UNIVERSITAT ROVIRA I VIRGILI

# VARIETY IN FRUIT AND VEGETABLE CONSUMPTION AND CARDIOVASCULAR HEALTH

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UNIVERSITAT
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# Variety in fruit and vegetable consumption and cardiovascular health

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**DOCTORAL THESIS**

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UNIVERSITAT ROVIRA i VIRGILI

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FAIG CONSTAR que aquest treball, titulat "Variety in fruit and vegetable consumption and cardiovascular health", que presenta Leyre López González per a l'obtenció del títol de Doctor, ha estat realitzat sota la meva direcció al Departament Bioquímica i Biotecnologia d'aquesta universitat.

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HAGO CONSTAR que el presente trabajo, titulado "Variety in fruit and vegetable consumption and cardiovascular health", que presenta Leyre López González para la obtención del título de Doctor, ha sido realizado bajo mi dirección en el Departamento de Bioquímica y Biotecnología de esta universidad.

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I STATE that the present study, entitled "Variety in fruit and vegetable consumption and cardiovascular health", presented by Leyre López González for the award of the degree of Doctor, has been carried out under my supervision at the Department of Biochemistry and Biotechnology of this university.

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"Si pudiésemos dar a cada individuo la cantidad adecuada de nutrición y ejercicio, ni muy poco ni demasiado, habríamos encontrado el camino más seguro hacia la salud"

**Hipócrates (460 a.C-370 a.C)**

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# ABSTRACT


## ENGLISH

In many epidemiological studies, fruits and vegetables (FV) have been positively associated with diet quality, lifestyle and human health. FV are low energy density foods rich in important dietary components, such as dietary fiber, micronutrients and phytochemicals, which may be responsible for their potential health benefits.

Most of the studies that have evaluated the associations between FV consumption and human health have only focused on the total amount of FV consumed per day. Nevertheless, some studies have suggested that a varied consumption of FV may be as important as the total amount of FV for human diet and health, since the different groups of FV vary in nutrient and phytochemical content. These studies have also indicated that beneficial properties of FV may be due to the interactions between their different dietary components, what is commonly known as synergistic effects.

Therefore, the aim of the present doctoral thesis is to evaluate the association between variety in FV consumption and different outcomes related to human diet and health, including diet quality, lifestyle and cardiometabolic risk. For this purpose, the present dissertation was conducted in the framework of the PREDIMED-plus study, an ongoing 6-year parallel group, multi-center randomized controlled clinical trial for the primary prevention of cardiovascular disease and mortality conducted in Spain since 2013 including 6.874 participants.

The results showed a significant inverse association between a varied consumption of FV (also in combination with higher amounts) and the risk of having inadequate intakes of dietary fiber and micronutrients. Besides, a varied intake of FV was also significantly associated with better diet quality and a healthier lifestyle. Regarding cardiometabolic risk, a varied consumption of FV (also in combination with higher amounts) was prospectively associated with a decrease in important cardiometabolic risk factors, including fasting blood glucose, body weight and waist circumference.

In conclusion, greater variety in FV consumption, in combination with higher amounts, would be beneficial for diet quality and cardiometabolic risk in elderly individuals at high cardiovascular risk.

## SPANISH

Estudios epidemiológicos han reflejado que las frutas y verduras se asocian con una mejor calidad de la dieta, un estilo de vida más saludable y un mejor estado de salud. Las frutas y verduras son alimentos de baja densidad calórica ricos en fibra alimentaria, vitaminas, minerales y compuestos bioactivos, que podrían ser los responsables de los efectos beneficiosos de las frutas y verduras sobre la salud.

La mayoría de estudios que han analizado la asociación existente entre el consumo diario de frutas y verduras y la salud humana se han centrado únicamente en la cantidad total de frutas y verduras consumidas diariamente. Sin embargo, algunos estudios han sugerido que el consumo variado de frutas y verduras podría ser tan importante para la calidad de la dieta y la salud humana, como la cantidad total, ya que los diferentes grupos de frutas y verduras poseen distinto contenido nutricional y de compuestos bioactivos. Además, estos estudios han indicado que los efectos beneficiosos de las frutas y verduras sobre la salud podrían deberse principalmente a las interacciones entre sus distintos componentes, lo que comúnmente se conoce como sinergia.

Por lo tanto, el objetivo principal de la presente tesis doctoral ha sido evaluar la asociación existente entre el consumo variado de frutas y verduras y distintos parámetros como la calidad de la dieta, el estilo de vida y el riesgo cardiometabólico. Por ello, esta tesis se ha realizado en el contexto del estudio PREDIMED-plus, un ensayo clínico aleatorizado y multicéntrico para la prevención de la enfermedad y mortalidad cardiovascular, que se está llevando a cabo en España desde 2013 con 6.874 participantes.

Los resultados obtenidos han mostrado una asociación inversa entre la variedad de frutas y verduras consumidas (también en conjunto con la cantidad) y el riesgo de ingesta inadecuada de fibra dietética, vitaminas y minerales. Además, la ingesta variada de frutas y verduras se ha asociado con una mejor calidad de la dieta y un estilo de vida más saludable. Con respecto al riesgo cardiometabólico, el consumo variado de frutas y verduras (también en conjunto con la cantidad) se ha asociado prospectivamente con la mejora de algunos factores de riesgo cardiometabólico como la glucemia basal, el peso corporal y el perímetro de la cintura.

En conclusión, una alta variedad en el consumo de frutas y verduras, en combinación con una alta cantidad, se asocia con una mejor calidad de la dieta y una disminución de los factores de riesgo cardiometabólico en individuos de edad avanzada que presentan alto riesgo de enfermedad cardiovascular.

## CATALÁ

Estudis epidemiològics han observat que el consum de fruites i verdures s´associa a una millor qualitat de la dieta, un estil de vida més saludables i un millor estat de salut. Les fruites i verdures són aliments de baixa densitat calòrica rics en fibra dietètica, vitamines, minerals i compostos bioactius, que podrien ser els responsables dels efectes beneficiosos de les fruites i verdures sobre la salut.

La majoria d´estudis que han analitzat l´associació existent entre el consum diari de fruites i verdures i la salut en humans s´han centrat únicament en la quantitat total de fruites i verdures consumides diàriament. No obstant, la varietat en el consum de fruites i verdures podria ser tan important per millorar la qualitat de la dieta i la salut, com la quantitat total, ja que els diferents grups de fruites i verdures tenen un diferent contingut nutricional i de compostos bioactius. A més, aquests estudis han indicat que els efectes beneficiosos de les fruites i verdures sobre la salut podrien ser deguts principalment a les interaccions entre els seus diferents components, la qual cosa comunament es coneix com a sinergia.

Per tant, l´objectiu principal de la present tesi doctoral ha estat avaluar l´associació existent entre el consum variat de fruites i verdures i diferents paràmetres com la qualitat de la dieta, l´estil de vida i el risc cardiometabòlic. L'estudi s´ha realitzat dins del context de l´estudi PREDIMED-plus, un assaig clínic, aleatoritzat i multicèntric per a la prevenció de la malaltia i mortalitat cardiovascular, que s´està duent a terme a Espanya des de 2013 amb 6.874 participants.

Els resultats obtinguts han mostrat una associació inversa entre la varietat de fruites i verdures consumides (també en conjunt la quantitat) i el risc d´ingesta inadequada de fibra dietètica, vitamines i minerals. A més, la ingesta variada de fruites i verdures s´ha associat a una millor qualitat de la dieta i un estil de vida més saludable. Respecte al risc cardiometabòlic, el consum variat de fruites i verdures (també en conjunt amb la quantitat) s´ha associat prospectivament amb la millora d´alguns factors de risc cardiometabòlic com la glucèmia basal, el pes corporal i el perímetre de la cintura.

En conclusió, una alta varietat en el consum de fruites i verdures, en combinació amb una alta quantitat, s'associa a una millor qualitat de la dieta, i una disminució dels factors de risc cardiometabòlics en individus d´edat avançada que presenten alt risc de malaltia cardiovascular.

# ABBREVIATIONS

**ACE**: Angiotensin-converting enzyme

**AESAN**: Spanish Agency for Food Safety and Nutrition

**AHA**: American Heart Association

**BMI**: Body mass index

**BP**: Blood pressure

**CHD**: Coronary heart disease

**CURES**: Chennai Urban Rural Epidemiology Study

**CVD**: Cardiovascular disease

**DBP**: Diastolic blood pressure

**DIF**: Dietary insoluble fibers

**DNA**: Desoxirribonucleic acid

**DRIs:** Dietary Reference Intakes for American population

**DSF**: Dietary soluble fibers

**EA**: eating disorders

**EFSA**: European Food Safety Authority

**EPIC**: European Prospective Investigation into Cancer and Nutrition

**EPIC-Norfolk**: Norfolk Component of the European Prospective Investigation into Cancer and Nutrition

**EU**: European Union

**FAO**: Food and Agriculture Organization

**FEN**: Spanish Nutrition Foundation

**FFQ**: Food Frequency Questionnaire

**FV**: Fruits and vegetables

**GBD**: Global Burden of Diseases, Injuries and Risk factors

**KoGES**: Korean Genome and Epidemiology Study

**HbA1c**: Glycated hemoglobin

**LMM**: Linear mixed-effects models

**MedDiet**: Mediterranean diet

**MetS**: Metabolic syndrome

**NHLBI**: National Heart, Lung and Blood Institute

**NF-kB**: Nuclear factor kappa B

**NRF2**: Nuclear factor erythroid 2-related factor 2 (NRF2)

**OR**: Odds Ratio

**PREDIMED-plus**: Prevención con Dieta Mediterránea Study

**SBP**: Systolic blood pressure

**SE**: Standard error

**SENC**: Sociedad Española de Nutrición Comunitaria (Spanish Society of Community Nutrition)

**TG**: Triglycerides

**TLGS**: Tehran Lipid and Glucose Study

**T2D**: Type 2 diabetes

**US**: United States

**USDA**: United States Department of Agriculture

**USDHHS**: United States Departments of Agriculture and Health and Human Services

**WC**: Waist circumference

**WCRF/AICR**: World Cancer Research Fund/ American Institute for Cancer Research

**WHO**: World Health Organization

**WHS**: Women´s Health Study

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# 1. INTRODUCTION

# 1. INTRODUCTION

## 1.1 Definition of fruits and vegetables

From a culinary perspective, fruits are pulpy seeded tissues with sweet or sour taste, while vegetables are edible parts of plants with less sweet or sour taste[1]. From a nutritional standpoint, fruits and vegetables (FV) are plant-based foods that differ widely in their energy and nutritional content[1]. FV are low caloric foods, because of its low content of fat and high in water. Besides, they are rich in carbohydrates, mainly sugars, and complex carbohydrates (starch and dietary fiber), but not in proteins or lipids. However, carbohydrate content of fruits varies among their different groups and subgroups. For instance, apples and pears contain high amounts of fructose, while apricots and peaches are rich in sucrose[2].

FV are good sources of minerals, as they provide greater amounts of potassium, calcium, and iron. Besides, fruits contain varying quantities of potassium, sodium, and magnesium. Related to vitamins, vegetables are rich in folic acid and vitamins A, C and K, whereas fruits are good sources of vitamin C, especially citrus and strawberries, beta-carotene (or vitamin A), like melon or apricots, and thiamin (vitamin B1), such as plums and dried fruits[2].

### *1.1.1 Classification of fruits and vegetables*

According to the edible part of the plant, FV can be classified in six different groups[3]:

- <u>Fruits</u>: including apples, apricots, grapefruit, lemon, mango, melon, oranges, pears, pineapples, strawberries, and watermelon. In this group are also placed avocado, cucumber, eggplant, green and red pepper, pumpkin, and tomato, commonly considered vegetables[3].

- <u>Stems, stalks, and flowers</u>: such as artichoke, asparagus, broccoli, cauliflower, and mushroms[3].

- <u>Seeds</u>: like corn, green beans, green peas, and soybeans[3].

- Tubers: as carrots, potatoes, radish, sweet potatoes, and turnips[3].

- Bulbs: like garlics and onions[3].

- Leaves: including Brussel sprouts, cabbage, lettuce, and spinachs[3].

FV can also be classified into nine different groups according to their color (**Table 1**), which indirectly reflects their phytochemical content[3,4].

**Table 1. Classification of fruits and vegetables (FV) in nine color groups, according to their phytochemical content.**

| NAME GROUP | PHYOCHEMICAL CONTENT | FRUITS AND VEGETABLES |
|---|---|---|
| Orange FV | Carotenoids (ß-carotene)<br>Ascorbic acid. | F: apricot, mango, orange, papaya, peach.<br>V: carrot, pumpkin, sweet potato. |
| Red-purple FV | Carotenoids (lycopene)<br>Flavonoids (anthocyanins) | F: cherries, grapes, pomegranate, strawberries, watermelon.<br>V: red cabbage, red pepper, tomato. |
| White FV | Flavonoids (anthoxanthins)<br>Organosulfur compounds (allicin) | F: apple, banana, white grapefruit, pear.<br>V: artichoke, cauliflower, cucumber, eggplant, garlic, mushrooms, onion, potato, soybeans. |
| Light green FV | Chlorophyll | F: melon, kiwi, lime.<br>V: cabbage, celery. |
| Green FV | Chlorophyll | V: asparagus, broccoli, Brussel sprouts, green peas lettuce. |
| Dark green FV | Chlorophyll | V: kale, spinach, turnip greens. |
| Yellow FV | Carotenoids<br>Ascorbic acid | F: lemon, pineapple.<br>V: avocado, sweet corn. |
| Blue-black FV | Flavonoids (anthocyanidins) | F: blackberries, blueberries, raisins, dates. |
| Pink FV | Flavonoids | F: pink and red grapefruit. |

Adapted from: Pennington JAT, Fisher RA. Classification of fruits and vegetables. Journal of Food Composition and Analysis. S23-S31. (2009).
Abbreviations: FV, fruits and vegetables; F, fruits; V, vegetables.

## 1.2 Current recommendations for fruit and vegetable intake

The *WHO/FAO report* recommends a minimum consumption of 400 grams per day (or 5 portions per day) of FV for the primary prevention of several chronic diseases (such as cardiovascular disease (CVD), diabetes and different types of cancer) and nutritional deficiencies in adult populations[5].

In United States (U.S), *Dietary Guidelines for Americans 2020-2025 (USDHHS/USDA)* strongly recommend a minimum consumption of 2.5 portions/day of vegetables and 2.0 portions/day of fruits in adults, in terms of FV quantity. Regarding FV variety, these guidelines provide weekly consumption recommendations for the different vegetable subgroups (1.5 portions of dark-green vegetables, 5.5 portions of red and orange vegetables, 1.5 portions of beans, peas and lentils, 5 portions of starchy vegetables and 4 portions of other vegetables) in portions/week for the adult population[6]. However, although there are no specific recommendations for the weekly intake of the different subgroups of fruits, these guidelines are focused on the intake of whole fruits and 100% fruit juices[6].

At the European (EU) level, dietary guidelines of the vast majority of countries recommend a minimum intake of 5 portions per day of FV in the adult populations[7].

Finally, the *Dietary guidelines for Spanish population* of the Spanish Agency for Food Safety and Nutrition (AESAN, 2008) and the Spanish Society of Community Nutrition (SENC, 2016) recommend a minimum consumption of 5 portions of FV per day[7,8]. In other words, the Spanish adult population should consume 3 or more portions of fruits and at least 2 portions of vegetables (one cooked and the other one raw) daily, including the different color varieties[8].

## 1.3 Consumption of fruits and vegetables

### 1.3.1 Global consumption of fruits and vegetables

In 2010, global mean intakes of FV (<300 g/day) were significantly low compared to the *WHO/FAO* current recommendations (≥400g/day), according to the GBD (Global Burden of Diseases, Injuries and Risk factors) study results. Specifically, between 1990 and 2010 fruit consumption was globally increased (+5.3 g/day), while vegetable consumption remained constant (+ only 1.8 g/day)[9].

Related to fruit consumption, global mean intake of fruits in 2010 was 81.3 g/day, a substantially lower amount compared to *WHO/FAO* recommendations for adult populations (≥240 g/day of fruits)[9]. In this sense, Jamaica, Malaysia, Greece, New Zealand, and Jordan reported the highest intakes of fruits, whereas Nepal, India, Ethiopia, Pakistan and Vanuatu the lowest ones. Besides, only in two countries, Jamaica and Malaysia, mean intakes of fruits were ≥300 g/day in adults (0.4% of the global adult population)[9].

Regarding vegetable consumption, global mean intake of vegetables in 2010 was 208.8 g/day, a significant lower amount in comparison with the *WHO/FAO* recommendations for adult populations (≥160 g/day of vegetables). Highest intakes of vegetables were found in African nations such as Zimbabwe, Swaziland, and Botswana, while the lowest ones were observed in Vanuatu, Hungary, Switzerland, Armenia, Georgia, and Philippines. Only 4 out of a total of 187 countries involved in the GBD study reported vegetable intakes ≥400 g/day[9].

### 1.3.2 Consumption of fruits and vegetables in United States

From 2010-2020, nearly the 85% of the U.S. population did not meet the daily recommendations for fruit intake. Only few adults of all ages met the daily recommended intake for fruits (2 portions/day). The majority of fruits consumed by this population were in form of fruit pieces, fruit juices and fruit salads[10,11].

In terms of vegetables, only about the 10% of the U.S. population met the daily intake recommendations for vegetables (2.5 portions/day). Although vegetable consumption has slightly increased with age in the U.S. adult population during the past decade, it still remains low (< 2 portions/day)[10,11].

### 1.3.3 Consumption of fruits and vegetables in the European Region

The World Health Organization estimated a FV consumption below its recommendations (≥400 g/day) in half of the European countries[12].

