for trend 0.052), c-peptide (fully adjusted model: $p = 0.033$, p for trend 0.042), and TNF-α (fully adjusted model $p = 0.033$, p for trend 0.042). Our results evidenced that a reduction in VAT was associated with clinical improvements in several inflammatory and adiposity markers, mainly in insulin, c-peptide, and PAI-1 levels.

Introduction

The worldwide overweight and obesity incidence has increased at an alarming rate. In 2016, nearly 40% of adults were overweight and 13% were obese[1]. Overweight and obesity, especially excessive visceral adipose tissue (VAT), is closely related to the inflammatory response due to the imbalance in the secretion of adipokines and cytokines with anti- and/or pro-inflammatory properties[2]. Moreover, strong evidence highlights that body mass index (BMI) does not differentiate physiological and pathological states, especially in senior populations[3,4]. In this sense, VAT content demonstrates a more specific capacity to identify individuals with altered glucose

metabolism and lipid profile, mainly regarding modified triglyceride (TG) and high-density lipoprotein cholesterol levels[3,5-7].

Adipose tissue is an endocrine and paracrine organ with the capacity to produce cytokines, such as interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and adipokines or adipocytokines (e.g., leptin, adiponectin, resistin, and visfatin, among others)[8,9]. These molecules influence body weight homeostasis and are linked to inflammation, coagulation, and fibrinolysis. Even though the inflammatory response observed in obesity is not yet fully understood, the macrophages infiltration into adipose tissue is increased in obese individuals, and this in turn, may contribute to the persistence of the chronic inflammation observed in obesity[10]. Under normal physiological conditions, adipocytes mainly produce anti-inflammatory adipokines, such as adiponectin. However, excess VAT is associated with higher secretion of pro-inflammatory adipocytokines and cytokines, leading to systemic inflammation and obesity-related metabolic disturbances, such as insulin resistance, dyslipidemia, hypertension, oxidative stress, and atherosclerosis[11,12]. This excess adipose tissue and inflammatory response can be reversed by lifestyle modification, including physical activity promotion and following a healthy dietary pattern, such as the Mediterranean Diet (MD)[11,13,14]. MD is rich in polyphenols, plant-derived bioactive compounds characterized by the presence of aromatic ring and attached hydroxyl groups, which are present in the main key-foods of this dietary pattern (extra virgin olive oil (EVOO), nuts, vegetables, wine, vegetables, fruits and whole-grain cereals). Its intake has been associated to anti-inflammatory and anti-oxidative properties, which may also improve obesity-related inflammatory response[15].

Our objective was to evaluate whether changes in VAT after a 12-month intervention were associated with greater improvements in obesity-related inflammatory and adipokine marker concentrations in older overweight or obese individuals with metabolic syndrome (MetS).

1. Experimental section

2.1. Study design

The present study was a longitudinal analysis of data collected during the first year of the Prevención con Dieta Mediterránea (PREDIMED) Plus study, an ongoing large-scale, multicenter, parallel-group, intervention randomized controlled trial conducted in 23 centers of the National Spanish Health System designed to assess the effect of an energy-restricted MD and physical activity promotion on cardiovascular morbidity and mortality in individuals with MetS[16].

Volunteers were men (aged 55–75) and women (60–75) free of documented history of cardiovascular disease (CVD) with overweight or obesity (BMI ≥27 or <40 kg/m^2) who met at least three or more criteria of MetS: abdominal obesity (waist circumference >102 cm for men or ≥88 cm for women), hypertension, hypertriglyceridemia, low HDL cholesterol levels and hyperglycemia, or diagnosis of type 2 diabetes mellitus[17]. The recruitment period lasted from September 5, 2013, to October 31, 2016. The study protocol and the eligibility and exclusion criteria can be found at http://predimedplus.com. After randomization, the participants were assigned to an intervention or control group. The intervention arm promoted an energy-restricted MD and physical activity (PA) and provided behavioral support, whereas the control group received usual health care for CVD prevention and advice on following a traditional MD without energy restriction or PA promotion. The trial protocol was approved by the local institutional ethics committees and was registered under International Standard Randomized Controlled Trial number 89898870 (ISRCT: http://www.isrctn.com/ISRCTN89898870). All participants provided written informed consent before joining the study.

For the present analysis, out of the total sample of 6,874 randomized participants, only 1,569 participants from 7 recruiting center underwent total body dual X-ray absorptiometry (DXA) scans at baseline. Thirty-seven participants were excluded because they reported energy intake values outside the predefined limits measured by food frequency questionnaire (FFQ) (<3,347 kJ [800 kcal]/day or >17,573 kJ [4,000 kcal]/day for men; <2,510 kJ [500 kcal]/day or >14,644 kJ [3,500 kcal]/day for women) at baseline and during follow-up[18]. Participants were also excluded if they had missing data on dietary information (n = 159) or lacked DXA data (n = 178) at baseline and after 1 year of follow-up. Ultimately, 1,195 participants had available data on body composition parameters measured by DXA. Of these, 1,157 participants had available data on VAT at baseline. For the present analysis, a random subsample of 117 participants with VAT measurements at baseline and after 1 year of intervention was selected for biomarkers analysis.

2.2. Inflammatory and adipokine biomarkers

Blood samples were collected after overnight fasting at baseline and the 1-year follow-up visit. These were centrifuged and stored at −80 ºC until analysis. The following biomarkers were analyzed: insulin, glucagon, IL-6, visfatin, ghrelin, glucagon-like peptide 1 (GLP-1), TNF-α, monocyte chemoattractant protein 1 (MCP-1), plasminogen activator inhibitor 1 (PAI-1), resistin, C-peptide, leptin, adipsin, and adiponectin. These

biomarkers were measured using a Bio-Plex assay (Bio-Rad Laboratories Inc., Hercules, CA, USA) based on multiplex technology. First, samples were incubated, and beads were suspended and covered with antibodies specific to the mentioned molecules. Second, samples were washed, and third, biotinylated detection antibodies were applied to the samples. Fourth, the samples were incubated with streptavidin–phycoerythrin. Finally, Bio-Plex 200 was used to read the fluorescent sign.

2.3. Body composition parameters

Direct measures of body composition were performed with a DXA scanner (GE Healthcare/DXA Lunar Prodigy Primo and Lunar iDXA; Madison, WI, USA) connected to enCore™ software. For VAT measurement, scans were reanalyzed using the validated CoreScan software application[19]. As described elsewhere[20], VAT (g) was subtracted from android fat mass. Body composition measures were preferably performed within two months from baseline and follow-up visit in. DXA scans were performed and calibrated daily by trained operators according to the standard protocols provided by the manufacturer. Participants were scanned wearing examination gown or light clothes.

2.4. Covariable assessment

Self-reported questionnaires were collected at baseline by trained staff and provided data on sex, age, smoking status, educational level, history of a medical condition, lifestyle habits, and medication use. Dietary information was assessed at baseline and after 1-year of follow-up by a validated 143-item semi-quantitative food-frequency questionnaire (FFQ) [21]. A 17-point score was used to assess MD adherence[22] at baseline and one-year follow-up visits. As described elsewhere[23], dietary polyphenol intake was estimated by multiplying polyphenol content in food (mg/100g of food) by the daily consumption of each food (g/day). Total polyphenol intake and polyphenol subclasses were calculated as the sum of all individual polyphenol intakes from the food sources reported from the FFQ. The validated Registre Gironí del Cor (REGICOR) short self-reported physical activity questionnaire was used to assess total leisure-time PA (MET min/week)[24] and the validated Spanish version of the Nurses' Health Study questionnaire to assess sedentary behaviors[25] at baseline and after 1-year of follow-up. Sociodemographic and lifestyle variables were categorized as follows: educational level (three categories: primary, secondary, or high school), physical activity level (three categories: low, moderate, or high), BMI (three categories: 27.0–29.9 kg/m^2 or overweight, 30.0–34.9 kg/m^2 or obesity class I, and ≥35 or obesity class II kg/m^2), and smoking status (three categories: never, former, or current smoker).

2.5. Statistical analysis

The baseline characteristics of study participants are expressed as means and standard deviation (SD) for continuous variables and counts and percentages for categorical variables. Variables with a skewed distribution (as assessed with the Kolmogorov test) were transformed to their logarithm for analysis. Differences in baseline characteristics by tertiles of VAT changes at 1 year versus baseline were analyzed using one-way ANOVA. VAT changes were allocated into tertiles of change after 1 year, with tertile 1 (reduction in VAT) as the reference category.

The differences in inflammatory and adipokines parameters at baseline and after 1 year of follow-up between tertiles of VAT changes were assessed by multivariate linear regression models. The minimally adjusted model included for age, sex, intervention group, recruitment center, smoking status (three categories: never smoker, smoker, and former smoker), type 2 diabetes diagnose (yes/no), changes in BMI (1-year versus baseline) and baseline levels of each parameter (pg/mL). The fully adjusted model was further adjusted for educational level (three categories: primary, secondary, or high school), physical activity level (three categories: low, moderate and active), cholesterol-lowering treatment (yes/no), energy intake (kcal/day), saturated fatty acid intake (g/day), trans fat intake (g/day), and fiber intake (g/day).

The differences in energy intake and nutrient density at baseline and after 1-year of follow-up according to tertiles of VAT changes were assessed by multivariate linear regression. The full-adjusted model included age, sex, intervention group, recruitment center, smoking status (three categories: never smoker, smoker, and former smoker), type 2 diabetes diagnose (yes/no), educational level (three categories: primary, secondary, or high school), physical activity level (three categories: low, moderate, and active), cholesterol-lowering treatment (yes/no), and baseline levels of each nutritional parameter (g/day). To assess the linear trend (*p* for trend) across tertiles of VAT changes, the mean value was assigned to each tertile.

To account for multiple comparisons, we applied the Simes method to interpret the results. Statistical analyses were performed using Stata v16.0 (StataCorp LLC, Texas, USA), and statistical significance was set at $p < 0.05$. The PREDIMED-Plus longitudinal database generated on June 26, 2020 (202006290731_PREDIMEDplus) was used.

2. **Results**

Baseline participant characteristics

Of the 117 participants included, 59 were randomly allocated to the energy-restricted MD intervention, and 51.3% were women. **Table 1** shows the baseline characteristics of study participants according to each tertile of VAT changes. Tertiles were well-balanced regarding BMI, smoking status, age, energy intake, physical activity levels, and MD adherence. Medication use and educational level were also similar among the three tertiles. Significant differences were observed in sex, for which tertile 3 consisted of significantly more women compared with tertile 1 ($p<0.001$), and waist circumference ($p = 0.036$).

Inflammatory and adipokine parameters

The baseline and 1-year mean changes for inflammatory and adipokines parameters are shown in **Table 2**. Significant differences between tertiles of VAT changes (Tertile 3 (T3) vs. Tertile 1 (T1)) after 1-year of follow-up were observed in insulin (T3 vs. T1, fully adjusted model: β= 64.3 [95% CI: 17.7 to 110.9], p = 0.033, p for trend 0.042), PAI-1 (T3 vs. T1, fully adjusted model: β= 372.4 [95% CI: 84.4 to 660.4], p = 0.042, p for trend 0.052), c-peptide (T3 vs. T1, fully adjusted model: β= 1.43 [95% CI: 1.27 to 1.82], p = 0.033, p for trend 0.042), and TNF-α (T3 vs. T1, fully adjusted model: β= 1.50 [95% CI: 1.13 to 1.99], p = 0.033, p for trend 0.042). Tertile 3 (VAT increase after 1 year of follow-up) showed significant mean increases in PAI-1 (mean difference 215.9 pg/mL [95% CI: 8.72 to 423.1]), ghrelin (15.5 pg/mL [95% CI: 0.5 to 30.4]), resistin (369.4 pg/mL [95% CI: 152.8 to 585.9]), and leptin levels (4399 pg/mL [95% CI: 702.4 to 8095]). Moreover, significant reductions in glucagon (-24.2 pg/mL [95% CI: -41.0 to -7.3]), and insulin levels (-29.1 pg/mL [95% CI:-57.1 to -1.1]), were observed in Tertile 1.

Supplementary Table 1 shows the associations between inflammatory and adipokine parameters according to tertiles of changes in total fat mass (kg) after 1 year of follow-up. No significant differences were observed between tertiles of total fat mass changes after 1 year of follow-up. Tertile 3 (total fat mass increase after 1 year of follow-up) showed significant mean increases in c-peptide (mean difference 15.2 pg/mL [95% CI: 4.1 to 26.3]), and leptin (4044 pg/mL [95% CI: 593.2 to 7495]). Moreover, significant reductions in glucagon (-15.0 pg/mL [95% CI: -26.3 to -3.7]), were observed in Tertile 1.

Changes in dietary and polyphenol intake after 1 year of follow-up

The baseline and 1-year mean changes for dietary and main polyphenol family intake are shown in **Table 3**. Significant differences among tertiles of VAT changes after 1-year of follow-up were observed in energy intake (T3 vs. T1, fully adjusted model: β= 261.8 [95% CI: 66.0 to 457.6], p = 0.009, p for trend = 0.007), trans-fat (T3 vs. T1, fully adjusted model: β= 0.06 [95% CI: 0.01 to 0.11], p = 0.023, p for trend = 0.019), fiber (T3 vs. T1, fully adjusted model: β= -1.45 [95% CI: -2.86 to -0.05], p = 0.043, p for trend = 0.028), and lignans intake (T3 vs. T1, fully adjusted model: β= -0.10 [95% CI: -0.19 to -0.02], p = 0.018, p for trend = 0.013). Tertile 1 showed a significant mean decrease in energy (mean difference -224.3 kcal/day [95% CI: -409.0 to -79.7]), carbohydrate (-8.4 g day/1000 kcal [95% CI: -13.3 to -3.5]), saturated fatty acids (SFA) (-1.1 g day/1000 kcal [95% CI: -1.8 to -0.5]), and trans-fat (-0.1 g day/1000 kcal [95% CI: -0.1 to -0.0]). Moreover, a significant increase was observed in protein (2.8 g day/1000 kcal [95% CI: 0.4 to 5.2]), total fat (2.2 g day/1000 kcal [95% CI: 0.1 to 4.3]), monounsaturated fatty acids (MUFA) (3.4 g day/1000 kcal [95% CI: 1.8 to 5.0]), polyunsaturated fatty acids (PUFA) (0.9 g day/1000 kcal [95% CI: 0.3 to 1.6]) and fiber (2.3 g day/1000 kcal [95% CI: 1.2 to 3.4]). Regarding dietary polyphenol intake, a significantly difference between tertiles was observed for lignans (T3 vs T1 p = 0.018, p for trend= 0.013).

3. Discussion

The present study analyzed data from 117 participants recruited into the PREDIMED-Plus study after 1 year of follow-up; we found significant associations between changes in VAT and circulating levels of insulin, c-peptide, and PAI-1 levels.

It is well-established that VAT is associated with CVD and metabolic disturbances, including non-alcoholic fatty liver disease, but the mechanisms underlying these effects are still unclear[14]. This excess VAT may induce chronic low-grade systemic inflammation mediated by macrophage infiltration and the secretion of several proinflammatory cytokines.[8,9] Clinical studies with insulin-resistant obese participants described adipose tissue macrophage infiltration, initiating the recruitment of other immune cells to promote the secretion of several cytokines to regulate the inflammatory response. In addition, macrophage infiltration has been postulated as a potential mechanism underlying the insulin resistance, pro-inflammatory response, and metabolic dysfunction observed in obese patients[26,27]. Moreover, the activation of these macrophages induces the secretion of several proinflammatory mediators, such as MCP-1, IL-6, and TNF-a[26,28].

The beneficial effects observed in individuals with higher VAT reduction on some circulating proinflammatory parameters related to obesity, such as leptin and MCP-1, were also reported by Salas-Salvadó *et al.* in the same study population [29]. Human studies conducted in the context of bariatric surgery- or lifestyle interventions focus on weight loss suggest that although adipose tissue inflammation is not sufficient to induce insulin resistance on its own, it is a major contributor to systemic insulin resistance, and substantial improvements in insulin sensitivity are associated with a reduction in VAT [30,31]. Weight loss achievements through dietary intervention alone or energy-restricted diet and physical activity promotion resulted in decreased circulating IL-6, CRP, PAI-1, TNF-α, soluble TNF receptor, P-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and IL-18 in men and women of various age groups and BMIs [32].

Despite its physiological functions, leptin is considered a pro-inflammatory cytokine. Its secretion is proportional to fat depots, and its inflammatory response is mediated by the activation of monocytes, leukocytes, and macrophages to secrete IL-6, TNF-α, along with increases in reactive oxygen species (ROS) [33,34]. No differences in changes from baseline for inflammatory cytokines were observed. Similar results were reported by Paquette *et al.* in a randomized controlled trial with non-diabetic overweight and obese adults after dietary intervention with strawberry and cranberry polyphenols [35]. Similar to leptin, resistin may play a pro-inflammatory role, inducing the expression of pro-inflammatory cytokines by nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling and inflammation markers, such as MCP-1.