Furthermore, the European Food Safety Authority (EFSA) reported an average vegetable intake of 220 g/day in EU countries over the past decades, as well as a mean fruit intake of 166 g/day. As a result, the average consumption of FV in EU countries was 386 g/day. Besides, the same organization reported a higher vegetable consumption in Southern Europe, whereas a higher fruit consumption in Central and Eastern Europe. Finally, according to data provided by EFSA, only four European countries (Poland, Germany, Italy and Austria) met the WHO recommendations for FV intake (≥400 g/day)[13].

Finally, according to data provided by Eurostat, in 2019, a 33% of the EU population did not consume any portion of FV daily; the 12% consumed 5 or more portions/day; and the remaining 55% consumed 1-4 portions/day[14]. In this sense, the EU countries with the highest percentage of population consuming 5 or more portions of FV daily were Ireland (33%), Netherlands (30%), Denmark (23%) and France (20%), whereas Romania had the lowest percentage (2%), followed by Bulgaria (5%), Slovenia (5%) and Austria (6%)[14].

### 1.3.4 Consumption of fruits and vegetables in Spain

In Spain, FV consumption has decreased over the past years. In 2018, the average consumption of FV was 264 g/day, according to the 2018 Spanish Nutrition Foundation (FEN) report[15]. Specifically, the mean consumption of vegetables was 1.3 servings/day in 2018, while the mean intake of fruits was 1.5 servings/day[15].

According to data from the same report, in 2018 the most consumed fruits in Spain were oranges (56.3 g/day/person), bananas (31.0 g/day/person) and apples (30.9 g/day/person), whereas the most consumed vegetables were tomatoes (38.3 g/day/person)[15].

Furthermore, the "National Survey on Food Consumption among adult population, elderly and pregnant women" (ENALIA 2, 2014-2015)[16–18] reported an average fruit consumption of 175.2 g/day and a mean vegetable intake of 175.8 g/day in Spanish adults (18-74 years).

Moreover, according to data provided by the Spanish Ministry of Health, in 2020 only the 67.7% (71.2% of women and 63.9% of men) and 46.6% (52.0% of women and 41.0% of men) of the Spanish adult population consumed fruits and vegetables daily, respectively[19].

Finally, regarding the consumption of specific types of FV, in 2020 the most consumed fruits in Spain were citrus (orange, lemon, tangerine, grapefruit), exotic (banana, kiwi, avocado, pineapple) and pome fruits (apple, pear, grape) while the most consumed vegetables were tomato, cucumber, eggplant, zucchini, pepper, broccoli and cauliflower, according to data provided by the Spanish Ministry of Health[19]

## 1.4 Fruits and vegetables, nutrient adequacy and diet quality

Diet quality is a term used to quantify how healthy a dietary pattern is according to its main constituents, including food groups, macro and micronutrients and bioactive compounds[20]. Besides, diet quality enables to examine the potential associations between health status and whole foods, rather than just nutrients[20].

A systematic review of longitudinal, cross-sectional and case-control studies reported a total of 25 overall diet healthy pre-defined quality indexes, highlighting the Healthy Eating Index (HEI), the Healthy Diet Indicator (HDI), the Recommended Food Score

(RFS), the Diet Quality Index (DQI), the Healthy Food Index (HFI) and the Mediterranean Diet Score (MDS)[20].

Dietary quality indexes are based on the specific dietary guidelines of the country in which they have been developed[20]. They evaluate dietary patterns in terms of their alignment with national dietary guidelines and the variety of healthy choices they offer within the major food groups[20].

However, diet quality can also be assessed by selecting key nutrients (carbohydrates, vitamins, minerals, proteins, dietary fiber, among others), and comparing their intakes with the age and sex-specific requirements, according to the Dietary Recommended Intakes (DRIs) for American population[21], or other recognized recommendations.

Several epidemiological studies have demonstrated that higher intakes of FV were positively associated with nutrient adequacy and overall diet quality in adult populations.

Firstly, in an interventional study in Scottish adults[22], an increment of 300 or 600 g/day of FV was significantly associated with higher intakes of carbohydrate, sugar and dietary fiber. Besides, in a cross-sectional study in US adults[23], canned FV consumers had significantly higher intakes of total energy, sugars, dietary fiber and potassium, while lower intakes of total fat, saturated fat, monounsaturated fat and sodium.

However, most of these studies have only focused on FV quantity, without taking into consideration the variety or diversity in FV intake[24].

Regarding **diet quality** and FV variety, a systematic review with meta-analysis of randomized-controlled clinical trials showed that carbohydrate, dietary fiber and micronutrient intake in adults improved by increasing variety in FV consumption[25].

Furthermore, a cross-sectional study in an adult population revealed that a varied fruit consumption was associated with higher intakes of vitamins A and C[26]. Besides, another cross-sectional study in a female population reflected that a varied vegetable intake was significantly associated with higher scores on the HEI-2005 diet quality index[27].

Moreover, in another cross-sectional study in frail elderly subjects, a significant direct association was found between FV variety score and intakes of many nutrients (carbohydrates, dietary fiber, vitamins C, A and B6, calcium, copper, potassium and magnesium)[28].

## 1.5 Fruit and vegetable consumption and lifestyle

The association between lifestyle behaviors such as smoking habit, physical activity (PA), sedentarism or sleeping and FV consumption in adult populations has hardly been investigated in epidemiological studies.

Related to **lifestyle** and FV consumption, a systematic review with meta-analysis of prospective cohort studies showed a higher prevalence of being physically active and non-smoker, and drinking less alcohol, per each increment of 100g per day in FV [29]. However, no previous studies have assessed these associations considering the variety or diversity in FV intake.

## 1.6 Beneficial constituents of fruits and vegetables

FV are rich in minerals, like potassium, magnesium, and calcium, which are essential components of a healthy diet. Besides, they are good sources of bioactive compounds, which are phytochemicals that exhibit beneficial effects on human health due to their antioxidant, anti-inflammatory, anti-aging, anti-proliferative, anti-carcinogenic, anti-mutagenic, anti-microbial and cardioprotective properties[15,30].

The great variety of bioactive compounds found in FV that may have positive effects on human health include carotenoids (carotenes and xanthophylls), vitamins (C and E), polyphenols (stilbenes, anthocyanins, phenolic acids, lignans and flavonoids), bioactive peptides, sulfur compounds and dietary fiber[30].

### 1.6.1 Carotenoids

Carotenoids are a wide group of pigments synthesized by plants and other photosynthetic organisms. The most abundant carotenoids in orange and yellow FV are beta-carotenes, while dark green leafy vegetables and potatoes are good sources of lutein and zeaxanthin. Besides, tomato and other red/pink fruits, such as watermelon or grapefruits are rich lycopene (17).

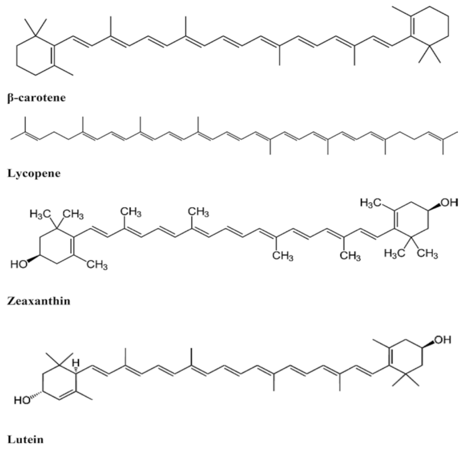


**Figure 1**. Chemical structure of some common carotenoids[32]

Regarding their health benefits, carotenoids are bioactive compounds capable of scavenging oxygen free radicals resulting from light induced lipid oxidation, thanks to the presence of terminal functional groups in their structure. Thus, in addition to acting as provitamins, carotenoids are powerful natural antioxidants abundant in a wide variety of FV[31].

### 1.6.2 Vitamins

FV are rich in vitamins C and E, which are essential micronutrients that exhibit a powerful antioxidant capacity[31,33].

Vitamin C or ascorbic acid is abundant in potatoes and cruciferous vegetables, including cauliflower, broccoli and cabbage, whereas vitamin E or alpha-tocopherol is presented in cruciferous vegetables, seeds and vegetable oils[31,33].

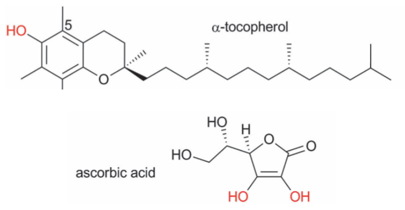


**Figure 2.** Chemical structure of vitamins C and E[34]

Related to their health benefits, both vitamins are potent antioxidants that act synergistically to prevent lipid oxidation (mainly LDL-cholesterol oxidation) by scavenging oxygen free radicals, protecting our cells from oxidative stress. Therefore, vitamins C and E are bioactive compounds with strong antioxidant and anti-atherogenic properties[31,33,35].

### *1.6.3 Polyphenols*

Polyphenols (or phenolic compounds) are plant secondary metabolites that may have beneficial effects on human health, as they could help to reduce the risk of chronic diseases such as diabetes, CVD and cancer[29].

FV are good sources of polyphenols, being blue/blackberries and spinach the fruits and vegetables with the highest content of these bioactive compounds, respectively[31,36,37].

Polyphenols are classified into five different subgroups, including flavonoids, tannins, phenolic acids, stilbenes and coumarins[31].

**Flavonoids** are the major group of polyphenols in FV that exhibit antioxidant, anti-mutagenic, anti-carcinogenic and anti-inflammatory properties[38]. In this sense, flavonoids are powerful inhibitors of key cellular enzymes such as lipoxygenase, cyclo-oxygenase and xanthine oxidase, which are involved in the inflammatory response or in the production of oxygen free radicals[38].

Flavonoids are also divided into six different subgroups, according to their different chemical structure, including flavones, flavonols, chalcones, anthocyanins, isoflavones and flavanols (**Figure 3**)[38].

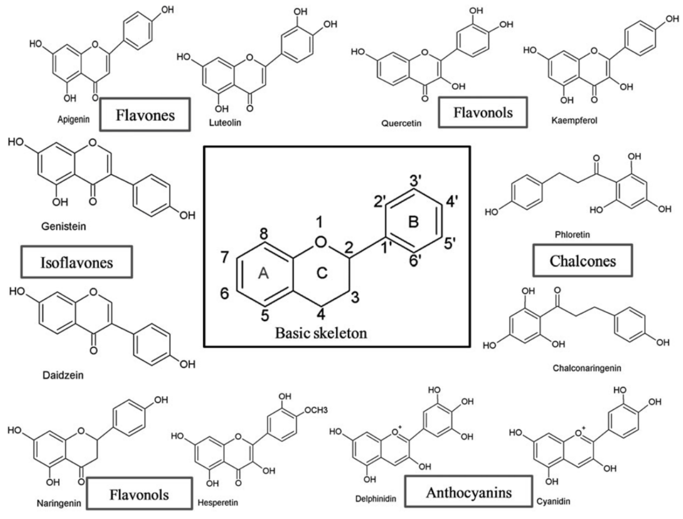


**Figure 3.** Chemical structure of the different classes of flavonoids[38]

The biological effects of flavonoids have been observed in several *in vitro* studies in which these compounds inhibited important enzymes involved in cell division and proliferation, as well as in the immune and inflammatory responses, platelet aggregation and cellular detoxification processes. Besides, some studies suggested that flavonoids may protect cells from oxidative stress and lipid peroxidation by scavenging oxygen free radicals, which reflects its antioxidant activity[39].

### *1.6.4 Bioactive peptides*

Bioactive peptides are phytochemicals that are absorbed throughout the intestine, which have proven antimicrobial, antioxidant, anti-hypertensive, anticoagulant, anticholesterolemic, anticarcinogenic, anti-inflammatory and anti-diabetic properties, among others[40].

Bioactive peptides with anti-hypertensive activities were identified in vegetables such as garlics, broccoli and pulses[41]. These peptides are also called angiotensin-converting enzyme (ACE)-inhibitor peptides, as they act on the renin-angiotensin system regulating blood pressure (BP)[42]. Antimicrobial peptides are rich in arginine, leucine and glycine amino acids. They interact with bacteria thanks to their hydrophobicity and cationic nature, as bacterial walls are negatively charged[40]. Bioactive peptides with antimicrobial activities were mainly isolated from chili pepper[43].

On the other hand, bioactive peptides with antioxidant activities are rich in lysine, cysteine, tryptophan, tyrosine, histidine and methionine amino acids. They were mainly identified in vegetables like potatoes, cauliflower and soybeans[40]. The antioxidant activity of these bioactive peptides derives from its ability to scavenge oxygen free radicals, protecting the body from oxidative stress[44]. Anticholesterolemic bioactive peptides were identified in *Brassica* vegetables and soybeans and contain hydrophobic amino acids like leucin, tyrosine and tryptophan. These bioactive peptides decrease cholesterol and triglyceride (TG) blood levels by enhancing the secretion of bile acids from the gallbladder, changing lipid metabolism and regulating steroid hormone and cholesterol receptors[40,44,45].

Finally, bioactive peptides with anticarcinogenic properties were identified in olive seeds and sweet potatoes. These peptides exhibit a potent anticancer activity due to their capacity to induce apoptosis, inhibit angiogenesis and initiate cell necrosis [40,46,47].

Therefore, bioactive peptides are phytochemicals that can be found in fruits (mango, passion fruit, tomato, plums) and vegetables (spinach, pulses, pepper, cruciferous and garlics) with powerful antimicrobial, anticarcinogenic, antihypertensive, antioxidant, anticholesterolemic properties that exert beneficial effects on people´s health[40].

### *1.6.5 Sulfur compounds*

Cruciferous vegetables (*Brassica* family) contain glucosinolates, which are plant-derived sulfur compounds with potent anti-cancer properties. Isothiocyanates like sulforaphane, which are metabolites of glucosinolates, are bioactive compounds with anti-inflammatory activity, since they decrease the secretion of nuclear factor kappa B (NF-kB) and other inflammation biomarkers. Besides, isothiocyanates induce the nuclear factor erythroid 2-related factor 2 (NRF2) synthesis, a regulatory protein that promotes the activity of antioxidant enzymes. Thus, regarding its health benefits, glucosinolates are a large group of plant-derived sulfur compounds that exhibit powerful anti-cancer and anti-inflammatory properties[35].

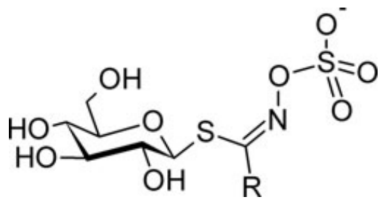

**Figure 4.** Chemical structure of a glucosinolate[48]

With regard to white vegetables, like onions and garlics, they are good sources of organosulfur compounds which are plant-derived sulfur metabolites that play an important role on cancer prevention by enhancing the activity of the immune system. Alliin and its metabolite allicin are the main organosulfur compounds found in garlics. These bioactive compounds are involved in the scavenging of oxygen free radicals, the enhancement of carcinogen detoxification, the modulation of DNA repair enzymes and the induction of apoptosis and xenobiotic-metabolizing enzymes. Therefore, organosulfur compounds (alliin and allicin) are bioactive compounds abundant in garlics and onions that play a major role on chemoprevention[35].

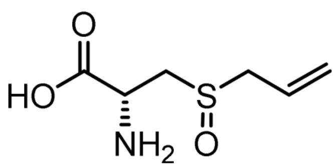

**Figure 5.** Chemical structure of alliin[49]

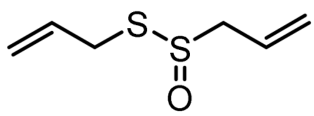

**Figure 6.** Chemical structure of allicin[49]

### *1.6.6 Dietary fiber*

Dietary fiber is a set of structural polysaccharides and other compounds that are not digested or absorbed in the small intestine, due to the lack of human enzymes responsible for their digestion. Consequently, these compounds are partially or totally fermented in the colon[50].

Dietary fibers are classified into two different groups according to their solubility in water and viscosity. Dietary soluble fibers (DSF), including mucilage, pectin, gums, beta glucans, inulin, fructo-oligosaccharides and some hemicellulose, are digested by the colonic bacteria and fermented in the colon. However, dietary insoluble fibers (DIF), such as cellulose, lignin and hemicellulose, are not digested by the gut bacteria, so they are partially fermented in the colon[51].

DSF absorb water and form a gel-like structure that accelerates intestinal transit, slows down gastric emptying and intestinal digestion and decreases nutrient absorption in the microvilli of the small intestine mucosa. On the contrary, since DIF do not absorb water, they slow down intestinal transit and increase the fecal volume, improving and relieving constipation[51,52].

Vegetables like carrots, onions, artichokes and broccoli, and fruits such as apples, pears, citrus and bananas are good sources of DSF, while DIF are only present in certain FV (tomato, cauliflower, potatoes, lettuce)[52].

DSF may attenuate postprandial blood glucose and lipid levels, due to its gel formation capacity that can affect the absorption of nutrients in the small intestine[53]. The hypocholesterolemic effects of DSF may be due to its viscosity that slows gastric emptying, and consequently decrease lipid absorption. Besides, DSF increase bile acid and cholesterol excretion, resulting in lower total and LDL-cholesterol plasma levels[53]. Finally, as DSF are fermented in the colon by the gut bacteria, they provide short-chain fatty acids (SCFAs), such as propionate, acetate and butyrate, that are rapidly absorbed by the colonic mucosal cells and decrease the hepatic synthesis of cholesterol, lowering plasma cholesterol levels[51]

The antidiabetic effects of DSF may be related to their capacity to increase satiety, and as a consequence weight loss, which in turn promotes the reduction of insulin resistance, and therefore the risk of T2D. Besides, DSF reduce postprandial glucose levels, due to its viscosity and gel-forming properties. Finally, higher concentrations of SCFAs, as a result of colonic fermentation of DSF decrease hepatic glucose output.[54]

On the other hand, as DSF slows down gastric emptying, they also increase the presence of micronutrients and antioxidants in the body and decrease caloric intake. Besides, DSF enhance the elimination of carcinogens thanks to their carcinogenic-binding effects[51]. Finally, the formation of SCFAs induced by the SBF increase the absorption of sodium and water by the colonic mucosal cells, what improves intestinal transit[51].

Last but not least, DSF have prebiotic functions, as they provide SCFAs to the colonic mucosa that can alter the human gut microbiota, by modifying the proportion of certain bacterial species, including *Prevotella*, *Firmicutes* and *Bacteroides*[53,40,42,43]. Specifically, the production of large amounts of SCFAs may modify the pH of the colon, which in turn alters the composition of the intestinal microbiota and the production of SCFAs[53]. In general, it has been demonstrated that DSF stimulate the proliferation and activity of health-promoting bacteria in the colon, while it decreases the growth of the pathogenic ones.[52,55,56]. Changes in the abundance of these bacteria in the intestinal lumen are responsible for the changes in the production of different metabolites that have been shown to be essential for health[52,57,58].

Therefore, dietary fibers, which are present in a wide variety of FV, are bioactive compounds with beneficial properties for human health, highlighting the improvement of the intestinal digestion and intestinal transit, the attenuation of glucose and cholesterol plasma levels, the reduction of caloric intake, the elimination of carcinogens and the proliferation of health-promoting gut bacteria[51,52,56,57].

Although DSF have been related to several metabolic benefits after consumption[53], epidemiological studies have also demonstrated that DIF consumption (especially in the form of whole cereals) is inversely associated to CVD and global mortality[59,60].

## 1.7 Health benefits of fruits and vegetables

FV consumption has been positively associated with human health, as previous studies have demonstrated that they could help to prevent the occurrence and development of major chronic diseases, such as CVD, type 2 diabetes (T2D) and several types of cancer[61].

Nowadays, it is well known that global dietary guidelines promote, in addition to a high intake of FV, the consumption of a wide variety of FV, due to the different nutrient profile and phytochemical content among their different groups and subgroups [62,63]. However, these associations have been mainly investigated considering total FV consumption, without taking into consideration the variety or diversity in the FV consumed.

### *1.7.1 Quantity in fruit and vegetable consumption and human health*

As above mentioned, several epidemiological studies have reported beneficial associations between FV consumption and human health, mainly considering the total amount of FV consumed per day. In most of these studies, quantity in FV consumption was estimated in grams or portions of FV consumed per day[64–68].

#### 1.7.1.1 Quantity in FV consumption and obesity

In the last decades, there has been an increase in the prevalence of overweight and obesity in the general population, which increases the risk of suffering from chronic diseases such as CVD, T2D and several types of cancer[69].

Epidemiological studies have reported beneficial associations between FV consumption and risk of overweight and obesity[70].

A systematic review with meta-analysis of prospective cohort studies[70] reported a borderline inverse association between high vegetable consumption and risk of overweight/obesity (0.93 (0.83-1.03) $I^2$=66%) in adult populations. Moreover, the same meta-analysis showed a significant inverse association between high fruit consumption and overweight/obesity risk (0.88 (0.80-0.96) $I^2$=76%)[70]. Finally, an increment of 100 g in fruit intake was significantly associated with a decreased risk of overweight/obesity (0.93 (0.86-1.00) $I^2$=89%) in the same population[70].

Regarding **metabolic syndrome (MetS)** and **abdominal obesity,** a longitudinal study in the framework of the Korean Genome and Epidemiology Study (KoGES)[71] found a lower risk of MetS in frequent fruit consumers (≥4 portions/day) compared to rare fruit consumers (<1 portion/day) in a Korean adult population. However, no significant associations were found between vegetable frequent consumers and MetS risk[71]. Besides, the same study reported a significant inverse association between high fruit consumption (≥4 portions/day) and abdominal obesity incidence[71].

Finally, a systematic review with meta-analysis of prospective cohort studies[72] found a significant decreased risk of **adiposity** with a high consumption of fruits (9% reduced risk), vegetables (17% reduced risk) and FV (17% reduced risk) in adult populations.

#### 1.7.1.2 Quantity in FV consumption and cardiometabolic risk factors

Several epidemiological studies have demonstrated the benefits of a diet rich in FV on some cardiometabolic risk factors, including triglycerides (TG), LDL-cholesterol, HDL-cholesterol, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose, glycated hemoglobin (HbA1c), body weight, waist circumference (WC) and body mass index (BMI)[69].

Regarding **TG**, an interventional study in elderly subjects[73] reported a significant decrease in TG levels with an increase of vegetable consumption up to 200 g/day in T2D patients. However, in a cross-sectional study conducted in the framework of the Prevención con Dieta Mediterránea (PREDIMED)-Plus study[74], no significant inverse associations were found between a high consumption of fruits (≥3 portions/day) and TG plasma levels in elderly subjects.

Related to **LDL-cholesterol**, a cross-sectional analysis in the National Heart, Lung, and Blood Institute (NHLBI) Family Heart study[75] showed a significant inverse association between a high consumption of FV and LDL-cholesterol plasma levels in adult and elderly subjects. In the same line, in a cross-sectional study in an elderly Mediterranean population with MetS[74], a significant reduction in LDL-cholesterol levels was found in participants with a high intake of whole fruits. Furthermore, in another cross-sectional study conducted in the framework of the Chennai Urban Rural Epidemiological Study (CURES)[76], a significant inverse association was reported between a high consumption of FV and total and LDL-cholesterol levels in healthy adults.

With regard to **HDL-cholesterol**, a meta-analysis of randomized controlled clinical trials[77] revealed a slight increase (0.18 (0.06-0.29), $p<0.001$) in HDL-cholesterol levels in overweight and obese individuals that consumed high quantities of FV.

Regarding **blood pressure**, in a systematic review and meta-analysis of randomized controlled clinical trials, a high consumption of FV was significantly associated with a reduction in DBP in adult patients with MetS[67]. Besides, in a 16-week interventional study[69], a significant reduction in SBP was found in obese men and women that consumed ≥500 grams/day of FV. Finally, in a cross-sectional study conducted in the framework of the CURES study[76], a significant inverse association was reported between a high consumption of FV and SBP in healthy adults.

Regarding **HbA1c** and **fasting blood glucose**, an interventional study in elderly subjects[73] found a significant decrease in HbA1c with an increase in total vegetable consumption of ≥150 g/day in participants with diagnosed T2D. Besides, in a prospective cohort study in diabetics subjects from the ADDITION-Cambridge Study[78], a significant reduction in HbA1c was observed with an increase in FV intake. Finally, in a cross-sectional study in an elderly Mediterranean population with MetS[74], a significant reduction in fasting blood glucose was found in participants with higher intakes of whole fruits.

Related to **WC**, in a cross-sectional study in an elderly Mediterranean population with MetS[74], a significant reduction in WC was found in participants with higher intakes of whole fruits. Besides, in another cross-sectional study conducted in the framework of the CURES study[76], a significant inverse association was reported between a high consumption of FV and WC in healthy adults. Moreover, in a longitudinal study from the ADDITION-Cambridge Study[78], a significant reduction in WC was observed with an increase of FV intake in participants with diabetes.

Regarding **body weight**, a longitudinal study that analyzed data from three large cohort studies (Nurses´ Health Study, Nurses´ Health Study II and Health Professionals Follow-up Study)[79] reported a significant association between an increased consumption of FV and a reduction in body weight (-0.24kg for fruits and -0.26 kg for vegetables).

Besides, in a prospective cohort study in Brazilian overweight adults[80], a significant inverse association was found between increasing the consumption of FV and body weight. Specifically, this study revealed that an increase of 100 g/day in the consumption of vegetables was significantly associated with a reduction of 500 grams in body weight after 6 months of follow-up. Also, the same study showed that an increase of 100 g/day in fruit intake was related to a 300 grams of weight loss after 6 months of follow-up[80]. In addition, another prospective cohort study conducted in the framework of the EPIC cohort[81], reported a significant inverse association between an increase of 100 g/day in FV intake and body weight changes at 6.5 years of follow-up in adult European subjects.

Finally, in a 16-week interventional study[69] a significant reduction in body weight, WC and BMI was found in obese men and women that consumed ≥500 grams/day of FV.

However, it is important to mention that a systematic review with meta-analysis of randomized controlled clinical trials found no significant associations between a high consumption of FV and changes in body weight (-0.16 (-0.78, 0.46) $I^2$=0.49) in adult populations[82].

#### 1.7.1.3 Quantity in FV consumption and cardiovascular disease

CVD is the main cause of death worldwide, being more prevalent in developed countries, mainly because of poor eating habits[76]. Elevated cholesterol, TG and BP levels can lead to the development of hypercholesterolemia, hypertriglyceridemia, and hypertension, conditions that are associated in epidemiologic studies to an increased risk of acute myocardial infarction, stroke, coronary heart disease (CHD) and other cardiovascular conditions[83].

A systematic review and meta-analysis of prospective cohort studies[84] showed a significant lower risk of **CVD** and **stroke** in adult and elderly subjects from 69 different countries with a high consumption of FV. Besides, the same meta-analysis reported a significant inverse association between a high consumption of fruits and/or vegetables and CVD, stroke and **CHD morbidity** in the same individuals[84].

With regard to **hypertension**, a longitudinal study from the Women´s Health Study (WHS) cohort[85] revealed a 14% decreased risk of hypertension in middle-aged and elderly woman who consumed ≥8 portions/day of FV, in comparison with women that consumed <2 portions/day. However, when FV were analyzed separately, the previous association remained significant only with a high consumption of fruits[85].

Furthermore, another longitudinal analysis in three prospective cohort studies (Nurses´ Health Study, Nurses´ Health Study II and Health Professionals Follow-up Study)[86] found a 11% lower risk of hypertension in adults and elderly subjects who consumed ≥6 portions/day of FV, compared to those consuming <1 portion/day.

Related to **hypertriglyceridemia**, a meta-analysis of cross-sectional studies[65] reported a significant inverse association between high intakes of fruits and hypertriglyceridemia prevalence in adult and elderly subjects. Besides, the same meta-analysis showed that an increase of 1 portion/day of fruits was significantly related to a 21% lower risk of hypertriglyceridemia[65]. Finally, a longitudinal analysis in the framework of the KoGES[71] Study revealed a significant inverse association between high fruit consumption (≥4 portions/day) and risk of hypertriglyceridemia in Korean adults. However, the same association was not significant with high consumption of vegetables (≥4 portions/day)[71].

#### 1.7.1.4 Quantity in FV consumption and Type 2 Diabetes

In the last decades, there has been an increase in the prevalence of T2D, which is a cause of premature death, as it increases the risk of suffering from other chronic diseases, such as CVD. Some epidemiological studies have reported lower risks of T2D with higher intakes of FV.

A systematic review with meta-analysis of prospective cohort studies[87] reported a borderline inverse association between a high consumption of vegetables (0.95 (0.89, 1.01) $I^2$=59%) and fruits (0.96 (0.93, 1.00) $I^2$=29%) and risk of T2D. Besides, an increment of 100 g/day of fruits was inversely associated with T2D risk (0.98 (0.97, 1.00) $I^2$=21%).

Besides, a case-cohort study in the European Prospective Investigation into Cancer (EPIC-Norfolk) cohort[88] revealed a significant inverse association between a high consumption of FV, separately and combined, and T2D incidence in adult and elderly subjects. Moreover, this study reported a lower risk of T2D with an intake of 3.5-7 portions/day of FV in the same study population[88].

Finally, a longitudinal study in the framework of the KoGES Study[71] showed a significant inverse association between high fruit consumption (≥4 portions/day) and risk of hyperglycemia in Korean adults. However, the same association was not significant with high vegetable consumption (≥4 portions/day)[71].

#### 1.7.1.5 Quantity in FV consumption and cancer

Cancer is the second cause of death worldwide. There is enough evidence that nutrition influences cell and molecular processes that promote the occurrence and development of this disease. Epidemiological studies have demonstrated the beneficial effects of FV consumption on cancer incidence and risk[89].

Firstly, a prospective cohort study in an elderly Dutch male population[90] revealed that consuming ≥200 g/day of fruits and ≥200 g/day of vegetables was associated with a 44% lower risk of cancer. Besides, the same study reported that individuals who consumed ≥200 g/day of fruits showed a 36% lower cancer incidence, compared to those who consumed less than 100 grams of fruits per day[90]. However, no significant associations were found between consuming ≥200 grams of vegetables per day and cancer incidence in the same elderly population[90].

Regarding the different cancer types, the most recent report of the WCRF/AICR (2018)[91] concluded that there is strong evidence of the protective effects of non-starchy FV on aerodigestive cancer. However, the same report stated that there is limited evidence for the beneficial effects of 1) non-starchy vegetables on mouth, pharynx, larynx, esophagus, lung and breast cancer, 2) fruits on esophagus and lung cancer, 3) citrus fruits on stomach cancer and 4) non-starchy FV on bladder cancer[91].

Furthermore, results from the EPIC cohort[92] revealed that the risk of upper gastrointestinal cancer was inversely associated with fruit consumption, while colorectal cancer risk was inversely associated with both FV consumption and dietary fiber intake. Besides, the same study found a significant inverse association between lung cancer risk and fruit intake[92]. In addition, other results from the EPIC cohort[93] showed a significant decreased colon cancer risk with a higher consumption of FV, although these associations did not remain significant with higher intakes of FV in a separate way. The same study did not find significant associations between rectal cancer risk and consuming higher amounts of FV[93].

Moreover, a systematic review with meta-analysis of prospective cohort studies found a significant decreased lung cancer risk with higher intakes of fruits and/or vegetables in adult populations[94]. Finally, a pooled analysis of 25 case-control worldwide studies showed a significant decreased gastric cancer risk with greater intakes of fruits and/or vegetable in adult populations[95].

### *1.7.2 Variety in fruit and vegetable consumption and human health*

As previously mentioned, variety in FV may play an important role on human health due to the different nutrient profile and phytochemical content among the different groups and subgroups of FV[63]. In the majority of epidemiological studies, variety in FV consumption was estimated by using variety scores or classifying FV into several groups and subgroups according to their color, which reflects their different phytochemical content[88,96–99].

#### 1.7.2.1 Variety in FV consumption and obesity

Although some epidemiological studies have reported beneficial associations between FV consumption and overweight/obesity risk or incidence, these studies have only focused on the amount of FV consumed[70].

Currently, there is no study that has determined the risk of overweight/obesity in adult and elderly subjects by taking into account the variety or diversity in the FV consumed. Therefore, further investigations are needed in order to determine the potential beneficial effects of a varied consumption of FV on overweight/obesity risk or incidence.

#### 1.7.2.2. Variety in FV consumption and cardiometabolic risk factors

Related to **triglycerides**, a cross-sectional analysis in the framework of The Japanese Elderly Intervention trial[73] reported a significant decrease in TG levels in elderly diabetic participants that consumed higher amounts of green vegetables (>130 g/day) compared to those who consumed lower amounts (<50 g/day). Besides, a longitudinal study from the Tehran Lipid and Glucose Study (TLGS)[97] revealed a significant inverse association between a higher consumption of green FV and TG plasma levels in adult men.
Regarding **HDL-cholesterol**, a longitudinal study from the TLGS[97], showed a significant inverse association between a higher consumption of yellow and green FV and 3-year changes in HDL-cholesterol in adult men. However, a cross-sectional study in the framework of the PREDIMED-plus Study[74], found a significant decrease in HDL-

cholesterol plasma levels with a high consumption of green fruits in elderly subjects at high CVD risk.

With regard to **LDL-cholesterol**, a longitudinal study from the TLGS[97] reported a significant inverse association between an increased intake of green, yellow and white FV and total cholesterol in adult subjects at 3 years of follow-up. Besides, a cross-sectional study in the framework of the PREDIMED-plus Study[74] showed a significant decrease in LDL-cholesterol levels with a higher consumption of orange fruits in elderly subjects with MetS.

Related to **HbA1c** and **fasting blood glucose**, a cross-sectional analysis in the framework of The Japanese Elderly Intervention Trial[73] reported a significant decrease in HbA1c levels in elderly participants with T2D that consumed higher amounts of green vegetables (>130 g/day) compared to those who consumed lower amounts of this subtype of vegetables (<50 g/day). Furthermore, in a cross-sectional study conducted in the framework of the PREDIMED-plus Study[74], a significant reduction in fasting blood glucose was found in elderly participants with higher intakes of green and red/purple fruits.

Regarding **WC** and **BMI**, a longitudinal study from the Tehran Lipid and Glucose Study[97] reported a significant inverse association between an increased consumption of red/purple FV and body weight, WC and abdominal fat in an adult population at 3 years of follow-up. Moreover, an interventional study in elderly subjects with T2D[73] reported a significant decrease in WC and BMI in participants that consumed high amounts of green vegetables (>130 g/day) in comparison with those consuming lower amounts (<50 g/day). Finally, in a cross-sectional study conducted in the framework of the PREDIMED-plus study[74], significant lower BMI and WC was observed in elderly Mediterranean subjects with MetS that consumed higher amounts of white fruits.

With regard to **body weight,** a study that analyzed data from three large cohorts[79] of adults reported a significant inverse association between body weight changes and the intake of cruciferous and green leafy vegetables after 4 years of follow-up. Besides, the same study revealed another significant inverse association between body weight changes and the consumption of berries and citrus fruits after 4 years of follow-up[79]. Finally, the same large study showed a significant inverse association between body weight changes and the consumption of other fruits (grapefruit, strawberries, apples, pears, prunes and blueberries) and vegetables (peppers and carrots) after 4 years of follow-up[79].