High serum adiponectin levels are negatively associated with pro-inflammatory markers, such as IL-6 or TNF-α, as well as insulin resistance [36]. Its anti-inflammatory properties are mainly mediated by the inhibition of NF-kB [37,38]. It should be noted that the secretion of adiponectin is suppressed in chronic obesity [39], and this might explain the non-significant changes we observed in adiponectin levels, except for TNF-α.

Expression of C-peptide has been directly linked with insulin resistance and CVD risk, and significant inverse associations were observed between C-peptide levels and physical activity in the same study population [40]. Interestingly, the association between physical activity levels and C-peptide are independent of body composition parameters [41,42], whereas our results suggest a potential association between VAT changes and C-peptide.

Regarding dietary factors, our results showed a significant reduction in energy, total fat, SFA, and trans-fat intake in participants who decrease their VAT deposits. These

nutrient intake changes can be explained by the reduction in the consumption of refined cereals, red meat, pastries, cakes, and sweets[29]. Dietary polyphenol intake has been associated with several CVD benefits and mediates inflammatory processes and ROS production [43,44]. In the case of inflammation, several meta-analyses evaluated the effects on inflammatory mediators after resveratrol supplementation, showing non-significant effects on TNF-α and IL-6 but significant reductions in high-sensitivity C-reactive protein [45,46]. In line with these findings, another meta-analysis assessing the effects of vegetable and fruit intake on inflammatory biomarkers observed similar effects on C-reactive protein and TNF-α [47]. In the case of protein, the type of protein (plant-based protein versus animal protein) may influence inflammatory status more than total protein intake [48]. Because several dietary components, such as β-carotene, lycopene, dietary fiber, and polyphenols, have beneficial effects, their combination, which is naturally found in fruits and vegetables, can enhance their anti-inflammatory properties [49].

The strengths of this study are that DXA has been considered the gold standard method for body composition measurement; thus, VAT was objectively measured with a validated imaging technique [50], and the assessment of fourteen biomarkers at baseline and after 1 year of follow-up. The main limitation of the present study is the prospective design, which does not allow attributing the conclusions to plausible causes. Moreover, the sample size is limited. Other limitations include potential residual confounding, reverse causation bias, and the lack of generalizability of the results to other populations.

In conclusion, a reduction in VAT was associated with improvements in several inflammatory and adipokines levels, mainly in insulin, C-peptide, and PAI-1 levels. These improvements may contribute to a reduction in cardiometabolic disturbances observed in obesity.

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Data sharing: There are restrictions on the availability of data for the PREDIMED-Plus trial, due to the signed consent agreements around data sharing, which only allow access to external researchers for studies following the project purposes. Requestors wishing to access the PREDIMED-Plus trial data used in this study can make a request to the PREDIMED-Plus trial Steering Committee predimed_plus_scommittee@googlegroups.com.

Table 1: Baseline characteristics according to tertiles of change in VAT (g) at 1-year in a sub-sample.

1 year – baseline VAT (g), median (min to max)	**Total**	**T1 -507g (-3114 to -283)**	**T2 -150g (-283 to 32.1)**	**T3 240g (32.1 to 1643)**	***P*-value**
N	117	39	39	39	
Age, years	65.2±4.6	63.8±4.9	65.7±3.9	66.2±4.7	0.049
Intervention arm, *n (%)*	59 (50.4)	22 (56.4)	14 (35.9)	23 (59.0)	0.083
Women, *n (%)*	60 (51.3)	10 (25.6)	22 (56.4)	28 (71.8)	<0.001
BMI, kg/m^2	32.8±3.1	32.8±3.2	32.3±2.7	33.2±3.3	0.376
Waist circumference, *cm*	107.2±8.6	109.9±8.0	105.0±9.8	106.8±7.4	0.036
Adherence to ER-MedDiet, 17p score	8.6±2.5	8.1±2.8	9.0±2.3	8.6±2.3	0.264
Total energy intake, *kcal/day*	2391±504	2454±549	2309±427	2409±530	0.436
Type 2 diabetes prevalence, n (%)	40 (34.2)	16 (41.0)	10 (25.6)	14 (35.9)	0.351
Current smokers, *n (%)*	21 (17.9)	9 (23.1)	7 (17.9)	5 (12.8)	0.084
Physical activity,(METS.min/week)	2753±2301	2342±2177	3183±2763	2735±1849	0.274
Medication, *n (%)*					
Antihypertensive agents	98 (83.8)	34 (87.2)	32 (82.0)	32 (82.0)	0.782
Cholesterol-lowering agents	63 (53.8)	20 (51.3)	23 (59.0)	20 (51.3)	0.739
Insulin	3 (2.6)	2 (5.1)	1 (2.6)	0 (0.0)	0.365
Metformin	32 (27.3)	13 (33.3)	9 (23.1)	10 (25.6)	0.578
Other hypoglycemic agents	32 (27.3)	14 (35.9)	8 (20.5)	10 (25.6)	0.305
Aspirin or antiplatelet agents	17 (14.5)	8 (20.5)	6 (15.4)	3 (7.7)	0.276
NSAIDS	25 (21.4)	6 (15.4)	5 (12.8)	14 (35.9)	0.024
Vitamin and minerals	8 (6.8)	3 (7.7)	2 (5.1)	3 (7.7)	0.877
Sedative or tranquilizer agents	29 (24.8)	8 (20.5)	10 (25.6)	11 (28.2)	0.731
Hormonal treatment (only women)	4 (3.4)	1 (2.6)	0 (0.00)	3 (7.7)	0.166
Educational level, *n (%)*					0.866
Primary school	62 (53.0)	17 (43.6)	23 (59.0)	22 (56.4)	
Secondary school	26 (22.2)	14 (35.9)	4 (10.3)	8 (20.5)	
University and other studies	29 (24.7)	8 (20.5)	12 (30.8)	9 (23.1)	

Continue variables are expressed as mean (±SD). Categorical variables are expressed as number (n) and percentage (%). Analysis of variance–one factor was used for continuous variables.

Table 2: Changes in inflammatory parameters according to tertiles of changes in visceral adipose tissue after 1-year of follow-up.

		T1	T2	T3	Minimally adjusted *P* value	Full-adjusted *P*-value	*P* for trend
		-507g (-3114 to -283)	-150g (-283 to 32.1)	240g (32.1 to 1643)	(T3 vs T1)	(T3 vs T1)	
Insulin, *pg/mL*	Baseline	163.4±82.7	116.2±60.6	146.9±88.7	0.890	0.691	0.811
	1-y Mean changes	-29.1 (-57.1; -1.1)	7.8 (-13.8; 29.4)	31.9 (-2.7; 66.4)	0.014	0.033	0.042
Glucagon, *pg/mL*	Baseline	186.7±56.9	181.2±46.1	168.7±67.2	0.220	0.382	0.336
	1-y Mean changes	-24.2 (-41.0; -7.3)	2.8 (-11.3; 16.8)	10.4 (-5.3; 26.1)	0.122	0.273	0.343
IL-6, *pg/mL*	Baseline	12.3±18.1	14.5±14.7	14.4±21.1	0.981	0.929	0.922
	1-y Mean changes	5.6 (-0.4; 11.6)	3.7 (-4.6; 12.0)	-0.0 (-7.6; 7.6)	0.595	0.965	0.922
Visfatin, *pg/mL*	Baseline	2886±2517	3341±2194	3592±2397	0.822	0.988	0.944
	1-y Mean changes	87.8 (-2046; 2221)	-52.6 (-693.2; 588.0)	72.6 (-318.3; 463.5)	0.673	0.871	0.861
Ghrelin, *pg/mL*	Baseline	111.4±79.4	105.5±55.4	86.9±51.0	0.140	0.079	0.066
	1-y Mean changes	6.8 (-5.8; 19.5)	16.2 (-1.2; 33.5)	15.5 (0.5; 30.4)	0.131	0.170	0.182
MCP-1, *pg/mL*	Baseline	160.9±176.6	224.3±202.2	195.8±213.9	0.706	0.594	0.582
	1-y Mean changes	-12.0 (-43.9; 19.7)	17.3 (-12.4; 47.0)	-3.71 (-28.3; 20.9)	0.059	0.170	0.224
GLP-1, *pg/mL*	Baseline	11.9±8.9	8.4±5.0	9.4±8.3	0.657	0.657	0.680
	1-y Mean changes	0.6 (-3.6; 5.0)	1.4 (-1.8; 4.5)	-1.9 (-6.1; 2.3)	0.434	0.973	0.922
TNF-α, *pg/mL*	Baseline	114.9±142.4	170.1±160.6	125.1±157.6	0.592	0.537	0.463
	1-y Mean changes	3.21 (-32.3; 38.7)	33.2 (2.3; 64.1)	12.2 (-16.4; 40.8)	0.028	0.033	0.042
PAI-1, *pg/mL*	Baseline	1541±559.9	1452±623.5	1358±674.0	0.092	0.037	0.038
	1-y Mean changes	-220.5 (-444.6; 3.50)	-48.1 (-197.1; 100.9)	215.9 (8.72; 423.1)	0.019	0.042	0.052
Resistin, *pg/mL*	Baseline	1885±1200	1568±1069	1316±683.4	0.028	0.026	0.023
	1-y Mean changes	-68.7 (-420.4; 283.0)	77.0 (-134.7; 288.7)	369.4 (152.8; 585.9)	0.122	0.017	0.182
C-peptide, *pg/mL*	Baseline	132.6±56.8	116.6±46.8	124.5±58.7	0.323	0.349	0.312
	1-y Mean changes	-17.2 (-33.2; -1.20)	9.6 (-2.9; 22.0)	16.1 (-0.9; 33.1)	0.019	0.033	0.042
Leptin, *pg/mL*	Baseline	16766±11445	18077±9702	18860±14878	0.466	0.433	0.380
	1-y Mean changes	-1769 (-4317; 779.1)	1528 (-1012; 4067)	4399 (702.4; 8095)	0.182	0.294	0.331

Adipsin, *pg/mL*	Baseline	3168±1290	3180±2054	2906±1276	0.745	0.690	0.661
	1-y Mean changes	-145.4 (-604.0; 313.0)	-189.1 (-690.1; 311.9)	-32.5 (-401.4; 335.4)	0.703	0.871	0.861
Adiponectin, *pg/mL*	Baseline	127332±66797	133653±87595	138053±77241	0.871	0.475	0.500
	1-y Mean changes	-1240 (-20767; 18288)	3750 (-20907; 28406)	-2076 (-22381; 18229)	0.367	0.342	0.331

N=117 participants (N=39 each tertile). Values are means±SD and mean changes are expressed as mean (95% IC). P-values and P for trend were respectively calculated by multavariate lineal regression models. Minimally adjusted model included age, sex, intervention group, recruitment center, smoking status (three categories: never smoker, smoker, and former smoker), type 2 diabetes diagnose (yes/no), changes in BMI (1-year versus baseline) and baseline levels of each parameter (pg/mL). The fully adjusted model was further adjusted for educational level (three categories: primary, secondary, or high school), physical activity level (three categories: low, moderate, and active), cholesterol-lowering treatment (yes/no), energy intake (kcal/day), saturated fatty acid intake (g/day), trans fat intake (g/day), and fiber intake (g/day). Linear trend (p for trend) was assessed across tertiles of VAT, and the mean value was assigned to each tertil. IL: Interleukin; MCP-1: Monocyte Chemoattractant Protein 1; GLP-1: Glucagon-Like Peptide-1; TNF-α: Tumor Necrosis Factor alpha; PAI-1: Plasminogen Activator Inhibitor-1.

Table 3: Changes in total energy intake and nutrient density according to tertiles of change in visceral adipose tissue after 1-year of follow-up

		T1	T2	T3	Unadjusted *P* value	Full-adjusted *P*-value	*P* for trend
		-509g (-3114 to -283)	-159g (-281 to 32.1)	242g (59.4 to 1643)	(T3 vs T1)	(T3 vs T1)	
Energy intake, *kcal/day*	Baseline	2454±549.5	2309±427.1	2409±529.9	0.699	0.178	0.170
	1-y Mean changes	-224.3 (-409.0; -79.7)	-81.6 (-204.9; 41.6)	-35.2 (-29.1; 188.7)	0.091	0.009	0.007
Carbohydrates, *g day/1000 kcal*	Baseline	95.6±14.6	98.1±13.8	100.2±17.0	0.182	0.418	0.432
	1-y Mean changes	-8.4 (-13.3; -3.5)	-9.5 (-14.2; -4.9)	-8.5 (-14.8; -2.2)	0.976	0.383	0.334
Protein, *g day/1000 kcal*	Baseline	40.7±6.9	42.2±7.0	39.8±8.3	0.593	0.170	0.153
	1-y Mean changes	2.8 (0.4; 5.2)	1.3 (-1.0; 3.6)	2.5 (-0.2; 5.2)	0.877	0.434	0.374
Total fat, *g day/1000 kcal*	Baseline	46.4±5.3	44.7±6.2	46.4±7.5	0.994	0.971	0.927
	1-y Mean changes	2.2 (0.1; 4.3)	3.4 (1.3; 5.5)	2.7 (0.1; 5.3)	0.785	0.866	0.860
MUFA, *g day/1000 kcal*	Baseline	24.6±4.3	23.5±4.7	24.3±4.7	0.809	0.435	0.490
	1-y Mean changes	3.4 (1.8; 5.0)	4.4 (2.6; 6.2)	3.5 (1.5; 5.5)	0.934	0.365	0.324
PUFA, *g day/1000 kcal*	Baseline	7.6±1.7	7.8±1.8	8.0±2.2	0.313	0.429	0.413

	1-y Mean changes	0.9 (0.3; 1.6)	0.8 (0.2; 1.5)	0.3 (-0.5; 1.1)	0.195	0.803	0.718
SFA, *g day/1000 kcal*	Baseline	11.8±2.1	10.9±1.8	11.5±2.2	0.566	0.778	0.941
	1-y Mean changes	-1.1 (-1.8; -0.5)	-0.5 (-1.1; 0.1)	-0.2 (-1.0; 0.5)	0.055	0.115	0.101
Trans fat, *g day/1000 kcal*	Baseline	0.3±0.2	0.2±0.1	0.3±0.1	0.768	0.950	0.853
	1-y Mean changes	-0.1 (-0.1; -0.0)	-0.1 (-0.1; -0.0)	-0.1 (-0.1; -0.0)	0.562	0.023	0.019
Fiber, *g day/1000 kcal*	Baseline	10.8±3.2	11.3±3.2	11.8±4.2	0.236	0.330	0.349
	1-y Mean changes	2.3 (1.2; 3.4)	1.9 (0.7; 3.1)	0.5 (-0.7; 1.8)	0.029	0.043	0.028
Alcohol, *g day/1000 kcal*	Baseline	5.3±5.9	5.1±6.3	3.2±5.2	0.104	0.856	0.777
	1-y Mean changes	0.3 (-1.2; 1.8)	0.3 (-0.8; 1.4)	-0.0 (-1.6; 1.6)	0.730	0.702	0.690
Total polyphenol, m*g day/1000 kcal*	Baseline	398.0±103.8	367.2±106.5	385.6±107.4	0.605	0.207	0.255
	1-y Mean changes	-12.2 (-40.0; 15.6)	-12.9 (-46.4; 20.6)	-42.3 (-80.3; -4.3)	0.199	0.122	0.125
Flavonoids, m*g day/1000 kcal*	Baseline	221.2±88.3	221.7±93.0	230.1±94.7	0.673	0.488	0.494
	1-y Mean changes	-9.2 (-43.0; 24.5)	-4.3 (-32.1; 23.6)	-27.6 (-59.2; 4.1)	0.403	0.309	0.288
Phenolic acids, m*g day/1000 kcal*	Baseline	136.0±61.1	113.6±50.2	118.5±53.4	0.165	0.252	0.332
	1-y Mean changes	-0.6 (-19.1; 17.8)	-13.6 (-29.7; 2.5)	-12.3 (-30.2; 5.5)	0.340	0.234	0.320
Lignans, m*g day/1000 kcal*	Baseline	0.7±0.3	0.7±0.2	0.6±0.2	0.664	0.141	0.131
	1-y Mean changes	0.0 (-0.0; 0.1)	0.0 (-0.0; 0.1)	-0.0 (-0,1; 0.0)	0.278	0.018	0.013
Stilbenes, m*g day/1000 kcal*	Baseline	1.1±1.3	1.0±1.7	0.6±1.3	0.146	0.670	0.617
	1-y Mean changes	0.2 (-0.3; 0.6)	0.5 (0.5; 1.0)	0.1 (-0.3; 0.4)	0.740	0.840	0.747

N=117 participants (N=39 each tertile). Values are means±SD and mean changes are expressed as mean (95% IC). P-values and P for trend were respectively calculated by multavariate lineal regression models. Full-adjusted model was adjusted for age, sex, intervention group, recruitment center, smoking status (three categories: never smoker, smoker, and former smoker), type 2 diabetes diagnose (yes/no), educational level (three categories: primary, secondary, or high school), physical activity level (three categories: low, moderate, and active), cholesterol-lowering treatment (yes/no), and baseline levels of each nutritional parameter (g/day). Linear trend (p for trend) was assessed across tertiles of VAT, and the mean value was assigned to each tertil. MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; SFA: Saturated fatty acids.