Furthermore, in a prospective cohort study in Brazilian overweight adults[80], it was shown a significant inverse association between dark and/or yellow FV consumption and body weight. In particular, this study revealed that an increase of 100 g/day in dark and/or yellow FV was significantly associated with a decrease in 1.5 kg in body weight at 6 months of follow-up[80].

However, results of these studies are limited and inconsistent, so further studies regarding FV variety and cardiometabolic risk factors are needed[74,97].

#### 1.7.2.3 Variety in FV consumption and cardiovascular disease

So far, there is enough evidence supporting the protective effect of FV quantity on CVD disease. However, only few epidemiological studies have demonstrated that not only quantity is important for CVD prevention, but also the variety intake[84].

A systematic review and meta-analysis of 18 prospective cohorts[84] indicated a significant inverse association between the consumption of certain types of fruits, mainly citrus and apples, and **CVD**, **stroke and CHD incidence** and mortality. Besides, the same meta-analysis reported a significant inverse association between the consumption of specific types of vegetables, including garlics, carrots, cruciferous and green leafy vegetables, and CVD, stroke and CHD incidence and mortality[84].

With regard to **hypertension**, a longitudinal analysis in three prospective cohort studies[86] found a lower risk of hypertension in adults and elderly subjects who consumed ≥4 portions/week of specific FV (broccoli, carrots, apples and raisins) in comparison to those who consumed <1 portion/month of these FV.

#### 1.7.2.4 Variety in FV consumption and type 2 Diabetes

Several epidemiological studies have reported lower risks of T2D with a high consumption of FV [78]. However, there is little evidence on the potential effects of a varied FV consumption on T2D incidence.

A case-cohort study in the EPIC-Norfolk cohort[88] revealed a significant inverse association between high values for FV variety scores, separately and combined, and T2D incidence, in adult and elderly individuals. Moreover, the same study reported a lower risk of T2D with a consumption of ≥12 different items/week of FV[88].

Therefore, given the lack of evidence, further studies are needed to clarify the role of a varied consumption of FV on T2D risk and incidence in adult populations.

#### 1.7.2.5 Variety in FV consumption and cancer

As previously mentioned, epidemiological studies have demonstrated that diets rich in FV may decrease the risk of developing different types of cancer[89]. This is probably due to the great diversity of bioactive compounds with antioxidant, anti-mutagenic, anti-carcinogenic and anti-inflammatory activities that can be found in the different groups and subgroups of FV[90].

In this sense, a prospective cohort study in an elderly Dutch male population[90] suggested a non-significant inverse association between a high variety in FV intake and cancer risk. Besides, the same study reported a significant inverse association between consuming a wide variety of vegetables and cancer risk[90]. However, no significant inverse associations were found between cancer risk and consuming a greater variety of fruits[90].

Related to the specific cancer types, a prospective study conducted in the framework of the EPIC cohort[99] found a significant inverse association between a great variety in the consumption of fruits and/or vegetables and esophageal cell carcinoma risk, but not with gastric and esophageal adenocarcinomas risk. Nevertheless, other results from the EPIC cohort[93] did not report a significant association between variety in FV intake and a decreased risk of colorectal cancer.

Finally, a systematic review with meta-analysis of prospective cohort studies found a significant decreased lung cancer risk with a high consumption of cruciferous and green leafy vegetables and citrus fruits[94].

# 2. JUSTIFICATION

# 2. JUSTIFICATION

Current worldwide recommendations for FV intake are mainly focused on quantity consumption expressed in grams or servings per day. However, FV differ in their nutrient profile and phytochemicals content, which could potentially have different effects on human health. In fact, in the last decades emerging evidence have demonstrated that not only quantity may contribute to reduce the burden of CVDs, but also variety, although evidence is currently limited.

Despite there are some epidemiological studies that have evaluated the association between FV consumption related to nutrient adequacy, diet quality and lifestyle, the majority of them have only focused on the total amount of FV consumed per day, without taking into account the varied intake of FV. The same is true for the many epidemiological studies that have demonstrated the beneficial effects of FV consumption on cardiometabolic risk factors and CVD risk and incidence, since few of them have taken into consideration the variety in FV intake. Of note, hardly any of these studies have been conducted in a middle-aged population with MetS who are already at high CVD risk.

Finally, it should be mentioned that no epidemiological study has evaluated neither the association between FV consumption and changes in cardiometabolic risk factors, nor the relationship between FV intake and nutrient adequacy, diet quality and lifestyle, cross-classifying both quantity and variety consumption.

Considering the limited evidence on the effect of consumption of a variety of FV on diet quality, lifestyle and several cardiometabolic risk factors, the present doctoral thesis aims to provide new insights regarding the potential beneficial role of FV on nutrient adequacy, diet quality, lifestyle and cardiometabolic risk factors in an elderly Mediterranean population at high CVD risk, giving special consideration to the varied consumption of these both food groups.

# 3. HYPOTHESIS AND AIMS

# 3. HYPOTHESIS AND AIMS

**Hypothesis 1**: A greater variety in FV consumption is associated with better diet quality and lifestyle in a Mediterranean population at high CVD risk.

- Objective 1.1: To determine the association between fruit and/or vegetable variety, diet quality and lifestyle.

- Objective 1.2: To assess the association between fruit and/or vegetable quantity, diet quality and lifestyle.

- Objective 1.3: To evaluate the association between the combination of variety and quantity in fruit and/or vegetable consumption, diet quality and lifestyle.

**Hypothesis 2**: A greater variety in FV consumption is associated with an improvement in several cardiometabolic risk factors in a Mediterranean population at high CVD risk.

- Objective 2.1: To analyze the association between one-year changes in fruit and/or vegetable variety and cardiometabolic risk factors.

- Objective 2.2: To evaluate the association between one-year changes in fruit and/or vegetable quantity and cardiometabolic risk factors.

- Objective 2.3: To assess the association between one-year changes in the combination of fruit and/or vegetable variety and quantity and cardiometabolic risk factors.

# 4. MATERIALS AND METHODS

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## 4.1 The PREDIMED-Plus study:

### *4.1.1 Main objectives of the study*

The present thesis was conducted in the framework of the PREDIMED-plus study, an ongoing 6-year parallel-group, multi-center, randomized controlled clinical trial for the primary prevention of CVD and mortality.

The main objective of this trial is to evaluate the effect of an intensive lifestyle intervention based on a low-calorie MedDiet, PA and behavioral therapy on CVD events, compared to a control group who receives advice and recommendations on a healthy MedDiet, but without caloric restriction.

The main outcomes of this trial are: 1) the **incidence of CVD events**, like non-fatal myocardial infarction, non-fatal stroke and CVD mortality, and 2) **weight loss** and long-term weight loss maintenance.

The secondary outcomes are the reduction of the **abdominal obesity** and **other conditions related to overweight/obesity**, such as acute coronary syndromes, heart failure, atrial fibrillation, peripheral arterial disease, venous thrombosis, T2D, overall cancer incidence, incidence of specific types of cancer (breast, colorectal, prostate, lung and stomach), bone fractures, gallstone disease, gout, neurodegenerative diseases (Parkinson disease and dementia), depression and eating disorders.

Besides, this trial also evaluates the effect of this intervention on **intermediate biomarkers** such as blood pressure, fasting blood glucose, serum lipid levels, kidney function, liver function, c-reactive protein, Hb1cA levels and alterations in the electrocardiographic tracing, as well as on **other parameters** like overall nutrient intake, cognitive function, quality of life, psychopathological symptoms and percentage of participants who need antihypertensive, antidiabetic or lipid-lowering drug treatment.

### 4.1.2 Study design

#### 4.1.2.1 Participants selection

Randomized participants of the PREDIMED-plus study were recruited between September 2013 and December 2016 in 23 Spanish centers like research institutes, universities, hospitals and primary health care centers located in Málaga, Sevilla, Navarra, Palma de Mallorca, Barcelona, Reus, Granada, Madrid, Vitoria, Valencia, Las Palmas de Gran Canaria, Córdoba, Alicante, Jaén and León.

##### 4.1.2.1.1 Eligibility criteria

**Inclusion criteria** for the PREDIMED-plus study were men (aged 55-75 years) and women (aged 60-75 years) with a BMI between 27 and 40 kg/m$^2$, who had not previously suffered from CVD or cancer. Moreover, participants had to meet at least three of the following MetS criteria, according to the American Heart Association (AHA), NHLBI and International Diabetes Federation[100]:

- Abdominal obesity (WC ≥88 cm in women and WC ≥102 cm in men).
- High TG levels (≥150 mg/dL or drug treatment for elevated TG).
- Low HDL-c levels (<50 mg/dL in women and <40mg/dL in men; or drug treatment for reduced HDL-c).
- High BP levels (SBP>130 mm Hg or SBP>85 mm Hg; or antihypertensive drug treatment).
- High fasting blood glucose (≥100 mg/dL; or drug treatment for elevated glucose).

**Exclusion criteria** for this study were the following ones:

- Previous history of CVD, including angina pectoris, myocardial infarction, stroke, peripheral arterial disease, ventricular arrhythmias, atrial fibrillation, heart failure, hypertrophic cardiomyopathy and aortic aneurism.

- Active cancer or history of malignant tumour in the last 5 years, except for nonmelanoma skin cancer.
- Obesity of endocrine origin, except for treated hypothyroidism.
- History of inflammatory bowel disease or small/large bowel resection.
- History of weight-loss surgery or intention to undergo bariatric surgery in the next 12 months.
- Inability to follow the recommended MedDiet, for religious reasons, swallowing problems or allergy to foods or components of the MedDiet.
- Incapacity to perform PA.
- Alcohol dependence syndrome or total daily alcohol intake >50 g.
- Drug abuse in the last six months.
- Cirrhosis or liver failure.
- Life expectancy less than one year.
- Severe psychiatric disorders: schizophrenia, eating disorders or depression.
- Immunodeficiency or HIV-positive status.
- History of vital organ transplantation.
- Concomitant treatment with immunosuppressive drugs or cytotoxic agents.
- Current use of weight loss medication.

The **recruitment process** was performed by family doctors of the primary health care centers who conducted a review of the clinical records in order to obtain the names of the potential participants that met the eligibility criteria. Afterwards, potential participants were interviewed by telephone to inform them about the study and to arrange a first screening visit with the trained study staff.

#### 4.1.2.1.2 Screening visit

In the PREDIMED-plus Study, three screening visits were performed.

In the **first screening visit,** participants were informed about the main objectives and characteristics of the study. Besides, inclusion and exclusion criteria questionnaires were administered to them. Participants who met the inclusion and exclusion criteria, and decided to participate in the study, signed the informed consent form.

Furthermore, an electrocardiogram was performed on the potential participants, as well as body weight, WC, BP and height measurements.

Finally, participants were provided with a 3-day prospective food record, a PA questionnaire, and a body weight and waist and hip circumference self-monitoring record, to be completed at home.

After two weeks, participants were interviewed by telephone (**second screening visit**) to assess their body weight change and to remind them the date of the next visit.

Finally, four weeks after the first visit, potential participants attended the **third screening visit** in which they returned all the questionnaires, as the run-in period ended. Besides, their weight and waist and hip circumferences were measured by trained dietitians. Finally, dietitians administered a 143-item food frequency questionnaire (FFQ).

Only participants who accomplished the four following criteria were selected for the study and randomly assigned to one of the two intervention groups:

- Attendance at the three screening visits.
- Adequate completion of the clinical-psychopathological and quality of life questionnaires.
- Adequate completion of the FFQ and PA questionnaire.
- Appropriate recording of at least three weight and three WC self-monitoring measures.
- Weight loss of at least 1.5 kg during the run-in period.

#### 4.1.2.1.3 Randomization

A total of 6.874 participants met the eligibility criteria and were randomly assigned to the intervention or control group, using a centrally assigned computer-generated random number sequence. Participants were assigned to the intervention group stratified by center, sex and age group, allowing the inclusion of couples in the same group, who were jointly randomized.

### 4.1.2.2 Intervention

PREDIMED-plus dietitians were primarily responsible for the dietary intervention, for which they have been specifically trained. The main objective of the intervention was to follow an energy restricted MedDiet (with 30% of calorie restriction) to promote weight loss, as well as PA promotion and behavioural support. The delivery of free extra olive oil and nuts increased the compliance and adherence to the intervention of the participants from both the intervention and control groups.

#### 4.1.2.2.1 Intervention group

Participants enrolled to the intervention group received 6 L of virgin olive oil and 3 kg of nuts per each six months.

Besides, dietitians promoted following a hypocaloric MedDiet, by increasing the intake of FV, extra olive oil, nuts, salads, whole grains, fiber-rich foods and low-fat yoghurts, while reducing the consumption of red meats and sausages, sugars and white bread, packaged fruit juices and sweetened beverages. The main characteristics of this hypocaloric MedDiet are summarized in **Table 2**:

**Table 2. Characteristics of the PREDIMED-plus hypocaloric MedDiet.**

| NUTRIENT | DAILY RECOMMENDED INTAKE |
|---|---|
| Calories | ~ 600 kcal/day (~ 30% of daily energy intake) |
| Total fat | 35-40% of total energy intake |
| Saturated fats | 9-10% of total energy intake |
| Monounsaturated fats | > 20% of total energy intake |
| Polyunsaturated fats | > 10% of total energy intake |
| Cholesterol | < 300 mg/day |
| Proteins | ~ 20% of total energy intake |
| Carbohydrates | 40-45% of total energy intake (low-glycemic index) |
| Sodium | < 100 mmol/day (~ 2.4 g of sodium or 6 g of sodium chloride) |
| Calcium | 1000-1500 mg |
| Dietary fiber | 30-35 g |
| ****A reduction between 500-1.000 kcal/day helped participants to lose between 0.5-1 kg/week.** | |

The adherence to the hypocaloric Mediterranean diet was achieved by the participants in the intervention group thanks to the dietary guidelines they received, aimed at meeting these following 17 objectives:

1. Culinary use of extra virgin olive oil for cooking, dressing and spreading.

2. Consumption of ≥ 3 servings/day of fruits.

3. Consumption of ≥ 2 servings/day of vegetables (at least one as a salad).

4. Consumption of ≤ 1 serving/day of white bread.

5. Consumption of ≥ 5 servings/day of whole grains and pasta.
6. Consumption of ≤ 1 serving/week of red meat, meat products or hamburger.
7. Consumption of < 1 serving/week of butter or cream.
8. Consumption of < 1 sweetened beverage or fruit juice with added sugar per week.
9. Consumption of ≥ 3 servings/week of legumes.
10. Consumption of ≥ 3 servings/week of fish or seafood.
11. Consumption of < 3 servings/week of sweets, cookies and pastries.
12. Consumption of ≥ 3 servings/week of nuts (including peanuts).
13. Consumption of chicken, turkey or rabbit meat instead of beef, pork, hamburgers or sausages.
14. Use sofrito ≥ 2 times/week (sauce made by tomato and onion, garlic or leek, at slow fire with olive oil).
15. Replace added sugar in beverages (coffee, tea) with non-caloric artificial sweeteners.
16. Consumption of < 3 servings/week of white pasta or rice.
17. Drinking between 2-3 glasses (men) or 1-2 glasses (women) of wine per day. It was only promoted in those participants who were alcohol consumers at the beginning of the study.

Furthermore, regarding PA promotion, participants were engaged to gradually increase their daily PA, being at least 45 min/day and 6 days/week. The PA program included light to moderate aerobic and endurance activities.

Finally, related to behavioral support, participants received problem-solving tools for excess caloric intake and sedentary lifestyles, especially in situations of depression, stress or anxiety, in order to train self-control over them.

Finally, participants in the intervention group assisted to group sessions (1 session every month), in which dietitians provided them the following information:

- Key aspects of the hypocaloric MedDiet diet, using a 17-item MedDiet adherence questionnaire.
- Description of typical low-calorie Mediterranean foods of each season.
- Weekly shopping list, according to the season of the year.
- Weekly meal plan (with detailed menus), adapted from the shopping list.
- Meal recipes for the suggested menus.

##### 4.1.2.2.2 Control group

Participants in the control group were advised to follow the PREDIMED study MedDiet recommendations (without calorie restriction), characterized by:

1. Use of extra virgin olive oil for cooking (≥ 4 tablespoon/day).
2. Consumption of ≥ 3 servings/day of fresh fruits (including natural fruit juices).
3. Consumption of ≥ 2 servings/day of vegetables (being one of them fresh).

4. Consumption of 2-3 servings/week of legumes.

5. Consumption of ≥ 3 servings/week of fish or seafood.

6. Consumption of ≥ 1 serving/week of nits or seeds.

7. Consumption of poultry instead of red or processed meats.

8. Use sofrito ≥ 2 times/week.

9. Optional consumption of ≥ 7 glasses/week of wine (only for habitual alcohol drinkers).

10. Avoid consumption of sugars, sweetened beverages, butter, fast food, and bakery products.

Although dietitians did not help them to lose weight, they provided them a list of general lifestyle recommendations in order to manage MetS.

Finally, participants in the control group assisted to group sessions (1 session every 6 months), in which dietitians provided them general dietetic recommendations for following the MedDiet, as well as other useful information such as a shopping list, menus, cooking recipes and descriptions of Mediterranean foods.