Supplementary 1: Changes in inflammatory parameters according to tertiles of changes in total fat mass after 1-year of follow-up.

	T1	T2	T3	Minimally adjusted *P* value	Full-adjusted *P*-value	*P* for trend

		-4.5 kg (-15.6 to -3.1)	-1.5 kg (-3.0 to -0.3)	0.4 kg (-0.1 to 7.9)	(T3 vs T1)	(T3 vs T1)	
Insulin, *pg/mL*	Baseline	145.9±75.6	150.0±94.6	129.8±67.3	0.163	0.188	0.184
	1-y Mean changes	-6.0 (-32.0; 20.0)	4.6 (-31.0; 39.9)	11.1 (-14.2; 36.4)	0.875	0.721	0.717
Glucagon, *pg/mL*	Baseline	197.8±61.6	174.6±47.3	164.4±57.6	0.774	0.864	0.863
	1-y Mean changes	-15.0 (-26.3; -3.7)	2.9 (-13.8; 19.8)	1.6 (-17.7; 20.9)	0.752	0.721	0.702
IL-6, *pg/mL*	Baseline	12.0±15.9	10.2±14.1	20.3±22.8	0.138	0.174	0.155
	1-y Mean changes	7.1 (0.5; 13.7)	7.0 (-0.3; 14.3)	-7.2 (-13.9; -0.5)	0.865	0.975	0.974
Visfatin, *pg/mL*	Baseline	3865±2800	3189±2302	2659±1751	0.836	0.580	0.590
	1-y Mean changes	163.1 (-1826; 2152)	-351.8 (-985.5; 281.9)	301.8 (-258.3; 861.9)	0.875	0.975	0.974
Ghrelin, *pg/mL*	Baseline	112.4±61.3	99.0±75.4	92.3±52.2	0.729	0.776	0.777
	1-y Mean changes	11.0 (-2.6; 24.5)	17.3 (-1.3; 35.9)	10.1 (-2.3; 22.5)	0.752	0.721	0.702
MCP-1, *pg/mL*	Baseline	172.6±195.1	184.5±170.7	225.2±226.5	0.477	0.869	0.861
	1-y Mean changes	-5.8 (-37.9; 26.2)	18.9 (-12.2; 50.0)	-11.7 (-33.0; 9.55)	0.875	0.975	0.974
GLP-1, *pg/mL*	Baseline	9.6±7.6	10.3±7.0	9.8±8.5	0.731	0.553	0.556
	1-y Mean changes	-0.3 (-5.1; 4.5)	1.9 (-1.2; 5.0)	-1.2 (-3.4; 1.0)	0.865	0.721	0.702
TNF-α, *pg/mL*	Baseline	112.7±131.5	150.4±156.4	148.7±174.3	0.250	0.546	0.527
	1-y Mean changes	21.3 (-18.7; 61.3)	21.5 (-8.9; 51.9)	4.6 (-15.6; 24.7)	0.752	0.721	0.702
PAI-1, *pg/mL*	Baseline	1550±668.2	1467±569.8	1335±613.8	0.536	0.492	0.496
	1-y Mean changes	-16.1 (-235.2; 203.0)	-70.9 (-283.6; 141.9)	39.9 (-143.3; 223.1)	0.752	0.721	0.702
Resistin, *pg/mL*	Baseline	1744±1095	1655±1114	1381±859.6	0.761	0.670	0.655
	1-y Mean changes	201.6 (-66.8; 470.0)	29.1 (-299.0; 357.3)	98.3 (-125.5; 322.0)	0.993	0.975	0.974
C-peptide, *pg/mL*	Baseline	130.7±59.2	126.6±59.3	116.2±41.8	0.346	0.483	0.472
	1-y Mean changes	-8.9 (-21.7; 3.9)	3.4 (-17.4; 24.1)	15.2 (4.1; 26.3)	0.752	0.721	0.702
Leptin, *pg/mL*	Baseline	21148±14526	17927±11428	14422±8884	0.563	0.780	0.780
	1-y Mean changes	-1626 (-4240; 987.9)	1789 (-924.0; 4501)	4044 (593.2; 7495)	0.836	0.721	0.725
Adipsin, *pg/mL*	Baseline	3365±1675	3074±1336	2815±1648	0.930	0.709	0.709
	1-y Mean changes	-445.8 (-916.3; 24.7)	18.5 (-485.6; 522.5)	72.5 (-261.3; 406.4)	0.752	0.721	0.702
Adiponectin, *pg/mL*	Baseline	134872±65979	131067±85069	133099±81013	0.545	0.294	0.294
	1-y Mean changes	-9839 (-31386; 11709)	4260 (-19586; 28106)	6012 (-12815; 24840)	0.752	0.721	0.702

N=117 participants (N=39 each tertile). Values are means±SD and mean changes are expressed as mean (95% IC). P-values and P for trend were respectively calculated by multavariate lineal regression models. Minimally adjusted model included age, sex, intervention group, recruitment center, smoking status (three categories: never smoker, smoker, and former smoker), type 2 diabetes diagnose (yes/no), changes in BMI (1-year versus baseline) and baseline levels of each parameter (pg/mL). The fully adjusted model was further adjusted for educational level (three categories: primary, secondary, or high school), physical activity level (three categories: low, moderate, and active), cholesterol-lowering treatment (yes/no), energy intake (kcal/day), saturated fatty acid intake (g/day), trans fat intake (g/day), and fiber intake (g/day). Linear trend (p for trend) was assessed across tertiles of VAT, and the mean value was assigned to each tertil. IL: Interleukin; MCP-1: Monocyte Chemoattractant Protein 1; GLP-1: Glucagon-Like Peptide-1; TNF-α: Tumor Necrosis Factor alpha; PAI-1: Plasminogen Activator Inhibitor-1.

Manuscript VI

Title: "Reliability and Concurrent and Construct Validity of a Food Frequency Questionnaire for Pregnant Women at High Risk to Develop Fetal Growth Restriction."

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Article

Reliability and Concurrent and Construct Validity of a Food Frequency Questionnaire for Pregnant Women at High Risk to Develop Fetal Growth Restriction

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Abstract: Accuracy of dietary assessment instruments such as food frequency questionnaire (FFQ) is crucial in the evaluation of diet–disease relationships. Test–retest reliability and concurrent and construct validity of a FFQ were evaluated in 150 pregnant women at high risk to develop fetal growth restriction randomly selected from those included in the improving mothers for better prenatal care trial Barcelona (IMPACT BCN). The FFQ and dietary records were performed at baseline and 34–36 weeks of gestation. Test–retest reliability of the FFQ for 12 food groups and 17 nutrients was moderate (ICC = 0.55) and good (ICC = 0.60), respectively. Concurrent validity between food, nutrients and a composite Mediterranean diet score (MedDiet score) and food records was fair for foods and nutrients (ρ average = 0.38 and 0.32, respectively) and moderate (r = 0.46) for the MedDiet score. Validation with biological markers ranged from poor (r = 0.07) for olives to moderate (r = 0.41) for nuts. A fair concordance between methods were found for nutrients (weighted κ = 0.22) and foods (weighted κ = 0.27). The FFQ-derived MedDiet score correlated in anticipated directions with intakes of nutrients and foods derived by food records. The FFQ showed a moderate test–retest reliability and reasonable validity to rank women according to their food and nutrient consumption and adherence to the Mediterranean diet.

Keywords: food frequency questionnaire; validity; reliability; Mediterranean diet; pregnancy; fetal growth restriction

1. Introduction

Monitoring the diet quality of pregnant women is crucial for the improved health of both themselves and their child. Good nutrition during pregnancy may help to prevent deficiencies that affect the health of both the mother and fetus [1,2]. Women following a Mediterranean diet (MedDiet) during pregnancy have displayed superior health for both themselves and their offspring [3]. The economic benefits of using food frequency questionnaires (FFQs), compared to more expensive methods such as 24 h recalls (24 hR) or food records [4], make them a popular tool to estimate food intake for different populations. However, the validity of an FFQ is limited to the target population in which it was validated. Thus, an FFQ validated in the general population [5] should not be administered to pregnant women.

The accuracy of an FFQ can be determined by comparing food and nutrient intake information collected by this instrument with those obtained using a reference method. Weighed food records are considered the gold standard method; however, the application of this method is often not feasible. Therefore, food records and 24 hR are widely used as the reference method. However, these instruments are not free from measurement errors caused by memory bias among others [4]. These markers are in contrast to auto reported data objective measures of food intake. It is, therefore, appropriate to include additional biological markers of food intake in the evaluation of the validity of an FFQ [6].

Most FFQ validation studies compare intake estimates collected by an FFQ with those collected by a reference method to assess their agreement in terms of reported intakes of individual nutrients and/or food groups. However, it should be acknowledged that these results cannot yield a generalized score composed of several specific nutrients and/or foods like the Mediterranean diet score (MedDiet score).

Given the great impact of diet quality on health [1,3,7], it is important that FFQs correctly estimate food and nutrient intake. However, most FFQ validation studies have not gone further than analyzing the presentation of data on test–retest reliability and concurrent validity of the questionnaire. Thus, this study aimed to determine the test–retest reliability indicating the stability of the FFQ over time through repeated measures at two different time points. Furthermore, we determined concurrent or relative validity, which indicates the amount of agreement between two different measures, and construct validity a measure of the concept that it is intended to measure. For this purpose, we compared dietary data obtained by the FFQ with those derived by food records and biological markers of food intake. Additionally, we analyzed these validity domains along with changes in adherence to the MedDiet.

2. Methods

2.1. Study Population

The present validation study was performed for the FFQ for women participating in improving mothers for better prenatal care trial Barcelona (IMPACT BCN) (NCT03846206). Eligible participants for the IMPACT trial were pregnant women with a high risk of developing fetal growth restriction (FGR) during pregnancy (odds ratio, OR >2), according to the criteria of the Royal College of Obstetricians and Gynecologists (RCOG) [8]. We randomly selected women attending their second-semester scan (from 19–23 weeks of gestation) to participate. Exclusion criteria were any of the following: fetal anomalies including chromosomal abnormalities, structural malformations or congenital infections detected prenatally; neonatal abnormalities diagnosed after birth; no possibility to perform additional visits; participation in another trial and maternal mental retardation or other mental or psychiatric disorders. After providing informed consent, they were randomized into three equally-sized intervention groups: (1) nutrition program based on MedDiet; (2) stress-reduction program based on mindfulness techniques and (3) control group with no specific intervention (usual care). From a total of 304 pregnant women who agreed to participate, we randomly selected 150 (50 per group). Complete data on plasma concen-

trations of vitamin B12, folic acid and linolenic acid and urine hydroxyl tyrosol were only available for 109 of the participants.

The study was approved by the Institutional Review Ethics Committee of the Hospital Clinic, Barcelona (HCB-2016-0830) and registered with the ClinicalTrials.gov identifier number NCT03166332. The study protocol was described elsewhere [9]. Signed informed consent was obtained for all participants.

2.2. Dietary Assessment

Dietary assessment was self-reported by the participants and performed at baseline (19–24 weeks) and at the final visit (34–36 weeks of gestation). The participants were asked about their dietary habits during the last year. Food consumption was estimated by a slightly modified and validated semiquantitative optical readable FFQ [5] administered by a trained interviewer. In a 151-item food list including alcoholic and non-alcoholic beverages (typical foods in Spain), participants indicated their usual consumption and chose from nine frequency categories, ranging from never or <1 time/month to ≥6 times/day. Food items were listed under 14 food groups: milk and dairy products, cereals and whole grains, vegetables, legumes, sausages, oils and fats, eggs, meat and fish, fast food, canned products, fruit, nuts, sweets and desserts and others (salt and sugar) and alcoholic and non-alcoholic beverages. The original questionnaire had 137 items. We added few food-items to our FFQ in order to adapt the habitual food products consumption in our geographical area, such as sweeteners, soy milk and other plant-based milks, avocado, dark chocolate, different snacks and non-alcoholic beverages.

Seven-day food records were collected at baseline and follow-up final visits. The participants filled a 7-day food diary of the previous 7 days before the meeting. Detailed information about their dietary intake, including the estimated grams or portions sizes was collected. Moreover, in the food diary the instructions with the portion sizes and how to provide this information in the food dairy was also described. Three days out of the seven-day food records, including two working days and one day in the weekend, were collected during each visit. Food consumption derived by the FFQ and food records was converted into energy and nutrient intake by the CESNID and Moreiras composition tables using traditional recipes [10,11].

2.3. Calculation of the Mediterranean Diet Score (MedDiet Score)

The MedDiet score was calculated according to the Trichopoulou and colleagues' method at baseline and follow-up [12]. The MedDiet score calculated at follow-up was used to assess concurrent validity. Additionally, biological markers analyzed at follow-up were used for the correlation with the corresponding food intake at follow-up. Median distribution of cereals, dairy products, fish, meat, legumes, vegetables, fruits and nuts, and the ratio between monounsaturated to saturated fatty acids (MUFA/SFA) were calculated. One point was assigned for intake of each food group of cereals, fruits and nuts, vegetables, legumes and fish and for MUFA/SFA equal or above the median and zero points for the consumption below the median. The consumption of dairy products and meat was reverse-coded. The final score ranged from 0 to 8 units. Alcohol intake was not included in the calculation because, for the participants who identified themselves as alcohol consumers (32.7%), the average intake of alcohol was below 1 g a day.

2.4. Biological Markers

Routine analyses were performed at the CORE laboratory of the Hospital Clinic of Barcelona, which fulfilled all the required quality criteria for the study. Blood samples were drawn in the morning (after a minimum of 10 h of fasting) for B12 vitamin and folic acid analyses. B12 vitamin and serum folic acid were measured by automated electro-chemiluminescence immunoassay system Advia-Centaur, Siemens (Siemens Tarrytown, NJ, USA), with reagents provided by the instrument manufacturer. The estimation of hydroxytyrosol and its glucuronides levels were measured following the method proposed

by Khymenets et al. [13] in the Department of Nutrition, Food Sciences and Gastronomy, XaRTA, School of Pharmacy and Food Sciences, University of Barcelona. Briefly, 0.5 mL of urine was acidified with H3PO4 at 4% and then extracted with solid-phase extraction (SPE) using Oasis® HLB 3 cc (60 mg) cartridges (Waters Corporation, Ireland). The extract was evaporated under an N2 curtain and reconstituted in 200 uL of 1 mM ammonium acetate at pH 5. The identification and quantification of these compounds were analyzed with UPLC-MS/MS analyses.

Plasma α-linolenic acid concentrations were assessed by gas chromatography [14]. The analysis was performed on a Shimadzu GC-2010 gas chromatograph equipped with a flame ionization detector (Shimadzu, Kyoto, Japan), with capillary columns of 10 m × 0.10 mm × 0.10 µm film thickness (Varian, Palo Alto, CA, USA). Determinations of individual fatty acids were estimated according to their retention time during gas chromatography and were quantified as a percentage by weight (% by weight) of total plasma fatty acids.

The limits of quantification for biological markers were 9.86–32.89 ng/mL, 10.0–16.8 ng/mL, equal or less than 90 pg/mL and equal or less than 0.70 ng/mL for hydroxytyrosol, fatty acids, vitamin B12 and folic acid, respectively.

We chose these biomarkers because they represent an objective measure of typical Mediterranean foods such as olive oil, olives nuts and fish.

2.5. Other Variables

Height and weight were measured at baseline and final visit. Information on demographic and socioeconomic variables and tobacco smoking were obtained through structured standard questionnaires administered by trained personnel. Socioeconomic status was defined as low if the subject had either never been in employment or had been unemployed for more than two consecutive years, medium if the subject had completed secondary studies and was currently in employment and high if the subject had completed university studies and was currently in employment.