#### 4.1.2.3 Data collection

At baseline, 6 months and annually, all participants attended to face-to-face interviews with trained dietitians, in which some anthropometric and biochemical measures were collected and several questionnaires were administered (see **Table 3**):

**Table 3. Data collection in the PREDIMED-plus Study visits.**

| | Baseline | 6 months | 1 Year | 2 Years | 3 Years | 4 Years | 5 Years | 6 Years | 7 Years | 8 Years |
|---|---|---|---|---|---|---|---|---|---|---|
| **Anthropometric measurements*** | X | X | X | X | X | X | X | X | X | X |
| **143-item semiquantitative FFQ** | | X | X | X | X | X | X | X | X | X |
| **MedDiet adherence questionnaire**** | X | X | X | X | X | X | X | X | X | X |
| **PA questionnaires ***** | X | X | X | X | X | X | X | X | X | X |
| **Accelerometry** | X | X | X | X | X | X | X | X | X | X |
| **Electrocardiogram** | | X | X | X | X | X | X | X | X | X |
| **BP measurements** | X | X | X | X | X | X | X | X | X | X |
| **Blood sample collection** | X | X | X | | X | | X | | X | X |
| **Urine sample collection** | X | X | X | | X | | X | | X | X |
| **Follow-up questionnaire** | | X | X | X | X | X | X | X | X | X |
| **Cognitive-neuropsychological tests[&]** | | | | X | | X | | X | | X |
| **Quality of life tests[¥]** | | | X | | X | | X | | X | |
| **Clinical-psychopathological tests[€]** | | | X | X | X | X | X | X | X | X |

*Includes weight, height, waist circumference and hip circumference.

**A 14-item MedDiet adherence questionnaire was used for the control group, while a 17-item MedDiet questionnaire was used for the intervention group.

***Regicor Short Physical Activity Questionnaire, Physical Activity Readiness Questionnaire (PAR-Q), Rapid Assessment of Physical Activity (RAPA1 and RAPA 2) and Nurse´s Health Study (NHS) Questionnaire.

[&]Mini-mental State Examination, Clock test, verbal and phonological fluency, trail making test, and inverse digit series.

[¥] SF-36.

[€] Beck´s Depression BDI-II, multidimensional scale of locus control over weight and diagnostic criteria for eating disorders.

Abbreviations: FFQ, Food frequency questionnaire.

#### 4.1.2.3.1 Dietary intake assessment

To assess dietary intake, a validated 143-item semiquantitative FFQ questionnaire[101] was administered to all participants in the third screening visit, as well as in each annual follow-up visit. Trained dietitians asked participants about the frequency of consumption of a wide ***variety*** of foods during the previous year in a face-to-face interview. To determine the total amount consumed of the different foods, information of all items in the FFQ was transformed into grams per day by multiplying serving sizes by its frequency of consumption (9 categories ranging from never to more than 6 times/day) and dividing the result by the assessed period. Finally, total energy and nutrient intake was estimated from all responses using Spanish food composition tables[102,103].

Besides, a validated 17-item MedDiet adherence questionnaire[104] was used in the intervention group to evaluate the compliance with the intervention, while a 14-item MedDiet adherence questionnaire was administered to the control group participants to determine their adherence to a non-hypocaloric MedDiet[104].

#### 4.1.2.3.2 Physical activity and other lifestyle parameters assessment

At baseline, six months and annual follow-up visits, the following questionnaires were administered to all participants in order to evaluate PA:

- Regicor Short Physical Activity Questionnaire[105–107]: a Spanish validated questionnaire adapted from the Minnesota Leisure Time Physical Activity Questionnaire (MLTPAQ) that includes questions related to the type of activity and its frequency in number of days and duration (in min/day).

- Physical Activity Readiness Questionnaire (PAR-Q)[108]: a PA aptitude questionnaire to detect the potential health and cardiovascular problems in healthy individuals that perform light, moderate or vigorous PA.

- Rapid Assessment of Physical Activity (RAPA1 and RAPA2)[109]: a 9-item questionnaire with questions related to PA level, ranging from sedentary to vigorous PA. Besides, it includes questions regarding strength training and flexibility.

- Nurse´s Health Study (NHS) Sedentary Lifestyle Questionnaire[110]: a validated questionnaire to evaluate sedentary behaviours, such as daily time spent watching TV or sitting (at work, leisure time or travelling).

In addition, steps walked every day were determined by using a pedometer in participants of the intervention group. Finally, PA and hours of sleep were quantified using an accelerometer, at baseline, 6 months and annually in a subsample of participants from both groups (control and intervention).

#### 4.1.2.3.3 Anthropometric measures

Anthropometric measurements were determined at baseline, 6 months and annually in all randomized PREDIMED-plus participants by trained dietitians:

- Body weight was measured with light clothes and no shoes using calibrated scales.

- Height was measured using a wall-mounted calibrated stadiometer.

- WC was measured twice midway between the last rib and the iliac crest using an anthropometric tape.

- Hip circumference was measured at the widest part of the hip using an anthropometric tape.

#### 4.1.2.3.4 Blood pressure determination

BP determination was carried out at baseline, 6 months and annually, as indicated in **Table 3**. BP (SBP and DBP) was determined three times (after 5 min rest) in seated participants using a validated semiautomatic oscillometer (Omron HEM-705CP, Netherlands) by trained dietitians. The mean of the three measurements were used for the statistical analysis.

#### 4.1.2.3.5 Biological samples and biochemical determinations

Biological samples were collected after an overnight fast at baseline, 6 months, and at years 1, 3, 5 and 7, as **Table 3** indicated, following a standardized protocol. PREDIMED-plus nurses were the responsible for the collection, processing and storage of all biological samples.
Regarding **blood samples**, a complete blood count was performed, as well as routinely biochemical measurements, including lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol and TG), fasting blood glucose, sodium, potassium, calcium, uric acid, urea, creatinine, albumin, c-reactive protein, erythrocyte sedimentation rate, Hb1Ac and hepatic function (bilirubin, alkaline phosphatase and transaminases).

**Urine samples** were collected at the time of blood extraction and biochemical determinations such as urine albumin and creatinine were performed.

All biochemical determinations were performed using standard enzymatic methods by laboratory technicians. Blood and urine samples were stored at -80ºC.

## 4.2 Study population for the analyses

For the present doctoral thesis, data from all randomized PREDIMED-plus participants was used to conduct both a cross-sectional and a prospective cohort study:

- Sample for the cross-sectional study considering FV consumption as an exposure and diet quality as an outcome:

  For these cross-sectional analyses, we selected a total of 6874 randomized PREDIMED-plus participants with baseline data. However, as diet quality was considered as an outcome and FV consumption as an exposure, we excluded 36 participants who did not complete the FFQ and 191 participants with extreme energy intake values (500-3500 kcal/day in women and 800-4000 kcal/day in men). Therefore, a final sample of 6647 participants was selected for these analyses.

- Sample for the prospective cohort study considering FV consumption as an exposure and cardiometabolic risk factors as different outcomes:
  A total of 6847 randomized PREDIMED-plus participants with baseline, 6 month and 1-year data were selected for these longitudinal analyses. Due to FV consumption was considered as an exposure, we excluded 36 participants without information from the FFQ and 191 individuals with extreme energy values (500-3500 kcal/day in women and 800-4000 kcal/day in men). Therefore, a final sample of 6647 individuals was used for these longitudinal analyses.

## 4.3 Statistical analysis

A detailed explanation of the statistical analyses conducted for the present doctoral thesis is reflected in each of the publications included in the results section. All statistical analyses were performed using the software Stata 14 (StataCorp, Texas, USA), using a level of significance $p<0.05$ for bilateral contrast.

It is important to take into consideration that a correction factor of 0,7 has been applied to some dietary exposure variables (related to vegetable intake) in the cross-sectional and longitudinal analyses, since vegetable consumption is overestimated in the PREDIMED-plus study.

For the cross-sectional study:

- Differences between categories in the baseline characteristics of the study population, as well as in dietary and lifestyle factors, were determined by using chi-square and ANCOVA with Bonferroni´s post-hoc tests for categorical and continuous variables, respectively.

- To determine the likelihood of meeting the DRIs recommendations or to determine the risk of inadequate intake of fibre, two or more, three or more and four or more micronutrients, according to categories of exposure, Odds Ratio (ORs) and 95% confidence intervals (CIs) were estimated using logistic regression models adjusted for several potential confounders.

For the prospective cohort study,

- The follow-up period was calculated as the interval between the dates of the baseline and one-year follow-up visits.
- To determine baseline, 6-month and 1-year characteristics of the study population, mean±standard errors (SE) were presented for continuous variables and numbers and percentages for categorical ones.
- Linear mixed-effects models (LMM) with specific random intercepts at recruitment centre, family members and patient level, a random slope for each visit (baseline, 6 months and 1 year) and with an unstructured correlation matrix were performed to examine changes (ß-coefficients) in different outcomes according to changes in different categories of exposure or in continuous exposures.

- To determine statistical interactions between categories of exposure and covariates, likelihood ratio tests were performed comparing the fully adjusted LMM with and without cross-product terms. For statistically significant interactions, a stratified analysis was conducted.

# 5. RESULTS

# 5. RESULTS

**Table 4** presents the list of the 2 publications included in the present doctoral thesis.

**Table 4**. Reference, impact factor, category, and journal rank of the two publications presented in this doctoral thesis[1]

| Reference | Impact factor | Category | Journal rank |
|---|---|---|---|
| **López-González L,** Becerra-Tomás N, Babio N, et al. Variety in fruits and vegetables, diet quality and lifestyle in an older adult mediterranean population. Clin Nutr. 202;40(4):1510-1518. doi: 10.1016/j.clnu.2021.02.024. | 7.324 | Nutrition & Dietetics | Q1 7/88 |
| **López-González L,** Becerra-Tomás N, Babio N, et al. One-year changes in fruit and vegetable variety intake and cardiometabolic risk factors changes in a middle-aged Mediterranean population at high cardiovascular risk. Eur J Clin Nutr. Epub ahead of print. doi: 10.1038/s41430-022-01124-3. | 4.016 | Nutrition & Dietetics | Q2 39/88 |

Q, Quartile
[1]Accessed Date: May 21, 2022 (Journal Citation Reports of the ISI web of knowledge, Clarivate).

## 5.1 Variety in fruits and vegetables, diet quality and lifestyle in an elderly Mediterranean population

Leyre López-González, Nerea Becerra-Tomás, Nancy Babio, Miguel Ángel Martínez-González, Andrés Díaz-López, Dolores Corella, Albert Goday, Dora Romaguera, Jesús Vioque, Ángel M. Alonso-Gómez, Julia Wärnberg, J. Alfredo Martínez, Luís Serra-Majem, Ramón Estruch, Francisco Tinahones, José Lapetra, Xavier Pintó, Josep A. Tur, José López-Miranda, Aurora Bueno-Cavanillas, Miguel Delgado-Rodríguez, Pilar Matía-Martín, Lidia Daimiel, Laura Álvarez-Álvarez, Josep Vidal, Clotilde Vázquez, Emili Ros, Zenaida Vázquez-Ruiz, Silvia Canudas; Rebeca Fernández Carrión; Olga Castañer, M Ángeles Zulet; Lucas Tojal Sierra; M Julia Ajejas Bazán; Carmen M López García; Marian Martín; Antonio García-Ríos; Rosa Casas; Ana M Gómez-Pérez; José Manuel Santos-Lozano; Estíbaliz Goñi; Patricia Guillem-Saiz; Camile Lassale; Itziar Abete; Itziar Salaverria Lete; Sonia Eguaras; Helmut Shroeder and Jordi Salas-Salvadó; on behalf of the PREDIMED-Plus Investigators.

**Key teaching points:**

- Little is known about the association between variety in FV consumption, nutrient adequacy, diet quality and lifestyle.

- For the first time, the present study has evaluated the association between a varied consumption of FV related to diet quality and lifestyle in an elderly Mediterranean population at high CVD risk.

- A cross-sectional analysis with 6647 elderly subjects at high CVD risk was conducted in the framework of the PREDIMED-plus study.

- Results revealed an inverse association between a varied consumption of fruits and/or vegetables (also in combination with higher amounts of total fruits and/or vegetables) and the risk of having inadequate intakes of dietary fiber and micronutrients.

- A varied consumption of fruits and/or vegetables was associated with better diet quality and a healthier lifestyle.

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Original article

# Variety in fruits and vegetables, diet quality and lifestyle in an older adult mediterranean population

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S U M M A R Y

*Background and aims:* Previous studies, mainly focused on quantity rather than variety, have shown beneficial associations between the amount of fruit and vegetable consumed, diet quality and healthy lifestyle. The aim is to evaluate the association between fruit and vegetable consumption, diet quality and lifestyle in an elderly Mediterranean population, considering both variety and the combination of quantity and variety (QV).
*Methods:* A cross-sectional analysis of 6647 participants (51.6% of males) was conducted in the framework of the PREDIMED-Plus study. A variety score was created as the sum of vegetables and/or fruits consumed at least once per month using food frequency questionnaires. Dietary Reference Intakes (EAR and IA values) were used to estimate the prevalence of inadequate intake of dietary fiber and micronutrients. Logistic regression models were performed to examine the association between fruit and vegetable consumption and not meeting the DRIs, by tertiles of fruit and vegetable variety and QV categories.
*Results:* Participants with higher fruit and vegetable variety score reported a significant higher intake of fiber, vitamins, minerals and flavonoids and were significantly more likely to be physically active and non-smoker. Besides, higher variety in fruit and vegetable consumption was associated with lower prevalence of having an inadequate intake of fiber [(0.13 (0.11–0.16)], two or more [(0.17 (0.14–0.21)], three or more [(0.15 (0.13–0.18)] and four or more [(0.11 (0.10–0.14)] micronutrients in our participants. Higher quantity and variety in fruit and vegetable consumption was associated with lower prevalence of having an inadequate intake of fiber [(0.05 (0.04–0.06)], two or more [(0.08 (0.06–0.10)], three or more [(0.08 (0.06–0.09)] and four or more [(0.06 (0.05–0.07)] micronutrients.
*Conclusion:* Greater variety in fruit and vegetable intake was associated with better nutrient adequacy, diet quality and healthier lifestyle in an elderly Mediterranean population.


## 1. Introduction

The World Health Organization (WHO) recommends a minimum consumption of 400 g or five portions per day of fruits and vegetables for the prevention of several chronic diseases such as type 2 diabetes, cardiovascular disease or different types of cancer [1]. In 2009, the same organization placed low fruit and vegetable consumption among the top ten selected risk factors for global mortality, even beyond tobacco smoking [1,2]. However, despite the recommendations, consumption of fruits and vegetables remains to be very low in the vast majority of countries [3,4].

Fruits and vegetables are low energy density foods because of their high water and dietary fiber and low energy content [5]. Besides, they are good sources of folate, potassium, magnesium, vitamins A, C, E and K, and phytochemicals, especially flavonoids, which may be responsible for their several health benefits [6].

Fruit and vegetable consumption has been associated with overall diet quality, lifestyle and human health [7]. With regard to diet quality, a systematic review with meta-analysis of seven randomized controlled clinical trials revealed that increasing fruit and vegetable consumption leads to a rise in carbohydrates, fiber and micronutrients intake, which implies an improvement in the overall diet profile [8]. In relation to lifestyle, another systematic review with meta-analysis of 72 prospective cohort studies reported that increasing 100 g/d of fruit and vegetable was significantly associated with an increased prevalence of being physically active or never smoker along with a decreased prevalence of being current and former smoker, as well as having a lower intake of alcohol [9].

However, these associations have been mainly investigated considering total fruit and vegetable quantity rather than its variety [5]. It is noteworthy that dietary guidelines also promote the consumption of a variety of fruits and vegetables, due to the different nutrient profile and phytochemical content among the different groups and subgroups of fruits and vegetables [10]. In this sense, a cross-sectional study in an adult's cohort (CSFII 94–96 cohort conducted by the USDA), reported that variety in fruit consumption was associated with higher intakes of vitamins A and C [11]. Another cross-sectional study in a low-income women cohort showed that variety in vegetable consumption was associated with a higher HEI-2005 diet quality score [12]. Finally, a cross-sectional study in a preschooler's cohort (2–5 year old children from the 2005–2010 NHANES cohort), demonstrated that variety of fruits and vegetables was associated with better diet quality scores [13].

Therefore, a) as few studies have investigated the association between fruit and vegetable consumption, diet quality and lifestyle; b) taking into account that variety has hardly been evaluated in elderly subjects; and c) none of the previous studies have assessed these associations in a Mediterranean population; the main aim of the present study was to analyze the association between fruit and vegetable variety, diet quality and lifestyle in an elderly Mediterranean population at high cardiovascular risk. The second objective was to assess the previous association considering the combination of quantity and variety in fruit and vegetable intake.

## 2. Material and methods

### 2.1. Design and study population

The present study is a cross-sectional analysis at baseline of the ongoing PREDIMED-plus study, a 6-year parallel-group, multicenter, randomized controlled clinical trial for the primary prevention of cardiovascular disease. Participants are men aged between 55 and 75 years and women 60–75 years, with a body mass index between 27 and 40 kg/m$^2$, who meet at least three MetS criteria defined according to the updated criteria of the AHA/National Heart, Lung and Blood Institute and the International Diabetes Federation [14]: abdominal obesity for European individuals (WC ≥ 88 cm in women and ≥102 in men), high triglycerides levels (≥150 g/dL; or drug treatment for elevated triglycerides), low HDL-cholesterol (<50 mg/dL in women and <40 mg/dL in men; or drug treatment for reduced HDL-c), high blood pressure (SBD ≥130 mm Hg or DBD ≥85 mm Hg; or antihypertensive drug treatment), or high plasma fasting glucose (≥100 mg/dL; or drug treatment for elevated glucose). Participants who have suffered chronic diseases before recruitment like CVD or active cancer were excluded from the study. Randomized participants were recruited by 23 Spanish centers from the National Health System like research institutes, universities, hospitals and primary health care centers [15].

Details of the study protocol are available at https://www.predimedplus.com/. This trial was registered in 2014 at the International Standard Randomized Controlled Clinical Trial (ISCRT; http://www.isrctn.com/ISRCTN89898870) with number 898988870 [15,16].