2.6. Statistical Analysis

Mean with standard deviation (SD) and proportions (%) were calculated for the characteristics of the subjects (shown in Table 1). Intraclass correlation coefficients between basal and follow-up data on FFQ-derived nutrients and food categories were determined for test–retest reliability. For the analysis of concurrent (relative) and construct validity we used data from the FFQ administer at follow-up and data from food records (reference method). The relative validity of the FFQ (test method) against the food records (reference method) was first assessed by calculating the Spearman correlation coefficient. Additionally, we determined Pearson correlation coefficients between biological markers and the corresponding food and nutrient intake. However, it should be observed that two highly correlated measures can still show considerable differences between the two measurements across their range of values. We thus calculated the absolute agreement of categorical variables between the two measurements by cross-classification and κ statistic of tertile distribution of food categories and nutrients. Values of κ were organized as follows: >0.8 for almost perfect agreement, between 0.61 and 0.80 for substantial agreement, 0.41 and 0.60 for moderate agreement, 0.21 and 0.40 for fair agreement and $\leq$0.20 for slight agreement [15]. The concurrent validity and absolute agreement of the MedDiet adherence scores derived by the FFQ (test method) and the dietary records (reference method) were also tested. Agreement between the scores obtained by the FFQ and dietary records was then assessed using the Bland–Altman method [16] and the intraclass correlation coefficient (ICC). These methods assess the agreement between two methods by calculating the mean of their differences and regressing that figure against the average score obtained by the two methods. A complete agreement between the methods would involve a mean proportional agreement of 100% and a mean difference of 0 between the scores derived by both measurements. Proportional bias represented by possible variations in the level of agreement between methods was also analyzed. We then fitted linear regression

models, with the mean instrument differences of the FFQ- and dietary records-derived MedDiet scores (FFQ–dietary records) constituting the dependent variable and the mean score of both ((FFQ + dietary records)/2)) constituting the independent variable. Finally, to assess construct validity, general linear modelling was used to estimate associations between energy-adjusted nutrient intakes derived from food records and Mediterranean diet adherence (tertile distribution) calculated from the FFQ. Linear trends were tested by including the categorized variable (tertile distribution of the scores) as continuous in this model. The polynomial contrast was used to determine p for the linear trends for continuous variables and a post hoc Bonferroni correction for multiple comparisons was conducted. The Statistical Package for the Social Sciences statistical software package version 21.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Differences were considered significant if p was <0.05.

Table 1. General characteristics of the participants (n = 150).

Variables	
Age, years (SD)	38.0 (4.0)
Weight, kg (SD)	65.8 (12.8)
Height, cm (SD)	162 (6)
Education, %	
- High	69.3
- Medium	26.7
- Low	4
Current smokers, %	6
Alcohol consumer, %	32.7 *
Energy intake, kcal/d	2412 ± 500

* Below a mean of 1 g a day.

3. Results

General characteristics of the study population are shown in Table 1. The mean of the MedDiet score at baseline and follow up was 4.0 ± 1.5 and 4.1 ± 1.6, respectively. Table 2 shows the test–retest reliability of the FFQ administered at 19–24 and 34–36 weeks of gestation. We found a significant test–retest reliability for each of the 12 food groups and 17 nutrients. The ICC ranged from moderate (cereal = 0.42) to very good (vegetables = 0.83) for foods and from moderate (MUFA = 0.48) to very good (Vitamin A = 0.86) for nutrients. The average correlation of foods and nutrients was 0.70 and 0.69, respectively. Test–retest reliability for the MedDiet score showed an ICC of 0.53, considerably lower than for foods and nutrients.

The analysis of concurrent validity (Table 3) yielded correlations ranging from poor to good for foods and nutrients. The average correlations of foods and nutrients between the two methods were 0.24 and 0.21, respectively. All correlations were significant with the exception for olive oil and vitamin B12 for non-standardized and olive oil, vitamin B12 and monounsaturated fat for standardized foods and nutrients (Tables 3 and 4). The degree of correlations ranged from poor (olive oil ρ = 0.12) to good (dairy products ρ = 0.63) for foods and from poor (vitamin B12 ρ = 0.10) to moderate (vitamin C ρ = 0.47) for nutrients (Table 3). Concordance between methods ranged from poor to fair for foods and nutrients. κ statistic showed a fair concordance between the methods for foods (weighted κ = 0.28) and nutrients (weighted κ = 0.21). The adjustment for energy intake did not meaningfully improve correlations or concordance between the methods (Table 4). The comparison of the biomarker α-linolenic acid was limited to nut consumption because there were no other relevant food sources α-linolenic acid included in the FFQ. Hydroxytyrosol was correlated with olive oil and olives, both principal sources of this bioactive compound. The comparison between biomarkers of food and nutrient intake with the corresponding data derived by the FFQ revealed a poor (r = 0.07; p = 0.59) to moderate (r = 0.41; p <0.001) concurrent validity for hydroxytyrosol and α-linolenic acid, respectively (Table 5).

Table 2. Test–retest reliability of the food frequency questionnaire (n = 150).

Food Groups [1] (g)	**ICC [2]**	**95% CI**
Cereals	0.26	0.11–0.41
Legumes	0.55	0.42–0.65
Vegetables	0.71	0.62–0.78
Fruits	0.53	0.41–0.64
Nuts	0.51	0.37–0.63
Dairy products	0.67	0.57–0.75
Fish	0.62	0.51–0.71
Meat	0.58	0.46–0.68
Processed meat	0.63	0.52–0.72
Olive oil	0.54	0.41–0.64
Animal fat	0.62	0.43–0.76
Baked goods	0.36	0.21–0.50
Nutrients [1] (units)	ICC	95% CI
Energy (kcal)	0.57	0.45–0.67
Carbohydrates (g)	0.58	0.46–0.68
Proteins (g)	0.62	0.51–0.71
Fat (g)	0.50	0.54–0.76
Saturated FA (g)	0.51	0.38–0.77
Monounsaturated FA (g)	0.48	0.35–0.59
Polyunsaturated FA (g)	0.49	0.36–0.60
Cholesterol (mg)	0.66	0.55–0.74
Fiber (g)	0.68	0.59–0.76
Potassium (mg)	0.67	0.57–0.75
Calcium (mg)	0.61	0.50–0.70
Vitamin C (mg)	0.62	0.51–0.71
Vitamin A (mcg)	0.76	0.68–0.82
Vitamin E (mg)	0.59	0.47–0.68
Niacin (mg)	0.62	0.51–0.71
Folic acid (mcg)	0.70	0.60–0.77
Vitamin B_{12} (mcg)	0.68	0.59–0.76
Score (units)	ICC	95% CI
MedDiet score	0.53	0.40–0.63

ICC = intraclass correlation coefficient; CI = confidence interval; FA = fatty acids; MedDiet = Mediterranean diet. [1] Variables were log-transformed with the exception of energy and the MedDiet score.[2] $p < 0.01$ for all.

Table 3. Concurrent validity and concordance of foods and nutrients derived by the food frequency questionnaire and dietary records (n = 150).

Food Groups (g/day)	**FFQ**			**24 h Recalls**			**Spearman's Correlation Coefficient [1]**	**Magnitude of Association**	**Weighted κ**
	Median	**P25**	**P75**	**Median**	**P25**	**P75**			
Cereals	94.3	77.1	113	146	119	180	0.29	Fair	0.23
Legumes	52.9	37.4	72.9	22.5	3.14	41.1	0.37	Fair	0.26
Vegetables	351	258	448	180	130	245	0.26	Fair	0.13
Fruits	374	255	501	204	137	283	0.53	Moderate	0.39
Nuts	17.1	6.0	32.0	4.33	0	10.0	0.46	Moderate	0.30
Dairy products	337	230	527	245	159	336	0.63	Good	0.42
Fish	78.8	53.1	108	60.0	34.6	80.0	0.40	Fair	0.29
Meat	107	74.3	149	60.0	35.0	91.7	0.30	Fair	0.24
Processed meat	40.6	21.4	60.1	40.4	22.3	59.2	0.38	Fair	0.29
Olive oil	50.0	50.0	50.0	35.0	30.9	35.9	0.12	Poor	-
Animal fat	0	0	5.14	0	0	3.35	0.47	Moderate	0.43
Baked goods	30.5	14.3	50.3	52.1	34.8	86.8	0.34	Fair	0.14

Table 3. *Cont.*

Food Groups (g/day)	FFQ			24 h Recalls			Spearman's Correlation Coefficient [1]	Magnitude of Association	Weighted κ
	Median	P25	P75	Median	P25	P75			
Nutrients (units/day)									
Energy (kcal)	2447	2133	2702	2001	1759	2198	0.29	Fair	0.16
Carbohydrates (g)	207	173	255	205	180	239	0.27	Fair	0.14
Proteins (g)	109	89.1	127	87.6	77.1	98.5	0.41	Moderate	0.28
Fat (g)	126	110	144	93.2	81.5	104	0.33	Fair	0.25
Saturated FA (g)	33.1	27.1	39.9	26.2	21.7	30.6	0.29	Fair	0.17
Monounsaturated FA (g)	62.2	53.5	71.5	42.3	38.6	47.0	0.28	Fair	0.17
Polyunsaturated FA (g)	20.8	16.4	27.5	13.8	11.2	17.2	0.37	Fair	0.25
Fibers (g)	34.0	26.9	42.0	21.8	18.6	25.3	0.42	Moderate	0.25
Cholesterol (mg)	313	270	395	294	240	350	0.26	Fair	0.17
Potassium (mg/day)	4530	3916	5517	2664	2213	3059	0.38	Fair	0.26
Calcium (mg)	1089	834	1371	869	727	1043	0.44	Moderate	0.30
Vitamin C (mg)	259	187	344	117	86.4	158	0.47	Moderate	0.29
Vitamin A (mcg)	1354	943	1754	1072	690	1433	0.28	Fair	0.16
Vitamin E (mg)	18.2	15.1	22.0	9.1	7.99	10.5	0.34	Fair	0.25
Niacin (mg)	24.9	21.2	29.1	52.5	45.3	61.3	0.27	Fair	0.19
Folic acid (mcg)	495	391	599	371	292	431	0.26	Fair	0.22
Vitamin B_{12} (mcg)	7.1	5.40	10.4	5.15	3.66	8.33	0.11	Poor	0.10

[1] $p < 0.05$ for all with the exception of olive oil and vitamin B12.

Table 4. Concurrent validity and concordance of foods and nutrients per 1000 kcal derived by the food frequency questionnaire and 24 h recalls (n = 150).

Food Groups (g/100 kcal/day)	FFQ			24 h Recalls			Spearman's Correlation Coefficient [1]	Magnitude of Association	Weighted κ
	Median	P25	P75	Median	P25	P75			
Cereals	39.5	33.2	47.7	73.0	59.2	87.2	0.25	Fair	0.19
Legumes	22.2	15.5	31.3	11.6	1.46	20.7	0.33	Fair	0.18
Vegetables	143	111	179	93.8	67.2	120	0.33	Fair	0.23
Fruits	149	106	200	103	71.8	141	0.50	Moderate	0.28
Nuts	7.71	2.83	12.7	2.18	0	5.0	0.47	Moderate	0.34
Dairy products	135	94.7	209	127	85.2	170	0.65	Good	0.45
Fish	33.8	21.9	45.9	28.8	17.3	41.3	0.43	Moderate	0.26
Meat	43.3	31.7	56.5	31.8	17.6	45.0	0.29	Fair	0.18
Processed meat	16.8	10.0	25.0	20.0	10.8	28.4	0.40	Fair	0.23
Olive oil	19.2	15.3	22.3	16.6	14.9	18.8	0.07	Poor	0.04
Animal fat	0	0	1.40	0	0	1.81	0.47	Moderate	0.42
Baked goods	12.5	6.11	20.1	27.8	16.5	43.1	0.36	Fair	0.20
Nutrients (units/day)									
Carbohydrates (g)	87.1	79.1	94.8	103	96.2	112	0.42	Moderate	0.27
Proteins (g)	44.1	39.2	50.4	43.3	38.9	48.9	0.58	Moderate	0.37
Fat (g)	52.2	48.2	56.2	47.0	43.9	49.7	0.25	Fair	0.14
Saturated FA (g)	13.7	12.3	15.3	13.3	11.4	14.7	0.39	Fair	0.26
Monounsaturated FA (g)	25.4	23.2	27.6	21.7	19.5	22.8	0.12	Poor	0.11
Polyunsaturated FA (g)	8.7	7.33	10.6	6.86	5.92	8.02	0.44	Moderate	0.32
Fibres (g)	14.2	12.0	16.3	10.9	9.31	12.7	0.37	Fair	0.23
Cholesterol (mg)	133	117	155	149	123	175	0.31	Fair	0.16
Potassium (mg/day)	1901	1667	2092	1314	1179	1544	0.39	Fair	0.26
Calcium (mg)	446	360	538	436	362	520	0.57	Moderate	0.40

Table 4. *Cont.*

Food Groups (g/100 kcal/day)	FFQ			24 h Recalls			Spearman's Correlation Coefficient [1]	Magnitude of Association	Weighted κ
	Median	P25	P75	Median	P25	P75			
Nutrients (units/day)									
Vitamin C (mg)	109	77.3	132	59.9	42.5	82.6	0.46	Moderate	0.26
Vitamin A (mcg)	538	415	699	550	341	752	0.28	Fair	0.23
Vitamin E (mg)	7.48	6.58	8.57	4.62	4.07	5.12	0.25	Fair	0.21
Niacin (mg/day)	10.2	9.02	11.4	27.1	22.6	30.8	0.31	Fair	0.18
Folic acid (mcg)	207	178	236	179	150	218	0.20	Poor	0.16
Vitamin B_{12} (mcg)	2.96	2.33	4.08	2.52	1.94	4.15	0.08	Poor	0.07

[1] $p < 0.05$ with the exception of olive oil, vitamin B12 and monounsaturated fat.

Table 5. Pearson correlations (r) between biological markers and related nutrient or food intake derived by the food frequency questionnaire (FFQ) (n = 109).

FFQ	Biological Marker	r	*p*-Value	r Adjusted [1]	*p*-Value
Nuts	α-linolenic acid	0.41	<0.001	0.23	0.010
Olive oil	Hydroxytyrosol	0.23	0.015	0.23	0.004
Olives	Hydroxytyrosol	0.07	0.587	0.05	0.615
Folic acid	Folic acid	0.25	0.010	0.24	0.012
Vitamin B_{12}	Vitamin B_{12}	0.09	0.333	0.10	0.304

All variables were log-transformed before analysis. [1] Standardized to 1000 kcal.

The mean ratings of the MedDiet score derived by the FFQ at baseline and follow-up 4.0 ± 1.5 and 4.1 ± 1.6, respectively, and for dietary records 4.0 ± 1.5. The FFQ significantly ($p < 0.001$) overestimated the MedDiet score (by 12%) compared to the corresponding MedDiet score derived by the reference method. However, no proportional bias was found (β coefficient 0.072; 95% CI-0.064, 0.204; $p < 0.287$) (Table 6 and Figure 1) across score ratings. The Pearson coefficient revealed a moderate and significant correlation (0.46 and $p < 0.001$) between the scores derived by the dietary records and FFQ. Additionally, the intraclass correlation coefficient, an indicator of the degree to which both instruments assigned the same absolute score ratings, showed the same degree of correlation (ICC = 0.46; $p < 0.001$). These findings indicate that the FFQ had a moderate ability to rank participants according to their adherence to the MedDiet. To analyze construct validity, we hypothesized a priori relationships between higher scores of the more favorable intake profiles for 17 nutrients. We found that intakes of 17 nutrients were associated in the anticipated direction with MedDiet score ratings derived by the FFQ, the associations were significant for nearly 50% of the nutrients (Supplementary Table S1).

Table 6. Correlation coefficients and between-method agreement of the Mediterranean diet adherence score.

	n = 150
Mean FFQ, unit (SD)	4.1 (1.6)
Mean dietary records, unit (SD)	4.0 (1.5)
Between-method difference, unit (SD) [1]	0.1 (0)
Proportional agreement, % (95% CI) [2]	113 (109, 124)
Upper LOA	3.2
Lower LOA	−3.0
Regression coefficient,[3] (95% CI)	0.072 (−0.061, 0.204)
Pearson correlation coefficient	0.46
Intra-class correlation coefficient	0.46
Absolute agreement, % [4]	57

Table 6. *Cont.*

	n = 150
Gross misclassification, % [5]	11
κ [6]	0.28

CI = confidence interval, FFQ = food frequency questionnaire, LOA = limits of agreement, MedDiet score = Mediterranean diet score. [1] Calculated as FFQ MedDiet score—dietary records MedDiet score. [2] Calculated as (FFQ MedDiet score/dietary records MedDiet score) × 100. [3] Regression coefficients (β) between the mean of the MedDiet score of both methods and the mean difference between both methods (independent variable). [4] Correctly classified tertiles of the MedDiet score derived by the FFQ and dietary records. [5] Opposite tertiles of the MedDiet score derived by the FFQ and dietary records. [6] Weighted κ between tertiles of the MedDiet score derived by the FFQ and dietary records.

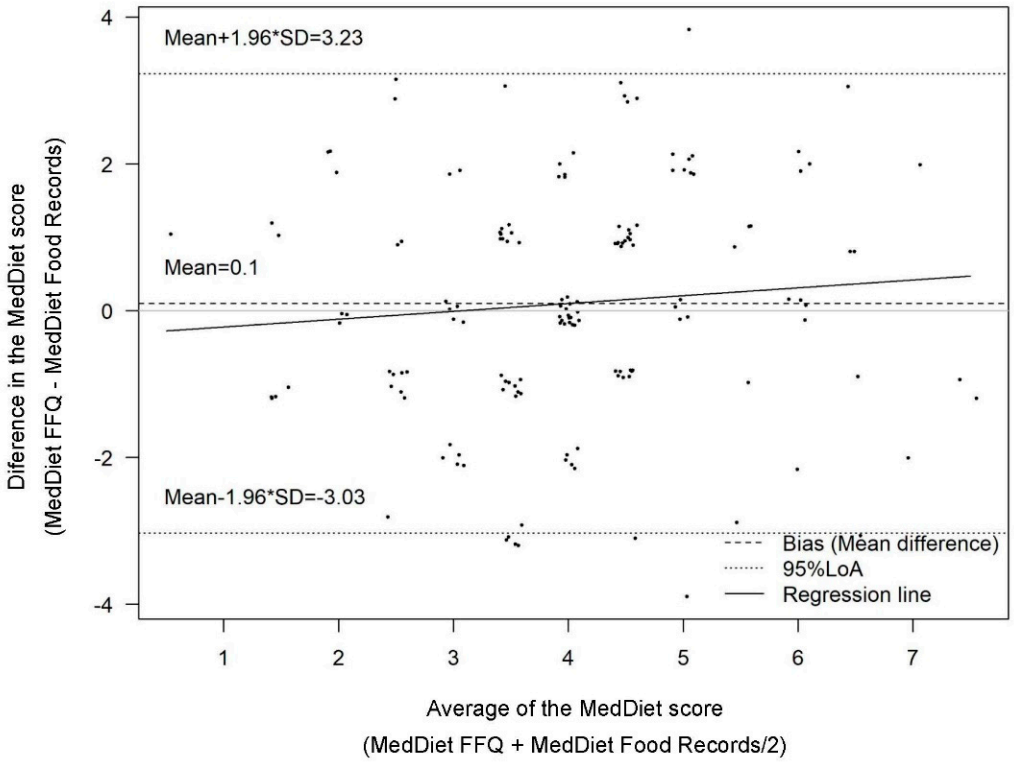

Figure 1. Bland-Altman plot for the agreement of the MedDiet score derived from the food frequency questionnaire and the reference method (n = 150).

4. Discussion

The results of this validation study show a good test–retest reliability and a fair to moderate validity of the FFQ for pregnant women. The questionnaire adequately ranked women according to their adherence to the MedDiet. Additionally, the construct of the MedDiet score was valid.

FFQs are useful tools for assessing long-term dietary intake in epidemiological studies [17]. Few FFQs capturing the complete diet have been developed and validated in European populations of pregnant women during the last 20 years [18–24]. Of those that have, only test–retest reliability and concurrent validity were analyzed, using 24 hRs or dietary records as the reference method. In this study, the average test–retest reliability for 12 food groups and 17 nutrients was moderate (ICC = 0.55) and good (ICC = 0.61), respectively. This is somewhat higher than that found by Vioque et al. [21] who reported moderate reliability for 29 nutrients (r = 0.51) and 17 foods (r = 0.41). A Finnish study found a higher average correlation (ICC = 0.65) for all foods and nutrients [18]. However, these comparisons are somewhat limited due to the different amounts of foods and nutrients considered and, in the case of Vioque et al. [21], different statistical methods used. Additionally, the test–retest reliability of diet in these studies is based on the assumption that

differences found between the two estimations are mainly due to measurement errors and less to alterations in dietary habits. In the present study one third of the pregnant women were allocated to a nutritional intervention program and have, therefore, changed their dietary habits during the test–retest period. This fact might partially explain the magnitude of the test–retest reliability of the present FFQ

In this study we found a poor (olive oil ρ = 0.12) to good (dairy products ρ = 0.63) concurrent validity of the FFQ for foods and from poor (vitamin B12 ρ = 0.10) to moderate (vitamin C ρ = 0.47) for nutrients compared with dietary records These findings are comparable with previous reports of the validity of FFQs in European pregnant women [22–27]. The poor concurrent validity of olive oil in the present study was somewhat surprising because it is a characteristic food of the Mediterranean diet and hence it is unlikely that the relatively short reporting time of the reference method was a reason for this finding. However, the objective measure of olive oil consumption by its corresponding biological marker revealed a better correlation. In contrast, a poor correlation of vitamin B12 derived by the FFQ with the corresponding data from the reference method and the biological marker were found for both. One might argue that this possibly reflects the difficulty in estimating meat servings but the correlations of meat and processed meat between methods were substantially better than that for vitamin B12. The poor correlation of vitamin B12 derived by the FFQ with its biological marker might be biased by dietary supplements containing vitamin B12.

Drawing a fair comparison between the concordance between the methods applied in this study and that of other publications presents a challenge, as most validation studies present proportional agreement instead of kappa statistics. The proportional agreement is easily understandable although it does not consider coincidental occurrences of agreement between two different measures. In this study, the concordance between food and nutrient intake derived by the FFQ and food records ranged from poor to moderate with an average weighted kappa of 0.21 and 0.28 for foods and nutrients, respectively. This finding indicates a fair overall concordance between the FFQ and reference methods.

Food records and 24 hRs are frequently used as gold standards in validation studies of dietary assessment [17]. These reference methods are not free from measurement errors, however, the type of error is independent of those from an FFQ [17]. The objective measurement of food and nutrient intake by corresponding biological markers yielded a more robust estimate of an FFQs validity. In this study, objective measurements of plasma levels of both folic acid and linolenic acid and urinary hydroxyltyrosol were fairly correlated ($p < 0.05$) with their corresponding FFQ-derived nutrient or food. Poor correlations were found for olives (r = 0.07; $p = 0.58$) and vitamin B12 (r = 0.09; $p = 0.30$). The magnitude of correlation was somewhat better for folic acid (0.12 vs. 0.25) but similar for vitamin B12 than that found by Vioque et al. [21]. Usually, validation studies compare intake estimates between the collected data from the desired instrument against a reference method to assess whether it correctly classifies reported intakes of nutrients and food groups. From these results, nothing can be deduced regarding the accuracy of the ranking for a composite score of multiple nutrients and/or foods like the MedDiet score; evidence for the validity of predefined indices, such as the MedDiet score, is scarce [22–24]. Benitez-Arciniega et al. [25] also reported a moderate (r = 0.48) correlation between a modified MedDiet score derived by an FFQ and repeated 24 h dietary recalls in a Mediterranean population. Stronger concurrent validity was found for the traditional and alternate MedDiet score in German women [27] and a multiethnic Asian population, respectively [27]. In comparison with this study, the agreement between test and reference method was considerably stronger in the Asian group but only slightly different in the German population.

Construct validity should also be considered when selecting a dietary assessment tool. We hypothesized that both the FFQ- and dietary records-derived dietary quality scores would show a positive correlation and that both of the FFQ-derived dietary quality indices would be positively associated with a favorable nutrient intake profile estimated by food records. Intakes of 17 nutrients were significantly associated in the anticipated direction

with MedDiet score ratings derived by the FFQ. These findings are in line with that of Benitez-Arciniega et al. [25] who reported good construct validity of two MedDiet scores by correlating nutrient intake derived by multiple food records with the MedDiet scores.

The strength of the present study is that validity was determined by correlations with foods and nutrients derived by a self-reported reference method and biological markers of food and nutrient intake. An additional strength is the inclusion of the validity of MedDiet adherence by a composite score. Finally, as in all validation studies, an inherent limitation is that reference methods such as multiple dietary recalls or records are themselves not free from error [17]. Food records for example requires motivated subjects and place a high burden on the participants. The ideal choice of the reference method is weighed food records, which is considered the "gold standard". However, the administration of food records or weighed food records may lead to participants changing their diet during a recording period. Food frequency questionnaires on the other hand are prone to memory bias because these questionnaires asked for the retrospective food intake. Furthermore, average consumption frequency of seasonal foods is especially critical and the fixed food list in fixed portion sizes are other sources of measurement error. Finally, the use of the FFQ to present data of absolute intakes of foods and nutrients is limited without prior calibration of these data by a reference method. This is especially the case for foods and nutrients with poor concurrent validity and concordance.

5. Conclusions

In conclusion, the present FFQ is a dietary assessment instrument with reasonable validity in its application to a population of pregnant women.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/nu13051629/s1, Table S1: Daily nutrient intake derived by 24 h recalls according to adherence to the Mediterranean diet calculated by the food frequency questionnaire.

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Review

Relationship between Mediterranean Dietary Polyphenol Intake and Obesity

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Abstract: Obesity is a multifactorial and complex disease defined by excess of adipose mass and constitutes a serious health problem. Adipose tissue acts as an endocrine organ secreting a wide range of inflammatory adipocytokines, which leads to systemic inflammation, insulin resistance, and metabolic disorders. The traditional Mediterranean diet is characterized by a high phenolic-rich foods intake, including extra-virgin olive oil, nuts, red wine, vegetables, fruits, legumes, and whole-grain cereals. Evidence for polyphenols' effect on obesity and weight control in humans is inconsistent and the health effects of polyphenols depend on the amount consumed and their bioavailability. The mechanisms involved in weight loss in which polyphenols may have a role are: activating β-oxidation; a prebiotic effect for gut microbiota; inducing satiety; stimulating energy expenditure by inducing thermogenesis in brown adipose tissue; modulating adipose tissue inhibiting adipocyte differentiation; promoting adipocyte apoptosis and increasing lipolysis. Even though the intake of some specific polyphenols has been associated with body weight changes, there is still no evidence for the effects of total polyphenols or some polyphenol subclasses in humans on adiposity.

Keywords: dietary intake; catechins; resveratrol; olive oil; wine; BMI

1. Introduction

The global overweightness and obesity epidemic is increasing at an alarming rate and constitutes a serious global public health problem, affecting over 27.5% of the worldwide adult population and 47.1% of children [1]. Between 1980 and 2013, the worldwide prevalence of overweight and obese individuals increased from 857 million to 2.1 billion [1]. There is some evidence that the obesity epidemic is leveling off in some populations, although the prevalence of excess weight remains high in many countries of the world. The health consequences associated with obesity have been widely recognized: overall mortality, cardiovascular disease (CVD), hypertension, type 2 diabetes mellitus (T2DM), hyperlipidemia, stroke, cancer, osteoarthritis, chronic kidney disease, and gynecological problems, among others [2]. The medium-to-long-term consequences of obesity lead to rendering the health system unsustainable and, consequently, an urgent priority must be given to finding solutions for this issue that should be based on the best scientific evidence available.

Obesity is a multifactorial complex disease defined by excess of adipose mass, which occurs through adipocyte hypertrophy and hyperplasia [3]. The adipose tissue is an endocrine organ that secretes a wide variety of inflammatory adipocytokines, such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), resistin, leptin, and adiponectin. Visceral adiposity is associated with a higher production of these inflammatory adipocytokines, leading to systemic inflammation, insulin resistance, and several obesity-related metabolic disorders [4]. This inflammation due to obesity can be reversed with weight loss, which causes a reduction in fat mass and proinflammatory adipokines. Moreover, the intake of foods rich in bioactive compounds such as omega-3 fatty acids and polyphenols have been described to decrease low-degree inflammation [3].

2. The Mediterranean Diet

The link between adherence to the traditional Mediterranean diet (MedDiet) and the risk of cardiovascular disease (CVD) are mediated by several mechanisms, including reduction in low-degree inflammation [5–7], high plasma concentration of adiponectin, improvement of endothelial function [8], diminution of oxidative stress [9], low concentration of atherogenic lipoproteins, and lower levels of oxidized low-density lipoprotein (LDL) particles [10]. The high-density lipoprotein (HDL) functionality was also improved by the MedDiet. Cholesterol efflux capacity, specifically the HDL esterification index and HDL antioxidant and anti-inflammatory capacity, and vasoprotective effects inducing nitric oxide synthesis by endothelial cells are increased [11]. Furthermore, there are other inflammatory biomarkers related to CVD and atherosclerotic process that may be modulated by lifestyle, such as C-reactive protein (CRP), IL-6, and homocysteine [7,12].

The MedDiet is characterized by a high intake of phenolic compounds, which are present in the main key foods of this dietary pattern: extra-virgin olive oil (EVOO), nuts, red wine, legumes, vegetables, fruits, and whole-grain cereals. Phenolic compounds, usually called polyphenols (Figure 1) [13], are important candidates responsible for the beneficial effects of the MedDiet. A continuous and prolonged polyphenol intake is related to blood pressure and adiposity lowering effects, improvements in lipid profile, and also anti-inflammatory effects, which all act as CVD protectors [14].

Mediterranean Diet and Weight Loss

Although the long-term health benefits of the MedDiet are well established, its efficacy for weight loss at ≥12 months in overweight or obese individuals remains controversial. A systematic review of five randomized clinical trials (RCTs) [15] studied the effect of the MedDiet on weight loss in overweight or obese individuals comparing MedDiet interventions with low-fat diets, a low-carbohydrate diet, and the American Diabetes Association (ADA) diet. In this review, the MedDiet showed greater weight loss than the low-fat diets (range of the mean values: −4.1 to −10.1 kg vs. −2.9 to −5.0 kg), but similar weight loss compared with the other two interventions (range of the mean values: −4.1 to −10.1 kg vs. −4.7 to −7.7 kg). Epidemiological evidence for the association between the adherence to a traditional MedDiet with reduction of body weight and waist circumference is unclear. In 2011, Esposito et al. published a meta-analysis of 16 RCTs, which shows that a greater adherence to the MedDiet causes more weight loss as compared with a control diet [5]. Moreover, in none of the 16 RCTs was MedDiet adherence correlated with weight gain. Many components of the MedDiet may favor weight loss due to the abundance of plant-based foods, which provide high dietary fiber intake with a low energy density and low glycemic load. However, the effect of the MedDiet on body weight was greater in association with an energy-restricted MedDiet plan (−3.88 kg) or physical activity improvements (−4.01 kg) [16].

Huo et al. studied the effect of a Mediterranean-style diet on T2DM patients in terms of glycemic control, weight loss, and cardiovascular risks factors. Body mass index (BMI) was decreased in participants who followed the MedDiet (mean difference, −0.29 kg/m^2; 95% CI, −0.46 to −0.12) compared with those in the control diets [16].