In this report, we cross-sectionally analyzed baseline data from all the PREDIMED-plus randomized participants. We excluded 36 participants who did not complete the Food Frequency Questionnaire (FFQ) as well as 191 participants who had energy intake values beyond the specified limits (500–3500 Kcal/day in women and 800–4000 Kcal/day in men) [17]. Therefore, a final sample of 6647 participants was analysed. The data were analysed using the available PREDIMED-Plus dataset, dated 26 June 2020.

### 2.2. Dietary assessment and determination of fruit and vegetable variety and quantity

To assess dietary intake, trained dietitians administered a 143-item semiquantitative FFQ at baseline. The reproducibility and validity of this FFQ has been previously described [18]. The FFQ contains 17 and 13 items related to vegetable and fruit consumption, respectively. In each item, a typical portion size was specified as well as nine potential categories of frequency consumption that ranged from never or almost never to more than six times per day. Besides, a 17-item MedDiet Questionnaire was used to evaluate adherence to an energy reduced MedDiet. Compliance with each item of the questionnaire was scored with one point while non-compliance with zero. A total score of 0 means no adherence while 17 points reflects maximum adherence.

For the present study, we have only focused on solid and raw fruits and vegetables, therefore fruit and vegetable juices were excluded. Besides, we did not consider potatoes and mushrooms as vegetables, because their nutritional composition differs from that of vegetables [19]. Finally, we did not consider dried fruits in our analyses because it includes more than one type of fruit, so it was not possible to distinguish to what type of raw fruit would be equivalent. Therefore, we included 10 items of fruit (oranges, bananas, apples, strawberries, cherries, melon, watermelon, kiwis, grapes and peaches) and 11 of vegetables (chards, cabbages, lettuce, tomatoes, carrots, green beans, courgette, pepper, asparagus, onions and garlics), which are commonly consumed in Spain.

Variety of fruit and vegetable intake was quantified as the sum of the total number of unique items consumed, irrespective of quantity, which corresponds to the at least 1–3 per month response category in the FFQ [19,20]. Continuous scores for variety in items consumed per month of fruit (0–10), vegetable (0–11) and both (0–21) were created. Other studies have demonstrated the reproducibility and validity of variety scores for nutritional adequacy in aged populations [21,22].

Finally, to determine the amount of fruit and vegetable consumption, information of the selected items of fruit and vegetable in the FFQ was transformed into grams per day, multiplying serving sizes by its frequency of consumption and dividing the result by the assessed period. After that, a continuous variable for fruit and vegetable quantity was created by summing up the total amount of fruits and vegetables in grams per day. Additionally, continuous variables for fruit and vegetable quantity were created separately by summing the total amount of fruits or vegetables in grams per day independently.

### 2.3. Determination of micronutrient intake and fiber

Using the FFQ, we estimated the dietary intake of fiber and 19 selected micronutrients, including calcium, phosphorus, magnesium, potassium, iron, iodine, selenium, zinc and vitamins B1, B2, B3, B6, B9, B12, A, C, D, E and K, using Spanish food composition database [23,24].

We used the Dietary Reference Intakes (DRI) for American people proposed by the Institute of Medicine to estimate the prevalence of inadequate intake of fiber and micronutrients in our participants [25]. Specifically, we took as reference the Estimated Average Requirement (EAR) value, which represents the daily amount of a nutrient that is estimated to meet the requirement for a specific criterion of adequacy of half of the healthy individuals of a specific age, sex, and life-stage. However, if there was insufficient scientific evidence available to estimate an EAR, the Adequate Intake (AI) value was used. The AI value is believed to cover the needs of all healthy individuals in a group, but insufficient data prevents from predicting with confidence the percentage of individuals who are covered by this intake [26]. In our analyses, we used the EAR cut-off points for most of the selected micronutrients and fiber, except for potassium and vitamin K, for which no estimated EAR is established, and therefore the AI cut-off points were used.

We evaluated the risk of inadequate intake of fiber, two or more, three or more and four or more of the micronutrients considered.

### 2.4. Lifestyle assessment

At baseline, trained dietitians collected information about age, sex, educational level, marital status, living alone and employment status. Besides, anthropometric variables (height, weight and waist circumference) were measured according to the PREDIMED-plus study protocol. Height and weight were determined with stadiometeres and calibrated scales, respectively. BMI was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured twice midway between the last rib and the iliac crest using an anthropometric tape. Abdominal obesity was estimated using the cut-off points established in the PREDIMED-plus study protocol (WC ≥ 88 cm in women and ≥102 in men).

Physical activity (PA) was assessed using the validated REGICOR questionnaire [27], which included questions related to the type of activity, its frequency in number of days and duration in min/day. Intensity of each type of activity was assigned taking into account the compendium of PA [28]: walking fast (5 MET) or slowly (4 MET), climbing stairs (7 MET), working in a garden or an orchard (5 MET), walking on the countryside (6 MET) and doing exercise outdoors, at home or in a gym (11 MET). According to its intensity, activities were categorized in light PA (<4.0 MET), moderate PA (4.00–5.50 MET) and vigorous PA (≥6.00 MET).

Sedentary behaviour was evaluated using the validated Nurse's Health Study questionnaire for sedentary behaviours [29], which contains questions about the average daily time spent watching TV or sitting (at work, leisure time or while travelling) during last year. Trained dietitians making the difference between weekdays and weekends when collecting the information.

Finally, participants reported their average daily sleeping time for weekdays and weekends over the past year, as well as their smoking habit (current smoker, former smoker or non-smoker).

### *2.5. Statistical analysis*

The database used for the statistical analysis was the PREDIMED-plus baseline database generated in June 2020. Participants were categorized into tertiles of fruit and/or vegetable variety. Chi-square and ANOVA tests were used to assess differences between tertiles in the baseline characteristics of the study population, as well as in dietary and lifestyle factors, but in this case an ANOVA adjusted for covariates (ANCOVA) was performed. Data were presented as mean ± SE or median and (IQR) for continuous variables, and numbers and percentage for categorical ones.

Logistic regression models were performed to examine the association between fruit and/or vegetable variety and not meeting the DRIs recommendations (EAR or IA values). We established the cut-off points ≥2, ≥3 and ≥ 4 unmet DRI in accordance with the number of micronutrients unmet. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated in two different models to determine the risk of inadequate intake of fiber, two or more, three or more and four or more micronutrients by tertiles of fruit and vegetable variety. In the first model, results were adjusted for sex (male/female), age (in years) and energy intake (in Kcal/day), while in the second one were additionally adjusted for physical activity (in Mets·min/week), smoking habit (current/former/never), living alone (yes/no), educational level (primary/secondary/tertiary school) and employment status (retired/employed/housekeeper/others), to control for potential confounders.

We also performed a complementary analysis jointly considering quantity and variety (QV) in fruit and vegetable consumption. In our study, the cut-off points for fruit and vegetable QV were population driven. For its determination, we calculated the average amount of fruits and vegetables consumed by our participants (661.72 g/d), as well as the average score for variety (15.14 g/day). A total amount of fruits and vegetables below 661.72 g/day was considered low quantity, while a total amount of fruits and vegetables equal or upper 661.72 g/day was regarded as high quantity. The same approach was used for low and high variety: a variety score below 15.14 was considered low variety in fruit and vegetable consumption, while a variety score equal or upper 15.14 was regarded as high variety in fruit and vegetable consumption. Consequently, participants were divided into four different categories: low QV (quantity<661.92 g/day and variety<15.14), low quantity and high variety (quantity<661.92 and variety≥15.14), high quantity and low variety (quantity≥661.92 g/day and variety<15.14), and high QV (quantity≥661.92 g/day and variety≥15.14). We followed the same approach to calculate fruit QV categories and vegetable QV categories, separately. Logistic regression models, adjusted for the same variables aforementioned, were performed to examine the association between QV categories of fruit and/or vegetable and not meeting the DRIs recommendations. All statistical analyses were conducted using the software Stata 14 (StataCorp, Texas, USA), and the level of significance was set at $P < 0.05$ for bilateral contrast.

## 3. Results

Baseline characteristics of the PREDIMED-plus study participants by tertiles of fruit and vegetable variety are shown in Table 1. Significant differences were observed in sex, age, educational level, employment status, marital status and abdominal obesity ($p < 0.05$), but not for BMI and living alone. Participants in the top tertile were older and more likely to be women. Similar results were found when participants were categorized into tertiles of variety of fruits and vegetables separately (Supplementary Table 1) and tertiles of quantity (grams/day) of fruit and vegetable (Supplementary Table 2). Furthermore, 85.3% of our population met the WHO recommendations for daily fruit and vegetable consumption (at least 400 g per day).

Table 2 shows the usual intake of nutrients, food group consumption and adherence to an energy-restricted MedDiet, by tertiles of fruit and vegetable variety. Individuals in the highest tertile of fruit and vegetable variety reported a significant higher consumption of fruits, vegetables, legumes, fish and nuts; whereas a significant lower consumption of cereals, olive oil, cookies and pastries and alcohol, compared to those in the lowest one. Besides, related to nutrient intake, individuals in the highest tertile of variety had a significant higher intake of dietary fiber, proteins, carbohydrates, vitamins, minerals and polyphenols, while a significant lower intake of total, monounsaturated and saturated fat and trans fatty acids. Individuals in the highest tertile of variety showed also a higher MedDiet adherence. Results remained essentially the same when variety in fruits and vegetables was analyzed independently (Supplementary Table 3) and when participants were categorized into tertiles of fruit and/or vegetable quantity (Supplementary Table 4 and Supplementary Table 5).

Lifestyle variables by tertiles of fruit and vegetable variety were shown in Table 3. Participants in the highest tertile were significantly less likely to be smokers compared to those in the lowest one. Related to PA, participants in the highest tertile spent significantly more time doing light, moderate and vigorous PA and were significantly more likely to meet the WHO exercise recommendations. With regard to sedentary behaviour, participants in the highest tertile of fruit and vegetable variety spent significantly less time watching TV. Results were similar when participants were categorized into tertiles of fruit and vegetable variety separately (Supplementary Table 6) and tertiles of fruit and/or vegetable quantity (Supplementary Table 7 and Supplementary Table 8).

Figure 1 shows the multivariate adjusted OR and 95% CI for the risk of inadequate intake of fiber, two or more, three or more and four or more micronutrients, according to tertiles of fruit and vegetable variety and the joint analysis between categories of fruits and vegetables in terms of quantity and variety. Participants in the top tertile of fruit and vegetable variety were significantly less likely to have an inadequate intake of fiber [0.13 (0.11–0.16)], and two or more [0.17 (0.14–0.21)], three or more [0.15 (0.13–0.18)] and four or more micronutrients [0.11 (0.10–0.14)]. Results remained essentially the same when variety in fruits (Supplementary Fig. 1) and vegetables (Supplementary Fig. 2) was analyzed independently of each other.

Compared to those in the category of low quantity and variety in fruit and vegetable consumption, individuals with high quantity

**Table 1**
Baseline characteristics of the study population according to tertiles of variety of fruit and vegetable consumption (n = 6647).

| | T1 (lowest) (n = 2523) | T2 (n = 2273) | T3 (Highest) (n = 1851) | *p*-value[a] |
|---|---|---|---|---|
| Age, years | 64.54 ± 5.00 | 65.24 ± 4.96 | 65.31 ± 4.66 | 0.004 |
| Women, n (%) | 1008 (39.95) | 1113 (48.97) | 1097 (59.27) | <0.001 |
| BMI, kg/m$^2$ | 32.63 ± 3.40 | 32.48 ± 3.44 | 32.51 ± 3.51 | 0.377 |
| Abdominal obesity, n (%) | 2372 (94.02) | 2116 (93.09) | 1760 (95.08) | 0.028 |
| Educational level, n (%) | | | | <0.001 |
| Primary school | 1135 (44.99) | 1154 (50.77) | 981 (53.00) | |
| Secondary school | 809 (32.07) | 634 (27.89) | 475 (25.66) | |
| Tertiary school[b] | 579 (22.95) | 485 (21.34) | 395 (21.34) | |
| Marital status, n (%) | | | | 0.035 |
| Married | 1913 (76.09) | 1737 (76.72) | 1430 (77.38) | |
| Widowed | 241 (9.59) | 241 (10.64) | 206 (11.15) | |
| Divorced/separated | 210 (8.35) | 167 (7.38) | 140 (7.58) | |
| Others[c] | 150 (5.97) | 119 (5.26) | 72 (3.90) | |
| Living alone, n (%) | 320 (12.71) | 282 (12.42) | 219 (11.83) | 0.682 |
| Employment status, n (%) | | | | <0.001 |
| Retired | 1414 (56.38) | 1285 (57.04) | 1021 (55.34) | |
| Employed | 583 (23.25) | 450 (19.97) | 327 (17.72) | |
| Housekeeper | 283 (11.28) | 351 (15.58) | 348 (18.86) | |
| Others[d] | 228 (9.09) | 167 (7.41) | 149 (8.08) | |

Data are presented as n (%) for categorical variables and mean ± Standard Error for continuous variables.
In the analysis, there were missing data for marital status in 21 participants (0.32%), for employment status in 41 participants (0.62%) and for living alone in 7 participants (0.11%).
[a] P-value for differences between tertiles of fruit and vegetable variety was calculated by Chi-square and ANOVA, as appropriate.
[b] Includes senior technician and university degree.
[c] Includes single and religious.
[d] Includes incapacity, sick leave, students and unemployed.

and variety of fruit and vegetable consumption (upper category) were significantly less likely to have an inadequate intake of fiber [0.05 (0.04–0.06)], two or more [0.08 (0.06–0.10)], three or more [0.08 (0.06–0.09)] and four or more micronutrients [0.06 (0.05–0.07)]. Results were similar when participants were categorized into QV categories of fruit (Supplementary Fig. 1) and vegetable (Supplementary Fig. 2) separately.

Finally, results remained essentially the same when participants were categorized into tertiles of fruit and/or vegetable quantity (Supplementary Fig. 3 and Supplementary Fig. 4).

## 4. Discussion

In the present study, individuals with greater variety in fruit and/or vegetable consumption showed better nutrient adequacy, diet quality and lifestyle. Furthermore, this study confirmed that greater variety in combination with greater quantity in fruit and/or vegetable consumption was more important for nutrient adequacy and diet quality than it was variety and quantity individually.

In line with previous cross-sectional studies [11,19] that have assessed the association between variety in fruits and/or vegetables and nutrient intake, we reported that individuals who had a greater variety in fruit and/or vegetable consumption had a significant higher intake of dietary fiber, carbohydrates, proteins, vitamins, minerals and flavonoids. This is the case of the Foote et al. study showing that fruit variety contributed to the intake of vitamins A and C, whereas no associations were found with regard to vegetable variety [11]. Griep et al. also found in a population based-cohort study of general population that greater variety in fruits and vegetables was associated with higher intakes of vitamins A and C, as well as of flavonoids and dietary fiber [19].

On the other hand, the present study also showed that individuals with higher scores of variety for fruit and/or vegetable consumption exhibited better compliance with the nutritional recommendations (DRIs) for fiber and micronutrients. Specifically, they were less likely to be below the EAR or AI values for fiber and 19 selected micronutrients. To the best of our knowledge, this is the first time that the association between variety in fruit and vegetable consumption and nutrient adequacy has been assessed using as reference the DRIs recommendations (EAR and IA values). In this sense, there is only one previous study that has assessed the prevalence of nutrient intake inadequacy in Europe, using a validated FFQ and the DRIs, but without focusing on any food group. That study reported a prevalence of inadequacy equal or below 10% of the population for iron, vitamin B12 and zinc, between 11 and 20% for vitamin C and beyond 21% for vitamin D, folic acid, iodine, selenium and calcium [11].

Nevertheless, there are some studies that have investigated the association between variety in fruits and/or vegetables and diet quality taking as reference diet quality scores, with their results mirroring ours. For instance, a paediatric cross-sectional study revealed that children who consumed greater variety of fruits and vegetables were more likely to have a better diet quality, measured with HEI-2010 scores (diet quality score and subscores in young children) [13]. Another cross-sectional study conducted in American low income women [11] showed that those who consumed greater variety of fruits and vegetables had better scores of diet quality and more healthful food attitudes (HEI scores and sub scores) [12].

In the present study we also assessed for the first time, nutrient adequacy and diet quality in a joint analysis considering quantity and variety in fruit and/or vegetable consumption together. Our study determined that greater variety combined with higher amounts of fruits and/or vegetables contributed more to improve nutritional adequacy and diet quality than greater variety or quantity separately. Individuals who consumed higher amounts and greater variety of fruits and/or vegetables were less likely to have an inadequate intake of fiber and micronutrients, taking as reference the DRIs recommendations, in contrast with those who had lower quantity and variety.