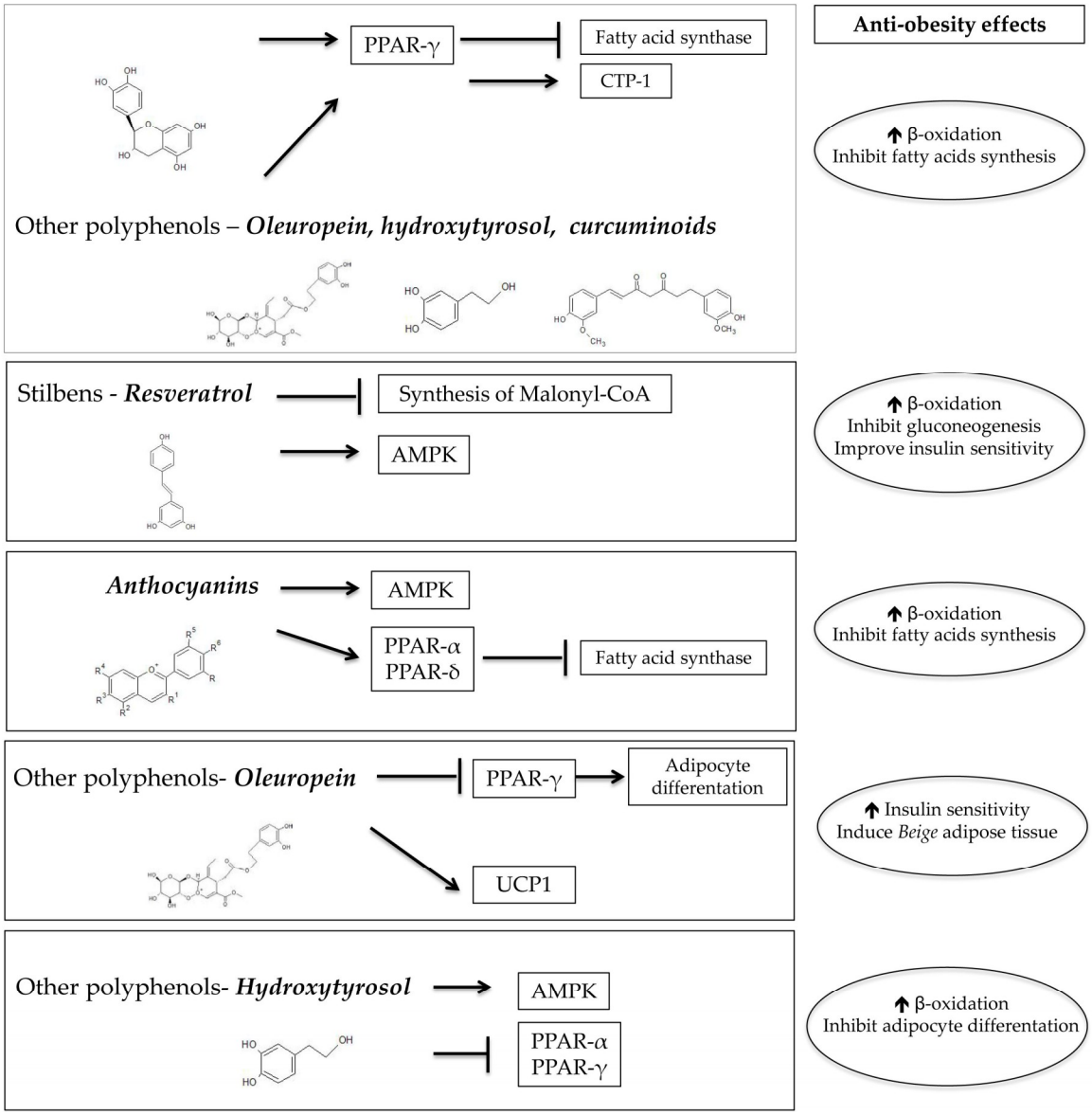

Figure 1. Molecular mechanisms of polyphenols involved in obesity. PPAR-γ: peroxisome proliferator-activated receptor gamma; CTP-1: tricarboxylate transport protein 1; AMPK: 5′-adenosine monophosphate-activated protein kinase; PPAR-α: peroxisome proliferator-activated receptor alpha; PPAR-δ: peroxisome proliferator-activated receptor delta; PPAR-γ: peroxisome proliferator-activated receptor gamma; → activation; ⊣ inhibition; and ↑ increase. ADC/ChemSketch (Advanced Chemistry Development, Inc., Toronto, ON, Canada) software was employed for chemical structures.

3. Dietary Polyphenol Intake

The effects of polyphenols depend on the amount and absorption of dietary polyphenols. Thus, to highlight the health benefits of polyphenols in humans, it is necessary to know the polyphenol content of the foods and the polyphenol subclasses' composition. Typically, polyphenol intake is currently evaluated using data extracted from food frequency questionnaires (FFQs). Recently, polyphenol intake has been measured using analysis of different biomarkers, mainly phase II enzyme-conjugated polyphenol metabolites, which are metabolites present in the bloodstream and urine and fecal samples. Unfortunately, there are thousands of potential biomarkers of polyphenol intake and there is no consensus yet [17]. On the other hand, Tresserra-Rimbau et al. studied the effect of dietary polyphenol intake on CVD, calculating the polyphenol consumption by matching FFQ data with the Phenol-Explorer database [14]. In this context, the effect of gut microbiota

has to be considered, as it metabolizes part of the dietary polyphenols and its metabolism can modify their absorption, bioavailability, and biological activity. The interindividual variability in gut microbiota, which determines polyphenol absorption, can explain the variety of health effects in the mentioned studies.

Polyphenol Intake in the Mediterranean Countries

The intake of dietary polyphenols and the main food sources depends on the dietary pattern and the native foods of each region, as described in Table 1. In the case of Mediterranean countries, the European Prospective Investigation into Cancer (EPIC) Nutrition cohort described the differences among the polyphenol intake of the European regions, estimating individual polyphenols and subclasses [18]. The estimation of polyphenol intake was performed by 24-h dietary recall of 36,027 adults, and the phenolic compounds data was obtained using the Phenol-Explorer database. Interestingly, the Mediterranean countries (including Spain, Greece, Italy, and the south of France) showed the lowest intake of total polyphenols (around 1011 mg/day) compared with non-Mediterranean countries and the United Kingdom (around 1284 and 1521 mg/day, respectively) [18]. Nevertheless, the profile of polyphenol subclasses was very different: Mediterranean countries showed the highest intake of stilbenes and flavonoids (49–62% of total polyphenols), followed by phenolic acids (34–44%). In relation to the main food sources, polyphenols in Mediterranean countries come mainly from coffee, fruits (the main source of flavonoids, representing 45% of the intake), wine, and vegetables oils (representing 26% of lignans intake), whereas in the non-Mediterranean countries, polyphenols come from coffee, tea, and wine (40.9%, 17.4%, and 4.6% of total polyphenols, respectively) [18].

Another cohort from France, called SUpplémentation en VItamines et Minéraux AntioXydants (SU.VI.MAX), quantified the polyphenol intake by 24-h dietary records and the Phenol-Explorer database in 4942 subjects. The mean total polyphenol intake (TPI) was 1193 mg/day, with hydroxycinnamic acids being the highest consumed polyphenol subclass, followed by proanthocyanidins [19]. The main food sources of hydroxycinnamic acids were coffee, potatoes, and apples, whereas for proanthocyanidins, were fruits, cocoa products, and red wine.

An observational study focusing on the nutritional habits characterizing the Mediterranean lifestyle, performed in Sicily in southern Italy, named the Mediterranean healthy Eating, Aging, and Lifestyle study (MEAL), estimated the polyphenol intake of 2044 subjects by FFQs and the Phenol-Explorer database. The main objective of the study was to describe the polyphenol intake differentiating the subjects by their level of adherence to the MedDiet, as measured by the MEDI-LITE score [20]. Additionally, Godos et al. described the intake of polyphenol subclasses and the major food sources in the MEAL study population [21]. Total polyphenol intake was 664 mg/day, of which the main intakes by subclass were phenolic acids, followed by flavonoids (363 and 259 mg/day, respectively). Nuts were the main food source of polyphenols, accounting for around 28% of total polyphenol intake, followed by coffee, cherries, red wine, and tea. Despite the fact that the adherence to the Mediterranean diet was high, the intake of total polyphenols was lower than the other areas described. The study concluded that the most consumed subclasses were flavonoids among the individuals with the highest adherence to the MedDiet, with fruits, vegetables, and red wine being the main food contributors [22].

Table 1. Profile of the dietary polyphenol subclasses' intake among the Mediterranean countries.

Mediterranean Area	Polyphenol Subclass (% of TPI) [a]	Main Food Sources (% of TPI) [a]
Spain, Greece, Italy, and south of France [17]	Phenolic acids (49), flavonoids (45), other polyphenols (0.6), stilbenes, and lignans (<0.7)	Coffee (36), fruits (25), red wine (10)
France [18]	Phenolic acids (54), flavonoids (42)	Coffee (44), tea (7), apples (7), red wine (6)
Spain [23]	Flavonoids (54), phenolic acids (37), other polyphenols (8.7), stilbenes, and lignans (<0.3)	Coffee (18), oranges (16), apples (12), olives and olive oil (11), red wine (6)
Sicily (Italy) [20,21]	Phenolic acids (53), flavonoids (37), lignans (0.4), stilbenes (0.3)	Nuts (28), coffee (7), red wine (6), tea (5)

TPI; Total polyphenol intake. [a] Dietary polyphenol intake was determined by the Phenol-Explorer Database (http://phenol-explorer.eu/, accessed on July 2018) for all the areas described.

The PREDIMED cohort (PREvención con DIeta MEDiterránea), comprised of a Spanish population at high cardiovascular risk, studied the effect of dietary polyphenol intake and the incidence of cardiovascular events [14]. Tresserra-Rimbau et al. described the intake of polyphenol subclasses' intake and the major food sources of the PREDIMED study subjects also using FFQs and the Phenol-Explorer database. Similar to the Italian population, the main intakes by subclass were flavonoids (443 mg/day), followed by phenolic acids (304 mg/day) [23]. Fruits were the main total polyphenols contributor, accounting for around 44%. Within the flavonoids, flavanols were strongly related to CVD prevention (HR = 0.4 (0.23–0.72)) and were mostly consumed from red wine (32%) and apples (31%) [14]. This study concluded that a higher intake of flavanols was associated with a 60% reduction of cardiovascular event and mortality risk. Despite the fact that the main phenolic acids subclass consumed was hydroxycinnamic acids, the intake of hydroxybenzoic acids was related to a lower incidence of CVD (HR = 0.47 (0.26–0.86)). It should be pointed out that increased intake of lignans was also related to CVD prevention (HR = 0.51 (0.30–0.86)), even though their intake was lower than 1 mg/day.

The main key foods of the MedDiet in the PREDIMED cohort were EVOO and nuts. EVOO and olives provide around 11% of the total polyphenol intake. The phenolic profile of EVOO and olives is unique, with 98% of the polyphenols being inside the 'other phenolic acids' and 'other polyphenols' subclasses. Among these subclasses, oleuropein is associated with antidiabetes, antiatherosclerosis, and anti-inflammation properties [24]. This characteristic phenolic profile has resulted in health benefits, a claim which was recognized by the European Food Safety Authority (EFSA) [25].

4. Antiobesity Effects of Dietary Polyphenols

Evidence for polyphenols' effect on obesity and weight control in humans is inconsistent due to the heterogeneity among study design, study populations, intervention period, and polyphenol supplements. These potential effects are summarized in Table 2. Some intervention clinical trials with polyphenol-enriched foods, such as an apple juice, showed a significant reduction in body fat mass but not in body weight, BMI, or waist circumference [26]. However, a recent double-blinded, randomized, parallel clinical trial conducted in 17 type 1 obesity participants (BMI between 30.1 and 33.3 kg/m^2) with a polyphenol supplement of 370 mg of total polyphenols showed a significant reduction in body weight, BMI, and waist and hip circumference compared with a placebo group after 12 weeks of intervention [27]. Moreover, only a few studies have studied the relationship between TPI from diet and weight control. Guo et al. [28] analyzed the association between body weight and TPI using a urine biomarker in a high cardiovascular risk population in a long-term study. After five years of follow-up, they showed an inverse association between total polyphenol excretion (TEP) and BMI, body weight, and waist circumference [28].

Similarly, a study conducted in the Mediterranean area demonstrated that higher dietary intake of flavonoids is inversely associated with an excess of weight and obesity [29]. Studies conducted in non-Mediterranean areas have shown an effect of polyphenol intake on weight control, but other clinical trials did not find any relationship between polyphenol intake and weight loss or changes in body composition (CITA).

A longitudinal study from a Netherlands cohort that included 4280 participants aged 55–69 years over 14 years of follow-up showed an association between a higher flavonoids intake and a lower increase in body mass index (BMI) in women ($p < 0.05$) [30]. Within the flavonoids, catechins are related with benefits in anthropometric parameters and body composition. More evidence that includes some studies with green tea extracts rich in catechins, epigallocatechin gallate (EGCG), showed a significant reduction in body weight, waist circumference, body fat mass, and visceral and subcutaneous fat [31]. Based on a meta-analysis of 11 studies, Hursel et al. concluded that catechin or an EGCG–caffeine mixture contained in green tea had a minimal effect on weight loss and weight loss maintenance [31]. Therefore, the clinical significance of the small changes seen in the body composition parameters indicates that green tea has no significant effect on weight loss and weight loss maintenance [32].

Resveratrol, a phenolic compound found in grapes, red wine, and some berries, also has potential antiobesity effects by inhibiting adipocyte differentiation and decreasing proliferation, mediated by adipocyte apoptosis and decreasing lipogenesis, promoting lipolysis and β-oxidation [30]. However, evidence about the effect of resveratrol intake on weight loss and weight loss maintenance is limited and the effects only seem to be achieved through dietary supplementation. Tome-Carneiro et al. performed several randomized, parallel, dose–response, placebo-controlled studies with a grape supplement rich in resveratrol and other grape polyphenols [33,34]. The effects were statistically significant for CVD risk factors: reduction in LDL-cholesterol, oxidized LDL, and thrombogenic plasminogen activator inhibitor type 1 (PAI-1), and increase in adiponectin and anti-inflammatory cytokines; however, they were not significant for adiposity parameters. Thus, the antiobesity potential and the optimal dose of resveratrol remain to be studied.

Despite the fact that the spice turmeric is not a characteristic food of the MedDiet, curcumin, a yellow-colored polyphenol from the curcuminoids subclass, is known for its health benefits such as anti-inflammatory, anticarcinogenesis, antiobesity, antiangiogenesis, and antioxidant activities [35]. The antiobesity properties of curcumin are similar to resveratrol, through inhibiting adipocyte differentiation, lipogenesis, reducing proinflammatory cytokines' synthesis in the adipose tissue, and promoting β-oxidation [35]. Similar to resveratrol, clinical trials to investigate the antiobesity properties of curcumin are limited. Ramirez-Bosca reported improvements in serum lipid profile through an increase in HDL-cholesterol and Apo A, as well as a decrease in LDL-cholesterol, ApoB, and the ApoB/ApoA ratio [36] with a supplement dose of 10 mg of a curcumin extract daily over 30 days.

Evidence from in vitro and experimental models suggests the potential effects of polyphenols on obesity, obesity-related inflammation, and other metabolic disorders. These studies show significant reduction of body weight by increasing basal metabolic rate, increasing β-oxidation, lowering triglycerides synthesis, and improving insulin sensitivity. Obese individuals have been reported to be more dependent on glucose oxidation rather that fat oxidation [37]. The mechanisms involved in weight loss where polyphenols may have a role are: inducing satiety; stimulating energy expenditure by inducing thermogenesis in brown adipose tissue; modulating adipose tissue by inhibiting adipocyte differentiation and promoting adipocyte apoptosis; modulating lipolysis; and activating β-oxidation [38]. Relative to metabolic disorders, an in vitro study about the effect of white tea EGCG showed improvements in cellular glucose metabolism mediated by glucose transporters (GLUTs) and a potential hypocholesterolemic effect stimulating LDL receptor binding activity [39].

Gut Microbiota and Prebiotic Potential of Dietary Polyphenols

The gut microbiota is, nowadays, strongly associated with several complex diseases, especially when this microbiota is imbalanced, also known as dysbiosis. This dysbiosis may be disrupted by lifestyle, such as excessive sanitation, diet, sedentarism, antibiotics, and so forth. Related to the topic of this review, the microbiota has a role in the host's metabolism, energy extraction, fat deposition, inflammatory status, gut barrier integrity, and also satiety [40]. The roles of the molecules generated from bacterial fermentation are crucial to establishing the causal relevance of the gut microbiota and health benefits.

Short-chain fatty acids (SCFAs) are formed from the fermentation of oligosaccharides, proteins, and peptides [41], with the main SCFA products being acetate, propionate, and butyrate. The consumption of complex carbohydrates from fruits and vegetables is associated with higher microbial production of SCFAs [42]. The contribution of SCFA products against obesity has been linked to decreasing weight gain by preventing fat accumulation [43–45]. Fernandes et al. showed that obese subjects present higher SCFA products in stool samples than lean subjects because of the differences in their colonic fermentation [42]. The before-mentioned SCFA main products display different mechanisms to induce satiety: butyrate acts on intestinal cells, increasing GLP-1 production [46], and propionate increases intestinal gluconeogenesis [45], both pathways leading to improvements in glucose homeostasis and increasing satiety.

Besides the microbial products, the gut microbiota is crucial for the metabolism and degradation of some other compounds. Branched-chain amino acids (BCAAs) are elevated in obesity and T2DM, which are contributing to the development of obesity-related insulin resistance. A reduction in BCAA level is strongly correlated with improvements in insulin sensitivity, more so than weight loss [47]. Interestingly, the composition of the gut bacteria, specifically the invasion of *Bacteroides* spp., may improve the efficiency of BCAA degradation [48].

Nevertheless, the main tool to balance the gut microbiota is diet. This notion is promoting the use of prebiotics, which are mainly dietary components such as nondigestible carbohydrates. Other dietary compounds not absorbed by the small intestine, such as polyphenols, are accumulated in the large intestine, thus being exposed to the enzymatic activities of the gut microbiota [49]. In vitro studies suggested that polyphenols may act as prebiotics by enhancing the growth of beneficial bacteria such as *Lactobacillus* spp. and *Bifidobacterium* spp. [50]. Related to the SCFAs, polyphenols from plum were reported to decrease fecal SCFAs in obese rats and, consequently, prevent weight gain in association with the changes in the bacterial composition of the gut microbiota by increasing *Faecalibacterium* spp., *Lactobacillus* spp., and *Bacteroidetes* spp. proliferation [50]. The potential prebiotic effect of proanthocyanidin on *Akkermansia muciniphila* is well described by Anhê et al. [51]. The pathways through which proanthocyanidins can enhance *Akkermansia* proliferation are: increasing mucus secretion to the intestinal lumen by goblet cells; proanthocyanidins and other polyphenols may use free oxygen radicals in the intestinal lumen, creating an environment only favorable for strict aerobic species; antimicrobial effects of polyphenols may help to degrade competitive bacteria of *Akkermansia*.

Relative to proanthocyanidins, a dietary supplement of grape seed extract in six female pigs caused a change in the distribution of the microbiota, increasing *Lachnospiraceae*, unclassified *Clostridales*, *Lactobacillus*, and *Ruminococcacceae* [52]. The same experimental models used by Quifer-Rada et al. described the molecular mechanisms of the potential hypocholesterolemic effects of proanthocyanidins shown in human studies [53]. The grape seed extract increases biliary excretion and reduces micellar solubility, which translates to a higher excretion of cholesterol in feces [54].

Table 2. Potential health benefits on body weight by Mediterranean diet polyphenols.

Phenolic Compound	Potential Health Benefits	References
Total polyphenols	↓ Body weight, BMI, and waist and hip circumferences	[26]
Total polyphenols	Prebiotic effect ↑ *Lactobacillus* spp., *Bifidobacterium* spp., *Faecalibacterium* spp., and *Bacteroidetes* spp. proliferation	[49]
Total polyphenols	↓ SFCAs excretion	[49]
Flavonoids	↓ BMI	[27]
Epigallocatechin gallate (EGCG) and green tea extracts	↓ Body weight, fat mass, and visceral and subcutaneous fat	[31]
Proanthocyanidins	↑ Proliferation of the *Akkermansia muciniphila* spp.	[50]
Proanthocyanidins	↓ Total cholesterol levels ↑ Biliary excretion and micellar solubility	[52]
Resveratrol	↓ Adipocyte proliferation ↓ Lipogenesis ↑ Lipolysis and β-oxidation	[28]

[1] BMI: Body mass index; SFCAs: Short-chain fatty acids; ↓ significant decrease; and ↑ significant increase.

5. Mechanism Involved

Catechins, mainly green tea EGCC, promote β-oxidation by regulating the expression in adipose tissue of peroxisome proliferator-activated receptor gamma (PPAR-γ) and fatty acid synthase (FAS), while increasing the levels of CPT-1, a protein that facilitates the transport of fatty acids to the mitochondria, which is a limiting step for β-oxidation [54].

In the case of resveratrol, its involvement in regulating β-oxidation has been studied by increasing 5′-adenosine monophosphate-activated protein kinase (AMPK) activity through preventing the degradation of intracellular cyclic adenosine monophosphate (cAMP) [55]. The AMPK function is to regulate glucose transport and fatty acid metabolism. Therefore, its activation may lead to fatty acid oxidation and suppression of hepatic gluconeogenesis as well as improvements in insulin sensitivity. Other studies revealed that resveratrol could mediate the expression of PPAR-γ [56] or promote β-oxidation by inhibiting the synthesis of malonyl-CoA [57], which is a precursor and promoter of fatty acid synthesis.

Curcumin contains polyphenols, and there is substantial evidence about its effectiveness in stimulating β-oxidation, inhibiting fatty acid synthesis, and decreasing fat storage [38]. The molecular pathways are similar to EGCG in the upregulation of CPT-1, but also entail the reduction of lipid biosynthesis by the downregulation of fatty acid synthesis enzymes [58].

Within the flavonoids, anthocyanins have been reported as having a role as antiobesity agents. Anthocyanins are widely found in fruits, such as apples with peel, strawberries, blueberries, blackberries, and blood oranges. To induce fatty acid oxidation, the postulated pathways are the modulation of AMPK synthesis and regulation of the expression of genes participating in β-oxidation [59].

Regarding EVOO polyphenols, tyrosol derivates, such as oleuropein, are involved in energy metabolism and adiposity [60], reducing the expression of PPAR-γ, compromising adipocyte differentiation, and improving insulin sensitivity [61]. Another interesting mechanism studied by Oi-Kano et al. in experimental models showed an increase in uncoupling protein 1 (UCP1) expression, which translates to the formation of "beige" adipose tissue, leading to a decrease of visceral fat mass [62]. Hydroxytyrosol and its derivatives constitute around 90% of the total polyphenol content of EVOO [63]. In vitro studies reported that hydroxytyrosol downregulates the expression of PPAR-α and -γ, which is translated to a reduction in adipocyte size [64]. Additionally, an increase in AMPK and lipase (hormone-sensitive and phosphorylated lipase) was observed in adipocytes exposed to

hydroxytyrosol [65]. Furthermore, these effects were not reported to have an impact on body weight and adiposity in humans [65].

There are several mechanisms of action involved and each polyphenol presents different pathways, as shown in Figure 1.

However, more randomized clinical trials are needed to verify if the ability of polyphenols to act as antioxidants and anti-inflammatory mediators, through suppressing the effects of oxidative stress and inflammation, can be translated to antiobesity effects.

6. Conclusions

The characteristic phenolic profile of the MedDiet differs from other dietary patterns, especially in the Mediterranean countries, where EVOO and olives are food sources that provide unique phenolic compounds with health benefits.

The health effects of polyphenols depend on the amount consumed and their bioavailability, which is low, and systemic concentrations of phenolic compounds may reach the millimolar range. As previously mentioned, the gut microbiota might be the most remarkable factor for the absorption and metabolism of dietary polyphenols. Moreover, bioavailability can be also modulated by the effects of culinary techniques, dietary patterns, or alteration of phase I/II metabolism by pharmacological or dietary agents.

However, the essential step towards the understanding of the protective effects of polyphenols against overweightness, unhealthy body composition, obesity-related inflammatory processes, and metabolic syndrome status is to estimate their consumption by dietary recalls (through 24-h dietary recall or FFQs) or other methods such as measurements of urine concentration of key polyphenols, in order to identify the compounds most likely to provide the greatest protection.

Even though the intake of some specific polyphenols has been associated with body weight improvements, there is still no evidence for the effects of total polyphenols or some polyphenol subclasses. Further randomized controlled trials are needed to confirm the promising protective effects of polyphenols on weight gain, obesity, and CVD. This research field might be useful for setting food and health counselling goals for overweightness and obesity, and additionally, to establish dietary recommendations for individuals and population groups and desired minimum levels of polyphenol intake.

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EDITORIAL

Ultra-processed food consumption and disease: the jury is still out

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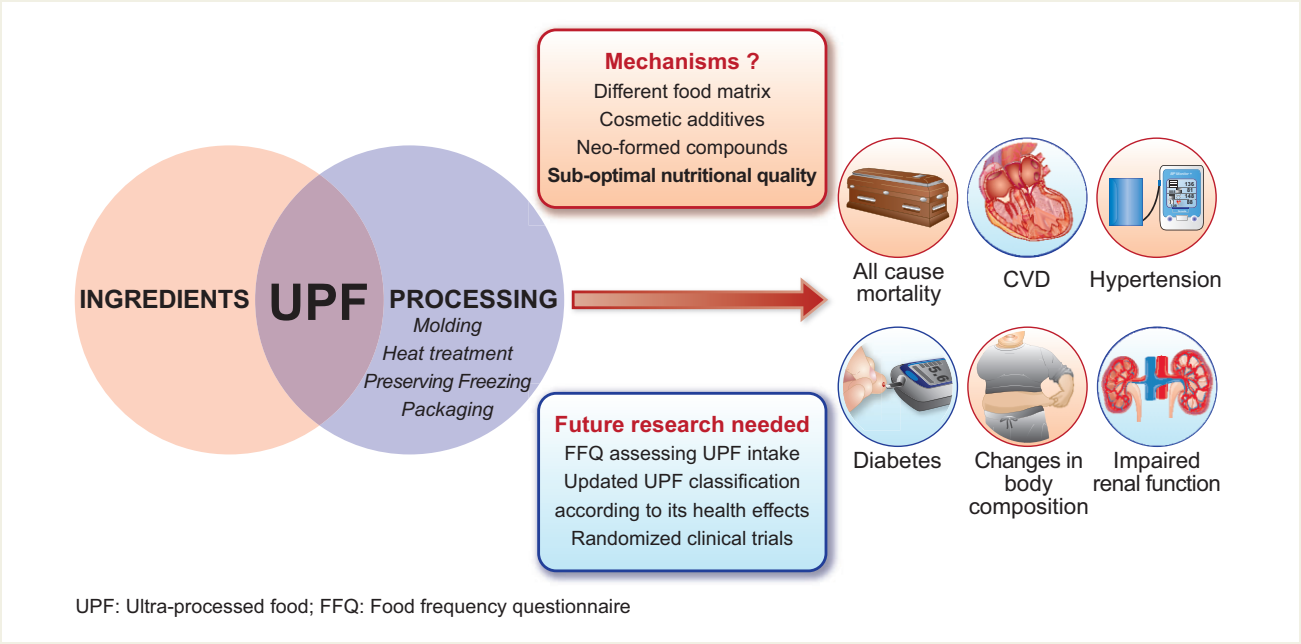
This editorial refers to 'Ultra-processed food intake and all-cause and cause-specific mortality in individuals with cardiovascular disease: the Moli-sani Study', by M. Bonaccio *et al.*, https://doi.org/10.1093/eurheartj/ehab783.



Graphical Abstract Simplified schematic representation of the effects of ultra-processed foods (UPF) consumption on health. Previous studies have suggested that high UPF consumption increases all-cause mortality and incidence of cardiovascular disease (CVD), hypertension, diabetes, obesity and kidney disease. However, more studies are required to know the mechanisms implicated in such adverse effects, mainly randomized clinical trial using questionnaires specifically designed to capture the different types of UPF.

As the world's population grows and tends to concentrate in large cities, eating fresh and local products becomes more difficult. In this setting, the collaboration of science and technology is crucial to ensuring that food can be available to everyone. Humans have processed food since ancient times to maintain its organoleptic and nutritional properties, in addition to reducing biological (mainly microbial) risks and thus extending the conservation period. Food processing, such as fermentation, has also allowed the creation of new

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and healthier foods and beverages, including miso, kefir, and bread. However, some food processing can be accompanied by partial or total loss of essential nutrients, such as vitamins or amino acids, or the formation of toxic substances such as heterocyclic amines.[1] Another concern is that during food processing, salt, sugar, or unhealthy fats are often added to improve palatability or extend shelf life, but these can have detrimental effects on health. Therefore, in recent decades, researchers have examined to what extent food processing is harmful to long-term health. Many have analysed the effects of consuming foods that have undergone a high degree of processing (ultra-processed foods; UPF) on the incidence of common non-communicable chronic diseases, mainly cardiovascular disease (CVD) and cancer (*Graphical Abstract*).[2]

Bonaccio *et al*. in Pozzilli, Italy, evaluated the association of UPF intake on mortality in people with a history of CVD in the Moli-sani Study, published in this issue of the *European Heart Journal*.[3] They studied 1171 participants (mean age 67 ± 10 years) who were followed-up for a median of 10.6 years. Those with a higher intake of UPF (>11.3% of total food) had increased hazard ratios for all-cause and CVD mortality by 38% and 65%, respectively, compared with those with a low intake (<4.7%), probably due to altered renal function. Interestingly, no other studies have analysed the effects of UPF intake on CVD risk or all-cause mortality including CVD mortality in patients who have had a CVD event. The same research group observed when analysing the entire Moli-sani cohort (22 245 participants) that high UPF intake was associated with a 58% increase in hazard ratio for CVD mortality.[4] Other studies have also reported that people with high UPF intake showed an increased risk of all-cause[5] and CVD mortality, as well as several metabolic conditions, such as obesity, diabetes, hypertension, and hyperlipidaemia.[6] Thus, recent epidemiological studies provide compelling evidence that high UPF intake is harmful regarding several conditions, especially those related to cardiovascular risk.

Only one randomized intervention study has demonstrated that 14-day exposure to ultra-processed diets vs. unprocessed diets caused weight gain among 20 US adults.[7] Almost all related epidemiological studies have analysed large cohorts recruited >10 years ago and used food classification systems based on the degree of processing and consumption of UPF, with intake estimated from validated semi-quantitative food frequency questionnaires (FFQs).

Unfortunately, most FFQs were not specifically designed to capture details of food processing, so some misclassification of foods as UPF is possible. The most common way to express UPF intake is by calculating the proportion (%) of UPF (g/day) in the total food and beverage intake (g/day). However, identifying UPF from FFQs is not easy. Items such as fruit juices, milkshakes, meatballs, hamburgers, or pizza can be consumed as artisanal or industrial varieties, but only industrial varieties are classified as UPF. Likewise, for yoghurts and wholegrain cereals, FFQs usually do not distinguish between plain, sweetened, or flavoured varieties. Thus, these foods may be classified as group 1 (minimally processed foods according to NOVA classification) when some products should be classified as UPF (NOVA group 4).

Therefore, one of the main issues is the high heterogeneity of foods considered to be UPF. The most known classification system, NOVA (from The Public Health School of Sao Paulo, Brazil), uses the term UPF to define food and drink products that are formulated with five or more ingredients and have undergone several industrial procedures, with no domestic equivalent.[8] The food processing identified by NOVA involves physical, biological, and chemical processes after foods are separated from nature and before they are consumed or used as ingredients. Notably, when products are made primarily of group 1 (unprocessed or minimally processed) or group 3 (processed) foods but also contain cosmetic or sensory-intensifying additives, such as plain yoghurt with added artificial sweeteners, or bread with added emulsifiers, they are classified as group 4. Likewise, when alcoholic drinks are identified as foods, those produced by the fermentation of group 1 foods followed by the distillation of the resulting alcohol, such as whisky, gin, rum, or vodka, are classified as group 4.

Also controversial are the ingredients used in some food production, such as isolated compounds extracted from food or food wastes, including casein, lactose, whey, and gluten, in addition to more processed ingredients such as hydrogenated or interesterified oils, hydrolysed proteins, and inverted sugars. The variety of foods included in the UPF group is thus considerable, including myriad products with different effects on health. Therefore, relating the degree of processing to health effects cannot be done independently of the composition of the food. Notably, the term 'ultra-processed' refers only to food processing and should not imply low nutritional quality since this depends mainly on the final composition of the product.

Moreover, most UPF are generally low in fibre and micronutrients, while their composition is rich in refined carbohydrates, added sugars, saturated and trans-fatty acids, and sodium. Therefore, at least part of their detrimental effects may be attributed to their nutritional composition of mainly refined carbohydrates (simple sugars). Substantial scientific evidence has demonstrated the effects of these compounds on the incidence of several chronic diseases.

On the other hand, several classes of food additives are only found in UPF, including dyes and other colourants, colour stabilizers, flavours, flavour enhancers, non-sugar sweeteners, and processing aids such as carbonating, firming, bulking and anti-bulking, de-foaming, emulsifying, and humectant agents. Of these additives, phosphates merit particular attention. Unlike organic phosphorus, which is present in plant-based foods (with low phosphorus availability due to its phytate content), inorganic phosphate is present in many UPF as an additive, and it is highly bioavailable. Its intake may promote nephropathy or exacerbate existing chronic disease. People with high consumption of UPF may consume 250–1000 mg/day more phosphates compared with those with low UPF consumption.

Finally, we should consider the neo-products formed during food processing, particularly during heat treatments, such as acrylamides, which may promote disease. The effects of packaging should also be considered as sources of phthalates and bisphenols, which have been associated with nephropathy.[9,10]

Interestingly, as well as their effects on CVD morbidity and mortality, UPF has shown a substantial effect on renal function. A low UPF intake prevents chronic kidney disease and, conversely, a high UPF intake provokes impaired renal function, independently of hypertension, diabetes, obesity, or other chronic conditions.[11] In the study of Bonaccio *et al*.,[3] nearly 40% of the increase in all-cause and CVD mortality observed in the high UPF consumers was explained by altered renal function. This effect may be attributed to an increase in

sodium intake (data not observed in the study), low fibre intake, and more probably a deleterious health effect of some additives, especially the phosphate content of UPF and the phthalates and bisphenols from their packaging. Other mechanisms should also be considered, such as the effects of UPF on changes in gut microbiota composition and their proinflammatory effects.