Finally, as we have shown with the present study, it has been reported that healthy lifestyles are usually positively associated with higher consumption of fruits and vegetables at population level [30]. In this sense, a meta-analysis of 51 cross-sectional studies revealed that cigarette smoking was associated with unhealthy dietary patterns like lower intakes of fiber, vitamins C and

**Table 2**
Food groups consumption, mean energy, nutrients and polyphenols intake, and adherence to energy-restricted Mediterranean diet according to tertiles of variety in fruit and vegetable consumption.

| | T1 (Lowest) (n = 2523) 11.51 ± 2.49 | T2 (n = 2273) 16.04 ± 0.80 | T3 (Highest) (n = 1851) 18.97 ± 0.95 | *p*-value[a] |
|---|---|---|---|---|
| **Food groups, g/day** | | | | |
| Fruits | 286.87 ± 3.83[c,d] | 374.22 ± 3.97[c,e] | 439.41 ± 4.48[d,e] | <0.001 |
| Vegetables | 237.70 ± 2.28[c,d] | 308.16 ± 2.36[c,e] | 383.87 ± 2.67[d,e] | <0.001 |
| Legumes | 18.34 ± 0.22[c,d] | 20.85 ± 0.23[c,e] | 23.73 ± 0.26[d,e] | <0.001 |
| Cereals | 154.23 ± 1.32[d] | 152.64 ± 1.37[e] | 142.00 ± 1.54[d,e] | <0.001 |
| Dairy products | 346.36 ± 3.90 | 343.75 ± 4.05 | 348.82 ± 4.57 | 0.704 |
| Meat | 147.31 ± 1.08 | 147.31 ± 1.12 | 148.61 ± 1.26 | 0.684 |
| Olive oil | 40.14 ± 0.33[d] | 40.52 ± 0.34[e] | 38.73 ± 0.38[d,e] | 0.001 |
| Fish | 92.63 ± 0.93[c,d] | 103.56 ± 0.96[c,e] | 113.63 ± 1.08[d,e] | <0.001 |
| Nuts | 12.42 ± 0.33[c,d] | 15.23 ± 0.34[c,e] | 17.92 ± 0.39[d,e] | <0.001 |
| Cookies and pastries | 30.08 ± 0.55[c,d] | 25.79 ± 0.57[c,e] | 23.70 ± 0.65[d,e] | <0.001 |
| **Total energy, kcal/day**[b] | 2244.78 ± 10.38[c,d] | 2377.10 ± 10.88[c,e] | 2514.88 ± 12.13[d,e] | <0.001 |
| **Alcohol, g/day** | 12.98 ± 0.26[c,d] | 10.45 ± 0.27[c,e] | 9.09 ± 0.31[d,e] | <0.001 |
| **Macronutrient %TE** | | | | |
| Protein | 16.24 ± 0.05[c,d] | 16.83 ± 0.05[c,e] | 17.44 ± 0.06[d,e] | <0.001 |
| Total fat | 40.10 ± 0.13[c,d] | 39.54 ± 0.14[c,e] | 38.90 ± 0.15[d,e] | <0.001 |
| MUFA | 20.73 ± 0.09[d] | 20.55 ± 0.10 | 20.24 ± 0.11[d] | 0.003 |
| PUFA | 6.76 ± 0.04 | 6.85 ± 0.04 | 6.87 ± 0.04 | 0.064 |
| SFA | 10.32 ± 0.04[c,d] | 9.85 ± 0.04[c,e] | 9.56 ± 0.05[d,e] | <0.001 |
| Trans-FA | 0.25 ± 0.01[d] | 0.21 ± 0.01[e] | 0.20 ± 0.01[d,e] | <0.001 |
| Carbohydrate | 40.03 ± 0.14[c,d] | 40.74 ± 0.14[c,e] | 41.07 ± 0.16[d,e] | <0.001 |
| **Protein g/kg/day** | 1.10 ± 0.01[c,d] | 1.16 ± 0.01[c,e] | 1.21 ± 0.01[d,e] | <0.001 |
| **Fiber g/day** | 22.77 ± 0.14[c,d] | 26.43 ± 0.15[c] | 30.34 ± 0.17[d] | <0.001 |
| **Micronutrients** | | | | |
| Vitamin A (μg/d) | 958.61 ± 12.29[c,d] | 1110.26 ± 12.75[c,e] | 1297.91 ± 14.37[c,e] | <0.001 |
| Vitamin B1 (mg/d) | 1.54 ± 0.01[c,d] | 1.64 ± 0.01[c,e] | 1.77 ± 0.01[d,e] | <0.001 |
| Vitamin B2 (mg/d) | 1.91 ± 0.01[c,d] | 2.00 ± 0.01[c,e] | 2.17 ± 0.01[d,e] | <0.001 |
| Vitamin B3 (mg/d) | 38.68 ± 0.15[c,d] | 40.71 ± 0.16[c,e] | 43.43 ± 0.18[d,e] | <0.001 |
| Vitamin B6 (mg/d) | 2.13 ± 0.01[c,d] | 2.35 ± 0.01[c,e] | 2.59 ± 0.01[d,e] | <0.001 |
| Vitamin B9 (μg/d) | 306.96 ± 1.59[c,d] | 355.44 ± 1.65[c,e] | 407.48 ± 1.86[d,e] | <0.001 |
| Vitamin B12 (μg/d) | 9.37 ± 0.08[c,d] | 9.92 ± 0.09[c,e] | 10.72 ± 0.10[d,e] | <0.001 |
| Vitamin C (mg/d) | 165.44 ± 1.50[c,d] | 205.73 ± 1.55[c,e] | 247.98 ± 1.75[d,e] | <0.001 |
| Vitamin D (μg/d) | 5.61 ± 0.07[c,d] | 6.27 ± 0.07[c,e] | 6.88 ± 0.08[d,e] | <0.001 |
| Vitamin E (mg/d) | 10.00 ± 0.07[c,d] | 10.56 ± 0.07[c,e] | 11.48 ± 0.08[d,e] | <0.001 |
| Vitamin K (μg/d) | 258.57 ± 3.55[c,d] | 326.79 ± 3.68[c,e] | 420.50 ± 4.15[d,e] | <0.001 |
| Calcium (mg/d) | 1002.73 ± 5.90[c,d] | 1030.72 ± 6.12[c,e] | 1082.01 ± 6.90[d,e] | <0.001 |
| Phosphorus (mg/d) | 1687.06 ± 5.89[c,d] | 1758.89 ± 6.11[c,e] | 1858.49 ± 6.89[d,e] | <0.001 |
| Magnesium (mg/d) | 392.60 ± 1.50[c,d] | 420.31 ± 1.56[c,e] | 458.43 ± 1.76[d,e] | <0.001 |
| Iron (mg/d) | 15.31 ± 0.05[c,d] | 16.54 ± 0.05[c,e] | 17.94 ± 0.65[d,e] | <0.001 |
| Potassium (mg/d) | 4053.59 ± 15.18[c,d] | 4512.60 ± 15.75[c,e] | 5005.21 ± 17.76[d,e] | <0.001 |
| Iodine (μg/d) | 280.76 ± 3.09 | 285.45 ± 3.20 | 292.17 ± 3.61 | 0.061 |
| Selenium (μg/d) | 113.41 ± 0.48[c,d] | 118.06 ± 0.50[c,e] | 120.78 ± 0.57[d,e] | <0.001 |
| Zinc (mg/d) | 12.84 ± 0.04[c,d] | 13.22 ± 0.04[c,e] | 13.58 ± 0.05[d,e] | <0.001 |
| **Polyphenols** | | | | |
| Total polyphenols (mg/d) | 800.24 ± 5.48[c,d] | 848.60 ± 5.69[c,e] | 903.98 ± 6.41[d,e] | <0.001 |
| Flavonoids (mg/d) | 452.60 ± 4.54[c,d] | 491.40 ± 4.71[c,e] | 542.36 ± 5.31[d,e] | <0.001 |
| Phenolic acids (mg/d) | 273.82 ± 2.55[d] | 282.64 ± 2.65 | 286.35 ± 2.99[d] | 0.004 |
| **MedDiet adherence** | 7.82 ± 0.05[c,d] | 8.70 ± 0.05[c,e] | 9.24 ± 0.06[d,e] | <0.001 |

Data are presented as adjusted means ± Standard Error.
Differences (p-value <0.05) between [c]Tertile 1 vs. Tertile 2, [d]Tertile 1 vs. Tertile 3, [e]Tertile 2 vs. Tertile 3 were indicated.
Abbreviations: MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; T, Tertile; TE, total energy intake; Trans-FA, trans fatty acid.
[a] P-value <0.005 for differences among categories of variety in fruit and vegetable consumption was calculated by ANCOVA with Bonferroni's post-hoc test, and adjusted for sex (male/female), age (in years) and energy intake (kcal/day).
[b] Total energy intake was only adjusted for sex and age.

E and beta-carotene [31]. Furthermore, another meta-analysis of 72 prospective cohort studies by Grosso et al. revealed that increasing 100 g/d of fruits and vegetables was significantly associated with lower prevalence of being smoker and higher prevalence of being physically active [9]. Finally, a cross-sectional study conducted in Brazil showed that lower levels of physical activity were significantly associated with lower consumption of fruits and vegetables in adolescents [32]. The present investigation determined that greater variety in fruit and vegetable consumption was highly associated with an increased prevalence of being physically active and non-smoker. However, no previous studies have investigated this association focusing on fruit and vegetable variety, as they have only considered fruit and vegetable quantity.

Fruits and vegetables are good sources of micronutrients and bioactive compounds, and are relatively low energy density foods [19]. We found that greater variety in fruit and vegetable consumption was associated with higher intakes of vitamins, minerals, dietary fiber and polyphenols. This is in line with a previous study that showed that variety in fruits and vegetables was significantly associated with nutrient adequacy [11]. Variety in fruits and vegetables may enhance higher intakes of total fruits and vegetables, and thereby greater intakes of vitamins and minerals [19]. Besides, variety in fruits and vegetables might be beneficial because it provides dietary fiber and a mix of phytochemicals (e.g. flavonoids), which may act synergistically to promote the normal body function [33]. Increasing dietary fiber and flavonoid intake may reduce the prevalence of several chronic diseases due to its beneficial effects on

**Table 3**
Lifestyle factors according to tertiles of variety in fruit and vegetable consumption.

| | T1 (Lowest) | T2 | T3 (Highest) | *p*-value[a] |
|---|---|---|---|---|
| **Smoking habit, % (n)** | | | | <0.001 |
| Current smoker | 395 (15.74) | 256 (11.31) | 172 (9.32) | |
| Former smoker | 1144 (45.58) | 1008 (44.54) | 724 (39.22) | |
| Never smoker | 971 (38.69) | 999 (44.14) | 950 (51.46) | |
| **Physical activity** | | | | |
| Light PA, min/day | 26.75 ± 0.68 | 26.11 ± 0.71[d] | 29.14 ± 0.79[d] | 0.013 |
| Moderate PA, min/day | 23.44 ± 0.86[b,c] | 29.54 ± 0.90[b] | 29.73 ± 1.00[c] | <0.001 |
| Vigorous PA, min/day | 11.39 ± 0.45[b,c] | 13.43 ± 0.47[b] | 14.14 ± 0.53[c] | <0.001 |
| **WHO Exercise Recommendations % (n)** | | | | 0.001 |
| No | 1873 (74.24) | 1577 (69.38) | 1307 (70.61) | |
| Yes | 650 (25.76) | 696 (30.62) | 544 (29.39) | |
| **Sedentary behavior** | | | | |
| Sedentary time, h/day | 6.07 ± 0.04 | 5.96 ± 0.04 | 6.05 ± 0.05 | 0.160 |
| TV- viewing time, h/day | 3.35 ± 0.03[b,c] | 3.23 ± 0.04[b] | 3.21 ± 0.04[c] | 0.009 |
| **Sleeping (hours/day)** | 7.07 ± 0.02 | 7.05 ± 0.03 | 6.99 ± 0.03 | 0.118 |

Data are presented as adjusted means ± Standard Error and n (%) for continuous and categorical variables, respectively.
In the analysis, there were missing data for smoking habit in 28 participants (0.42%).
Differences (p-value<0.05) between [b]Tertile 1 vs. Tertile 2, [c]Tertile 1 vs. Tertile 3, [d]Tertile 2 vs. Tertile 3 were indicated in continuous variables.
Abbreviations: PA, physical activity; T, tertile; WHO, World Health Organization.
[a] P-value <0.05 for differences among categories of variety in fruit and vegetable consumption was calculated by Chi-square and ANCOVA with Bonferroni's post-hoc test, and adjusted for sex (male/female) and age (in years), as appropriate.

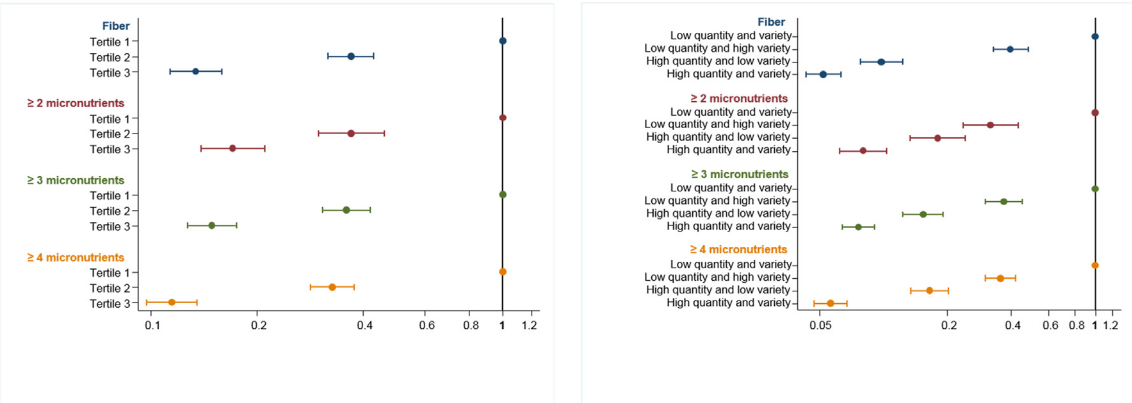


**Fig. 1.** OR and 95% CI for inadequate intake of fiber, two or more, three or more, four or more micronutrients by tertiles of fruit and vegetable variety and QV categories of fruit and vegetable consumption.

health. In this sense, dietary fiber increases bile acid excretion and short chain fatty acids production, decreases caloric intake and has carcinogenic binding effects. Besides, high-fiber foods increases the presence of antioxidants and micronutrients in our body [34]. On the other hand, flavonoids may have anti-inflammatory properties and are also known as potent antioxidants [35]. Plant-based dietary fiber and flavonoids may mediate its beneficial effects on human health particularly through their interaction with gut microbiota [36] Specifically, both may sinergically enhance the growth of beneficial bacteria within the gut, while inhibiting the growth of certain pathogenic bacteria [37]. All of this supports the current recommendation of eating a diet rich in fruits and vegetables [38].

### 4.1. Strengths and limitations

Our study has some strengths. First of all, the present investigation examined the association between fruit and vegetable variety, nutritional adequacy and diet quality from a different approach, such as the use of the DRIs recommendations. Besides, it is the first time that the association between fruit and vegetable consumption, nutrient adequacy and diet quality has been analyzed in a joint analysis considering quantity and variety in fruit and/or vegetable consumption. Another novelty of the study was the assessment of the association between lifestyle, which includes physical activity, sedentary behavior and smoking habit, and fruit and vegetable variety. To conclude, it is important to mention that the assessment of these previous associations in an elderly Mediterranean population at high cardiovascular risk is another added value for the present investigation as it is the first time that these associations have been reported for this population.

However, our study has some limitations. Firstly, it is a cross-sectional study, so causal inferences cannot be drawn, we have only described associations. Secondly, the sample consisted in a Mediterranean population at high cardiovascular risk, and therefore the results cannot be extrapolated to the general population or other populations. Thirdly, the use of a FFQ may difficult the real dietary intake assessment accurately, mainly for vitamins and minerals rather than macronutrients [39].

## 5. Conclusion

In conclusion, the current study demonstrated that greater variety of fruits and/or vegetables was associated with better nutrient adequacy, diet quality and healthier lifestyle in an elderly Mediterranean population at high cardiovascular risk.

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## Author contributions

All the principal PREDIMED-plus investigators contributed to study concept and design and to data extraction from the participants. LL-G, NB-T, NB and JS-S performed the statistical analyses. LL-G, NB-T, NB and JS-S drafted the manuscript. All authors reviewed the manuscript for important intellectual content and approved the final version to be published.

## Data availability

The datasets generated and analyzed during the current study are not expected to be made available outside the core research group, as neither participants' consent forms nor ethics approval included permission for open access. However, the researchers will follow a controlled data-sharing collaboration model, as in the informed consent participants agreed with a controlled collaboration with other investigators for research related to the project's aims. Therefore, investigators who are interested in this study can contact the PREDIMED Steering Committee by sending a request letter to predimed_scommittee@googlegroups.com. A data-sharing agreement indicating the characteristics of the collaboration and data management will be completed for the proposals that are approved by the Steering Committee.

## Ethical standards

All participants provided their written informed consent. The study protocol and procedures were approved in accordance with the ethical standards of the Declaration of Helsinki.

## Potential conflicts of interest

JS-S serves on the board of (and receives grant support through his institution from) the International Nut and Dried Fruit Council and the Eroski Foundation. He also serves on the Executive Committee of the Instituto Danone, Spain, and on the Scientifc Committee of the Danone International Institute. He has received research support from the Patrimonio Comunal Olivarero, Spain, and Borges S.A., Spain. He receives consulting fees or travel expenses from Danone, the Eroski Foundation, the Instituto Danone, Spain, and Abbot Laboratories.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2021.02.024.

## References


[1] World Health Organization (WHO). WHO | Promoting fruit and vegetable consumption around the world. Information sheet. Global Strategy on Diet, Physical Activity & Health; 2015. Available at, https://www.who.int/dietphysicalactivity/fruit/en/#:%7E:text=A%20recently%20published%20WHO%2FFAO,alleviation%20of%20several%20micronutrient%20deficiencies%2C. [Accessed 20 July 2020].

[2] Wang X, Ouyang Y, Liu J, Zhu M, Zhao G, Bao W, et al. Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. BMJ 2014;349(July):1–14.

[3] Micha R, Khatibzadeh S, Shi P, Andrews KG, Engell RE, Mozaffarian D. Global, regional and national consumption of major food groups in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys worldwide. BMJ Open 2015;5(9):e008705.

[4] Afshin A, Sur PJ, Fay KA, Cornaby L, Ferrara G, Salama JS, et al. Health effects of dietary risks in 195 countries, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2019;393(10184):1958–72.