In conclusion, since most studies relating UPF intake to increased all-cause and CVD morbidity and mortality risk are based on FFQs not designed for this purpose, new trials are needed. Relating the type and degree of food processing to health cannot be done independently of the nutritional composition of the final food. Similarly, it is important not to associate UPF with foods of low nutritional quality, because this depends on not solely the intensity and complexity of processing but also the final composition of the food itself. The use of additives is subject to regulation derived from risk analysis, so their use cannot alone be linked to nutritional damage. Not all UPF can be considered the same (for instance, decaffeinated coffee and processed cakes, pies, and pastries), but we should be aware of the low nutritional quality of most, especially those rich in sugar, saturated fat, and salt.

Conflict of interest: none declared.

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DISCUSSION

This thesis presents six manuscripts about the associations of dietary polyphenol intake and MetS criteria, including diabetes status, body composition parameters, inflammatory markers and adipokines levels. We first described dietary polyphenol intake and main food sources and evaluated the associations between polyphenol subclasses intake and MetS criteria. Second, we examined the association between dietary polyphenol intake and T2D according to sex and BMI. Third, we assessed the association between changes in dietary polyphenol intake and T2D parameters, including fasting glucose and HbA1c levels after 12-months of follow-up. Fourth, we examined the associations between changes in dietary polyphenol intake and body adiposity parameters measured by DXA, including total fat mass, and regional adiposity, as trunk, leg, android and gynoid fat mass. Fifth, we evaluated whether changes in VAT after 12-months of intervention were associated with greater improvements on obesity-related inflammatory markers and adipokines concentrations, including insulin, glucagon, IL-6, visfatin, ghrelin, GLP-1 TNF-α, MCP-1, PAI-1, resistin, C-peptide, leptin, adipsin and adiponectin. Finally, we validated an updated version of the FFQ, determining test–retest reliability indicating the stability of the FFQ over time through repeated measures at two different time and concurrent or relative validity, which indicates the amount of agreement between two different measures, including biological markers of food intake.

Dietary polyphenol intake

MD is characterized by high dietary polyphenol intake, but significant differences in the main polyphenol subclasses and food sources were observed. In our study, the mean polyphenol intake was 846 mg/day, and the highest intake was observed for flavonoids (58% of total), followed by phenolic acids (33.1%). Total polyphenol intake was considerably lower than the intake observed in the Mediterranean countries of the EPIC Study (1011 mg/day) (154), the SU.VI.MAX cohort study (1193 mg/day) (337), and the data from other studies conducted in non-Mediterranean countries, such as the UK National Diet and Nutrition Survey Rolling Programme for participants with similar age (1053 mg/day) (338).

The main noteworthy difference between our results and those of other countries was the relevant contribution of seeds, olives and olive oil, and red wine (337), while coffee, tea, and cocoa products are the main polyphenol foods sources observed in non-Mediterranean countries (339–341). In addition to the differences observed according to geographical location and dietary habits, sociodemographic and lifestyle habits significantly influence the quantity and the profile of intake of polyphenol subclasses. The

intake of total polyphenols, particularly flavonoids and lignans, increased with age compared to younger participants (<65 years), although Grosso *et al*. reported the opposite observation (340). In addition, BMI was inversely associated with total polyphenol intake, mainly with lower flavonoid and stilbene intake. This finding was also reported in the TOSCA.IT and EPIC studies (154,342).

Metabolic syndrome components and T2D

The intake of polyphenol subclasses has been reported to have an impact on MetS components (343,344). Although evidence is still limited, it has been suggested that the benefits of dietary polyphenols regarding T2D may include anti-inflammatory, antioxidant, and glucose metabolism regulatory effects, such as the inhibition of α-amylases and α-glucosidases, protection against glucose toxicity in pancreatic β-cells (345), and modulation of GLUT4 receptors.

Even though flavonoids were the principal contributors of total polyphenol intake in our study, no associations were found with any of the MetS components, except for an inverse association with waist circumference. In our study, catechins were the main source of flavan-3-ols, because theaflavin intake was very low. Together with proanthocyanidins, these compounds are classified as flavanols. The main sources of flavanols in the PREDIMED-Plus cohort were cocoa and chocolate products, apples, plums, red wine, and tea.

Proanthocyanidins and catechins were strongly and inversely associated with T2D in overweight men and women, and obese women. In a previous longitudinal study conducted in a similar cohort (PREDIMED study), proanthocyanidins and catechins showed the same association with new-onset T2D when baseline glucose levels were taken out of the model but not in the fully adjusted model. In a large, prospective, case-cohort study, Zamora-Ros *et al*. concluded that all flavan-3-ol monomers, including catechins, as well as proanthocyanidins of low polymerization degree were associated with a lower risk of developing T2D (346). Data from a meta-analysis of 19 RCT on cocoa intake and cardio-metabolic risk suggested significant improvements on insulin-related outcomes (347). In this sense, similar findings were observed when the associations between dietary polyphenols and glucose and HbA1c levels were assessed. Interestingly, the improvements in HbA1c levels observed in the present study are similar to those arising from other dietary interventions in T2D patients, such as high-fiber diets or health education programs (348,349). According to the United States Food and Drug Administration, even a modest reduction in HbA1c levels (0.3 to 0.4%) reduces the risk of developing T2D (350).

Phenolic acid intake was associated with higher fasting plasma glucose levels and waist circumference. These results are opposite from those observed in the HAPIEE cohort study, which described the beneficial effects of phenolic acid on the overall risk of developing MetS and lowering BP (351). Nevertheless, it must be considered that the dietary intake of phenolic acids and total polyphenol in the mentioned study doubled the amount estimated in our results, probably because of the higher intake of tea and its contribution to phenolic acid intake compared to our study population (351). Hydroxybenzoic acids followed the same pattern as that of proanthocyanidins and catechins, showing inverse associations in overweight men and women but not in obese men. It may be explained by the fact that hydroxybenzoic acids are minor components of the phenolic acid group, and they have not received much attention in previous studies. Hydroxycinnamic acids, on the other hand, accounted for more than 90% of the phenolic acid group, coffee being the main food source. In our cohort, hydroxycinnamic acid intake was associated with higher prevalence of T2D in men and almost in women. This result contradicts other studies regarding coffee and their polyphenols, such as caffeic acid, or chlorogenic acid. Large observational studies have pointed towards a significant inverse association between coffee consumption and T2D, especially with decaffeinated coffee (352,353).

In Mediterranean countries, dietary intake of stilbenes is relatively high compared to other countries (354), with red wine being the main source (>90%). In this setting, higher stilbene intake was associated with higher HDL-c levels, but since HDL-c is the best-established cardiovascular protective factor by alcohol consumption, we cannot exclude that the alcohol content of red wine may interfere with this result (355). Stilbenes were strongly associated with lower odds of developing T2D in the PREDIMED study (356), however, we only found a mild association and a linear trend for overweight man in the present cohort. Nevertheless, whether stilbenes are feasible for the prevention and/or management of T2D is still controversial (317)

In western populations, the consumption of lignans is usually greater than isoflavones. Those in the highest quartile of lignan intake had lower odds of having T2D except for overweight women where no association was found. These results agree with the inverse association found a few years ago in the PREDIMED cohort (356). Alligned with these finding, lower T2D incidence was found in U.S. women with higher enterodiol and enterolactone levels in urine, which are gut microbiota metabolites of dietary lignans (357).

Interestingly, in our study we found an association between the intake of all polyphenol subclasses, except phenolic acids and lignans, and higher HDL-c levels. We also

observed that TG levels were inversely associated with stilbene and lignan intake. Despite the fact that the antioxidant properties of polyphenols for the prevention of LDL-c oxidation are well described, the effects of dietary polyphenols on the reduction of total cholesterol levels or TG are controverted (358).

Body composition

In weight-loss oriented interventions, such as the energy-restricted MD and physical activity promotion designed in the PREDIMED-Plus study, body composition is a key role of the intervention effectiveness. Lean body mass/total body fat ratio, which is consider as a more favorable body composition, suggests that lifestyle interventions for weight-loss should include physical activity promotion in order to prevent or delay age and weight-loss related sarcopenia. Moreover, DXA-derived indicators of body composition, especially abdominal obesity, showed higher ability to predict abnormal cardiovascular and metabolic risk parameters than classical anthropometric indicators (150). The magnitudes of the present associations were small per increased tertil or sex-specific z-score standard deviation. However, losing even small amounts of body weight are associated with health improvements (359,360). The benefits of dietary polyphenol associated with body composition include activation of lipolysis and β-oxidation, inhibit lipogenesis and adipocyte proliferation, and exert a prebiotic effect for gut microbiota (317). Even though significant associations were observed between several polyphenol subclasses and body composition parameters, more RCT are needed to clarify how its consumption and which polyphenol subclasses affects body composition.

Obesity-related inflammatory, adipokines and hormonal levels

It is well established that VAT is associated with CVD and metabolic disturbances, but the mechanisms underlying these effects are still unclear. Therefore, this excess of VAT may induce chronic low-grade systemic inflammation mediated by macrophage infiltration and the secretion of several proinflammatory cytokines (361,362). The beneficial effects observed in individuals with higher VAT reduction on some circulating proinflammatory parameters related to obesity, such as leptin and MCP-1 were also reported by Salas-Salvadó *et al*. in the same study population (148). Human studies conducted in the context of bariatric surgery- or lifestyle change induced weight loss-suggest that, while adipose tissue inflammation is a major contributor to systemic insulin resistance, it is not sufficient by itself, and substantial improvements in insulin sensitivity are associated with a reduction in VAT (363,364). Weight loss achievements through dietary intervention alone or energy-restricted diet and physical activity promotion resulted in decreased circulating IL-6, CRP, PAI-1, TNF-α, soluble TNF receptor, P-

selectin, ICAM-1, VCAM-1, and IL-18 in men and women of various age groups and BMIs (365).

In the case of c-peptide, which expression has been directly linked with insulin resistance and CVD risk, significant associations were observed between c-peptide and physical activity levels in the same study population (366). Interestingly, the association between physical activity levels and c-peptide are independent of body composition parameters (367,368), while our results suggest potential association between VAT changes and c-peptide.

A recent study that evaluated plasma cytokines production in insulin-resistant and insulin-sensitive participants with obesity, no significant differences were found in 24h plasma concentration for cytokines, except for PAI-1 (369). Plasma levels of PAI-1 were greater in insulin-resistant obese compared to insulin-sensitive obese individuals, and PAI-1 levels were associated with measurements of insulin sensitivity, suggesting a systemic metabolic function of adipose tissue PAI-1 production, but not for the rest of cytokines.

Dietary assessment

Food records and 24h food registries are frequently used as gold standard methods in validation studies of dietary assessment (370). These reference methods are not free from measurement errors; however, the type of error is independent of those from a FFQ (370). The objective measurement of food and nutrient intake by its corresponding biological markers yielded a more robust estimate of a FFQ validity. In our study, objective measurements of plasma levels of both folic and linolenic acid and urinary hydroxyltyrosol were fairly correlated ($p < 0.05$) with their corresponding FFQ-derived nutrient or food. Moreover, this validation includes the MD adherence by a composite score. While the FFQ estimates dietary polyphenol intake and the main food sources, the 14-items used in the PREDIMED study captured the highest dietary intake of polyphenols in comparison of other MD indexes (371). In this sense, the use of a consensus methodology and polyphenol database might facilitate this in future studies. Future large-scale clinical trials are needed to clarify the underlying mechanisms of action and to establish safe doses to ensure the described health effects.

Moreover, other endogenous and exogenous factors should be considered in dietary assessment, such as metabolomics, microbiomics and exposome to predict or understand the effect of a dietary intervention (372–374). Some between-person variability could be explained by genetic variations, especially on those genes involved in diet response, such as the genes involved in fatty acids and amino acids metabolism

(375). In this sense, Li *et al.* identify a metabolic signature to measure the adherence to the MD and predicts future CVD risk independently of known CVD risk factors (376). It was capable of measuring CVD risk independently of self-reported dietary data, which could be used as a complementary information to traditional dietary assessment, including FFQ, and minimizing the measurement errors inherent to the methodology (376). Additionally, this metabolic signature was reproducible between two study population, Spain and US, which individuals have different dietary habits and environments. Moreover, cross-sectional, and longitudinal studies, as the presented in the present thesis, can play an important role in future biomarker discovery. Comparison among low vs high consumers by using dietary assessment tools can lead to the identification of biomarkers, especially for habitual intake nutrients or foods (263).

Strengths and limitations

The major strengths of the present study are its large sample size, except for Manuscript V (N=117), the multicenter design, and the use of the Phenol-Explorer as the most comprehensive food composition database on dietary polyphenols (153). In prior studies, the FFQ was validated to evaluate total polyphenol intake in both clinical and cross-sectional studies (146,377). Moreover, the comprehensive data and repeated measures of MetS criteria, T2D and other potential confounders are strengths of the present work. In relation to Manuscript IV and V, unlike previous studies, we examined wider spectrum of body composition using DXA-scan rather than anthropometry. Moreover, VAT data was determined based on an automated algorithm from the DXA manufactures, avoiding predictive estimation that may underestimate this fat depot. Manuscript VI main strength is that validity was determined by correlation with foods and nutrients derived by the reference method and the determination of biological markers of food and nutrient intake. Our study has also some limitations. First, Manuscript I and II used a cross-sectional design which does not allow attributing conclusions to plausible causes. In order to establish causality, a RCT based on the intake of different polyphenol subclasses should be performed. Second, potential residual confounding and the lack of generalizability of the results to other populations than middle-aged to elderly people with MetS are limitations. Third, the use of the FFQ may have led to a misclassification of the exposure due to self-reported information of food intake and to the fact that some polyphenol-rich foods are grouped in the same item (e.g., spices). Nevertheless, the FFQ used has been validated in the adult Spanish population and showed good reproducibility and validity (146). Fourth, other factors that affect food polyphenol content, such as bioavailability, variety, ripeness, culinary technique, storage, region, and environmental conditions,

were not collected. Manuscript VI has several limitations, such as the administration of food records or weighed food records may lead to participants changing their diet during the recording period. FFQ, on the other hand, are prone to memory bias because these questionnaires asked for the retrospective food intake. Furthermore, average consumption frequency of seasonal foods is especially critical and the fixed food list in fixed portion sizes are other sources of measurement error. Finally, the use of the FFQ to present data of absolute intakes of foods and nutrients is limited without prior calibration of these data by a reference method.

CONCLUSIONS

The conclusions derived from this thesis are as follows:

1. The total dietary polyphenol intake of the participants from the PREDIMED-Plus study at baseline was 846 ± 318 mg/day, 621 ± 273 mg/day of aglycone intake, of which 58% were flavonoids, and 33% phenolic acids. The contribution of seeds, olives, olive oil, and red wine are the main difference compared to other non-Mediterranean countries.
2. Differences in total dietary polyphenol and polyphenol subclasses intake were observed according to BMI and physical activity, were individuals with higher BMI (>35 kg/m^2) and lower physical activity levels showed lower total polyphenol, flavonoids, and stilbene intake. Total polyphenol, flavonoids and lignans intake increased with age and higher educational level.
3. Higher intake of dietary polyphenol subclasses was inversely associated with MetS components, especially HDL-c levels.
4. The associations between dietary polyphenol intake and MetS were observed mainly with the polyphenol subclasses whose contribution to total polyphenol intake was lower, including other polyphenols, lignans and stilbenes.
5. Higher intake of proanthocyanidins, catechins, hydroxybenzoic acids and lignans were inversely associated with T2D prevalence in older adults with MetS. However, high hydroxycinnamic acids intake was directly associated with T2D. The association between dietary polyphenol intake and T2D depend on sex and BMI, being stronger in overweight subjects than in obese individuals.
6. After 12-months of follow-up, regular consumption of dietary polyphenols and certain polyphenols subclasses were associated with lower levels of fasting glucose and HbA1c.
7. The association between dietary polyphenol intake and fasting glucose and HbA1c levels were mediated by diabetes status, and prediabetic and diabetic participants showed greater improvements.
8. After 12-months of follow-up, flavonoids intake was inversely associated with VAT, especially catechins intake. In the case of phenolic acids, higher intake of hydrobxybenzoic acids was associated with lower VAT, while no significant effects were observed for total phenolic acids.
9. Lignans and stilbenes intake were inversely associated with total fat mass after 12-months of follow-up.
10. A reduction of VAT was associated with improvements in inflammatory parameters and adipokines levels, mainly in insulin, c-peptide and PAI-1, after 1

year of follow-up, while no significant differences were observed for total fat mass reduction.

11. Dietary estimation using an updated version of the FFQ showed a good test-retest reliability and a fair to moderate validity of the FFQ for a specific population.
12. The updated version of the FFQ showed a good validity to rank individuals according to their food and nutrient consumption and their adherence to the MD.

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