[5] Slavin JU of M, Lloyd Beate PNGR. Health benefits of fruits and vegetables. Adv Nutr 2012;3:506–16.
[6] Eichholzer M, Lüthy J, Gutzwiller F, Stähelin HB. The role of folate, antioxidant vitamins and other constituents in fruit and vegetables in the prevention of cardiovascular disease: the epidemiological evidence. Int J Vitam Nutr Res 2001;71:5–17.
[7] Angelino D, Godos J, Ghelfi F, Tieri M, Titta L, Lafranconi A, et al. Fruit and vegetable consumption and health outcomes : an umbrella review of observational studies. Int J Food Sci Nutr 2019:1–16. https://doi.org/10.1080/09637486.2019.1571021.
[8] Fulton SL, McKinley MC, Young IS, Cardwell CR, Woodside JV. The effect of increasing fruit and vegetable consumption on overall diet: a systematic review and meta-analysis. Crit Rev Food Sci Nutr 2016;56(5):802–16.
[9] Grosso G, Micek A, Godos J, Pajak A, Sciacca S, Galvano F, et al. Health risk factors associated with meat, fruit and vegetable consumption in cohort studies: a comprehensive meta-analysis. PloS One 2017;12(8):1–21.
[10] Mirmiran P, Bahadoran Z, Moslehi N, Bastan S, Azizi F. Colors of fruits and vegetables and 3-year changes of cardiometabolic risk factors in adults: tehran lipid and glucose study. Eur J Clin Nutr 2015;69(11):1215–9.
[11] Foote JA, Murphy SP, Wilkens LR, Basiotis PP, Carlson A. Dietary variety increases the probability of nutrient adequacy among adults. J Nutr 2004;134(7):1779–85.
[12] Keim NL, Forester SM, Lyly M, Aaron GJ, Townsend MS. Vegetable variety is a key to improved diet quality in low-income women in California. J Acad Nutr Diet 2014;114(3):430–5.
[13] Ramsay SA, Shriver LH, Taylor CA. Variety of fruit and vegetables is related to preschoolers' overall diet quality. Prev Med Reports 2017;5:112–7. https://doi.org/10.1016/j.pmedr.2016.12.003.
[14] Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; National heart, lung, and blood institute; American heart association; World heart federation. International. Circulation 2009;120(16):1640–5.
[15] Martínez-González MA, Buil-Cosiales P, Corella D, Bulló M, Fitó M, Vioque J, et al. Cohort profile: design and methods of the PREDIMED-Plus randomized trial. Int J Epidemiol 2019;48(2):387–8.
[16] Salas-Salvadó J, Díaz-López A, Ruiz-Canela M, Basora J, Fitó M, Corella D, et al. Effect of a lifestyle intervention program with energy-restricted Mediterranean diet and exercise on weight loss and cardiovascular risk factors: one-year results of the PREDIMED-Plus trial. Diabetes Care 2019;42(5):777–88.
[17] Forman M. Nutritional epidemiology. Am J Clin Nutr 1999 May 1;69(5):1020.
[18] Fernández-Ballart JD, Piñol JL, Zazpe I, Corella D, Carrasco P, Toledo E, et al. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br J Nutr 2010;103(12):1808–16.
[19] Oude Griep LM, Verschuren WMM, Kromhout D, Ocké MC, Geleijnse JM. Variety in fruit and vegetable consumption and 10-year incidence of CHD and stroke. Publ Health Nutr 2012;15(12):2280–6.
[20] Conklin AI, Forouhi NG, Suhrcke M, Surtees P, Wareham NJ, Monsivais P. Variety more than quantity of fruit and vegetable intake varies by socioeconomic status and financial hardship. Findings from older adults in the EPIC cohort. Appetite 2014;83:248–55.
[21] Drewnowski A, Renderson SA, Driscoll A, Rolls BJ. The dietary variety score: assessing diet quality in healthy young and older adults. J Am Diet Assoc 1997;97(3):266–71.
[22] Bernstein MA, Tucker KL, Ryan ND, ONeill EF, Clements KM, Nelson ME, et al. Higher dietary variety is associated with better nutritional status in frail elderly people. J Am Diet Assoc 2002;102(8):1096–104.
[23] Moreiras O, Carbajal A, Cabrera LCC. Tablas de composición de alimentos. 16o Edició. Ediciones Pirámide. Madrid, Spain. Madrid, Spain: Pirámide; 2013.
[24] Mataix J, García L, Mañas M, Martínez E. LlJ. Tabla de composition de alimentos. 4º Edición. Universidad de Granada. 2009.
[25] Institute of Medicine (US) Subcommittee on Interpretation and Uses of Dietary Reference Intakes, Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. DRI Dietary Reference Intakes: Applications in Dietary Assessment. Washington (DC): National Academies Press (US); 2000.
[26] Institute of Medicine of The National Academies [Internet]. Dietary reference intakes ( DRIs ): estimated average requirements food and nutrition board. 2011. Available from, http://nationalacademies.org/hmd/~/media/Files/Report Files/2019/DRI-Tables-2019/1_EARV.pdf?la=en%0Ahttp://iom.national academies.org/~/media/Files/Activity Files/Nutrition/DRIs/1_ EARs.pdf.
[27] Molina L, Sarmiento M, Peñafiel J, Donaire D, Garcia-Aymerich J, Gomez M, et al. Validation of the regicor short physical activity questionnaire for the adult population. PloS One 2017;12(1):1–14.
[28] Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR, Tudor-Locke C, et al. 2011 compendium of physical activities: a second update of codes and MET values. Med Sci Sports Exerc 2011;43(8):1575–81.
[29] Martínez-González MA, López-Fontana C, Varo JJ, Sánchez-Villegas A, Martinez JA. Validation of the Spanish version of the physical activity questionnaire used in the nurses' health study and the health professionals' follow-up study. Publ Health Nutr 2005;8(7):920–7.
[30] Johansson L, Andersen LF. Who eats 5 a day?: intake of fruits and vegetables among Norwegians in relation to gender and lifestyle. J Am Diet Assoc 1998;98(6):689–91.
[31] Popkin BM, Udry JR. Community and international nutrition. J Nutr 1998;128(January):701–6.
[32] Silva DAS, Silva RJ, dos S. Association between physical activity level and consumption of fruit and vegetables among adolescents in northeast Brazil. Rev Paul Pediatr 2015;33(2):167–73. English.
[33] Randall E, Nichaman MZ, Contant CFJ. Diet diversity and nutrient intake. J Am Diet Assoc 1985 Jul;85(7):830–6.
[34] Lattimer JM, Haub MD. Effects of dietary fiber and its components on metabolic health. 2010. p. 1266–89.
[35] Maleki SJ, Crespo JF, Cabanillas B. Anti-inflammatory effects of flavonoids. Food Chem 2019;(July):125124. https://doi.org/10.1016/j.foodchem.2019.125124.
[36] Klinder A, Shen Q, Heppel S, Lovegrove JA. Impact of increasing fruit and vegetables and flavonoid intake on the hum gut microbiota. 2016.
[37] Wan MLY, Ling KH, El-Nezami H, Wang MF. Influence of functional food components on gut health. Crit Rev Food Sci Nutr 2019;59(12):1927–36. https://doi.org/10.1080/10408398.2018.1433629.
[38] Jin J. Dietary guidelines for Americans. JAMA, J Am Med Assoc 2016;315(5):528.
[39] Jain M, Howe GR, Rohan T. Dietary assessment in Epidemiology. Comparison of a Food Frequency and a Diet History Questionnaire with a 7-Day Food Record 1996;143(9):953–60.

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**Supplementary Table 1. Baseline characteristics of the study population according to tertiles of variety of fruits and vegetables (n= 6647).**

| | Variety in fruit consumption | | | | Variety in vegetable consumption | | | |
|---|---|---|---|---|---|---|---|---|
| | T1 (lowest) (n=2361) | T2 (n=2761) | T3 (Highest) (n=1525) | *p*-value[a] | T1 (lowest) (n=3128) | T2 (n=2314) | T3 (Highest) (n=1205) | *p*-value[a] |
| **Age, years** | 64.69±4.92 | 65.17±4.90 | 65.13±4.87 | <0.001 | 64.71±5.05 | 65.23±4.82 | 65.28±4.64 | 0.11 |
| **Women, n (%)** | 1003 (42.48) | 1397 (50.60) | 818 (53.64) | <0.001 | 1309 (41.85) | 1196 (51.69) | 713 (59.17) | <0.001 |
| **BMI, kg/m$^2$** | 32.65±3.40 | 32.47±3.40 | 32.51±3.60 | 0.150 | 32.61±3.44 | 32.42±3.43 | 32.62±3.49 | 0.747 |
| **Abdominal obesity, n (%)** | 2216 (93.86) | 2607 (94.42) | 1425 (93.44) | 0.407 | 2926 (93.54) | 2176 (94.04) | 1146 (95.10) | 0.152 |
| **Educational level, n (%)** | | | | <0.001 | | | | 0.006 |
| Primary school | 1058 (44.81) | 1446 (52.37) | 766 (50.23) | | 1473 (47.09) | 1159 (50.09) | 638 (52.95) | |
| Secondary school | 756 (32.02) | 750 (27.16) | 412 (27.02) | | 955 (30.53) | 652 (28.18) | 311 (25.81) | |
| Tertiary school[b] | 547 (23.17) | 565 (20.46) | 347 (22.75) | | 700 (22.38) | 503 (21.74) | 256 (21.24) | |
| **Marital status, n (%)** | | | | <0.001 | | | | 0.188 |
| Married | 1782 (75.70) | 2145 (78.03) | 1153 (75.71) | | 2367 (75.96) | 1780 (77.19) | 933 (77.49) | |
| Widowed | 214 (9.09) | 292 (10.62) | 182 (11.95) | | 320 (10.27) | 249 (10.80) | 119 (9.88) | |
| Divorced/separated | 211 (8.96) | 180 (6.55) | 126 (8.27) | | 244 (7.83) | 179 (7.76) | 94 (7.81) | |
| Others[c] | 147 (6.24) | 132 (4.80) | 62 (4.07) | | 185 (5.94) | 98 (4.25) | 58 (4.82) | |
| **Living alone, n (%)** | 306 (12.99) | 323 (11.70) | 192 (12.60) | 0.361 | 406 (13.01) | 280 (12.10) | 135 (11.20) | 0.241 |
| **Employment status, n (%)** | | | | <0.001 | | | | <0.001 |
| Retired | 1346 (57.45) | 1538 (55.97) | 836 (55.18) | | 1749 (56.27) | 1304 (56.82) | 667 (55.44) | |
| Employed | 504 (21.51) | 549 (19.98) | 307 (20.26) | | 719 (23.13) | 419 (18.26) | 222 (18.45) | |
| Housekeeper | 280 (11.95) | 453 (16.48) | 249 (16.44) | | 376 (12.10) | 385 (16.78) | 221 (18.37) | |
| Others[d] | 213 (9.09) | 208 (7.57) | 123 (8.12) | | 264 (8.49) | 187 (8.15) | 93 (7.73) | |

Data are presented as n (%) for categorical variables and mean ± Standard Error for continuous variables.
In the analysis, there were missing data for marital status in 21 participants (0. 32%), for employment status in 41 participants (0.62%) and for living alone in 7 participants (0.11%).
[a] P-value for differences between tertiles of fruit variety and vegetable variety was calculated by Chi-square and ANOVA, as appropriate.
[b] Includes senior technician and university degree
[c] Includes single and religious.
[d] Includes incapacity, sick leave, students and unemployed

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**Supplementary Table 2. Baseline characteristics of the study population according to tertiles of quantity of fruits and vegetables (n= 6647).**

| | T1 (lowest) (n=2216) | T2 (n=2216) | T3 (Highest) (n=2215) | P-value[a] |
|---|---|---|---|---|
| **Age, years** | 64±5 | 65±5 | 66±5 | <0.001 |
| **Women, n (%)** | 863 (38.94) | 1115 (50.32) | 1240 (55.98) | <0.001 |
| **BMI, kg/m$^2$** | 32.60±3.43 | 32.57±3.45 | 32.47±3.46 | 0.416 |
| **Abdominal obesity, n (%)** | 2061 (93.01) | 2078 (93.77) | 2109 (95.21) | 0.007 |
| **Educational level, n (%)** | | | | <0.001 |
| Primary school | 983 (44.36) | 1108 (50.00) | 1179 (53.23) | |
| Secondary school | 702 (31.68) | 643 (29.02) | 573 (25.87) | |
| Tertiary school[b] | 531 (23.96) | 465 (20.98) | 463 (20.90) | |
| **Marital status, n (%)** | | | | 0.357 |
| Married | 1681 (76.20) | 1723 (78.03) | 1676 (75.77) | |
| Widowed | 228 (10.34) | 212 (9.60) | 248 (11.21) | |
| Divorced/separated | 184 (8.34) | 155 (7.02) | 178 (8.05) | |
| Others[c] | 113 (5.12) | 118 (5.34) | 110 (4.97) | |
| **Living alone, n (%)** | 271 (12.25) | 258 (11.65) | 292 (13.19) | 0.294 |
| **Employment status, n (%)** | | | | <0.001 |
| Retired | 1181 (53.66) | 1270 (57.73) | 1269 (57.55) | |
| Employed | 556 (25.26) | 434 (19.73) | 370 (16.78) | |
| Housekeeper | 247 (11.22) | 331 (15.05) | 404 (18.32) | |
| Others[d] | 217 (9.86) | 165 (7.50) | 162 (7.35) | |

Data are presented as n (%) for categorical variables and mean ± Standard Error for continuous variables.
In the analysis, there were missing data for marital status in 21 participants (0. 32%), for employment status in 41 participants (0.62%) and for living alone in 7 participants (0.11%).
[a] P-value for differences between categories of fruit and vegetable consumption was calculated by Chi-square and ANOVA, as appropriate.
[b] Includes senior technician and university degree
[c] Includes single and religious.
[d] Includes incapacity, sick leave, students and unemployed

SUPPLEMENTARY ONLINE MATERIAL 1

**Supplementary Table 3. Food groups consumption, mean energy, nutrients and polyphenols intake, and adherence to energy-restricted Mediterranean diet according to tertiles of fruit variety and vegetable variety**

| | Variety in fruit consumption | | | | Variety in vegetable consumption | | | |
|---|---|---|---|---|---|---|---|---|
| | T1 (Lowest) 5.23±1.91 | T2 8.56±0.50 | T3 (Highest) 10.00±0.00 | *p*-value[a] | T1 (Lowest) 5.55±1.45 | T2 8.48±0.50 | T3 (Highest) 10.29±0.45 | *p*-value[a] |
| **Food groups, g/day** | | | | | | | | |
| Fruits | 286.68±3.96[c,d] | 379.72±3.63[c,e] | 434.43±4.94[d,e] | <0.001 | 323.04±3.52[c,d] | 376.17±4.07[c,e] | 420.62±5.68[d,e] | <0.001 |
| Vegetables | 279.42±2.60[c,d] | 307.77±2.38[c,e] | 328.67±3.24[d,e] | <0.001 | 237.23±1.95[c,d] | 334.69±2.25[c,e] | 410.10±3.13[d,e] | <0.001 |
| Legumes | 19.31±0.23[c,d] | 21.13±0.21[c,e] | 22.10±0.28[d,e] | <0.001 | 18.67±0.20[c,d] | 21.51±0.23[c,e] | 24.43±0.31[d,e] | <0.001 |
| Cereals | 155.76±1.36[c,d] | 149.66±1.24[c,e] | 142.89±1.69[d,e] | <0.001 | 153.46±1.18[d] | 150.40±1.36[e] | 141.79±1.90[d,e] | <0.001 |
| Dairy products | 349.89±4.01[d] | 349.74±3.67[e] | 333.86±5.00[d,e] | 0.020 | 339.90±3.48[c] | 352.95±4.02[c] | 349.32±5.61 | 0.043 |
| Meat | 149.53±1.11[d] | 148.13±1.01[e] | 143.99±1.38[d,e] | 0.007 | 144.86±0.96[c,d] | 150.43±1.11[c] | 149.69±1.54[d] | <0.001 |
| Olive oil | 39.74±0.34 | 40.28±0.31 | 39.36±0.42 | 0.177 | 40.35±0.29 | 39.65±0.34 | 39.09±0.47 | 0.054 |
| Fish | 97.46±0.97[c,d] | 104.05±0.88[c] | 106.26±1.20[d] | <0.001 | 93.35±0.82[c,d] | 105.94±0.95[c,e] | 118.09±1.33[d,e] | <0.001 |
| Nuts | 12.84±0.34[c,d] | 15.33±0.31[c,e] | 17.38±0.43[d,e] | <0.001 | 13.30±0.30[c,d] | 15.43±0.34[c,e] | 18.10±0.48[d,e] | <0.001 |
| Cookies and pastries | 28.80±0.57[c,d] | 25.82±0.52[c] | 25.62±0.71[d] | <0.001 | 29.42±0.49[c,d] | 25.55±0.57[c,e] | 22.57±0.79[d,e] | <0.001 |
| **Total energy[b], kcal/day** | 2249.62±10.71[c,d] | 2373.72±9.89[c,e] | 2528.89±13.31[d,e] | <0.001 | 2291.30±9.41[c,d] | 2401.63±10.91[c,e] | 2487.31±15.16[d,e] | <0.001 |
| **Alcohol, g/day** | 13.06±0.27[c,d] | 10.39±0.25[c,e] | 9.03±0.34[d,e] | <0.001 | 12.03±0.24[c,d] | 10.60±0.27[c,e] | 9.24±0.38[d,e] | <0.001 |
| **Macronutrient %TE** | | | | | | | | |
| Protein | 16.64±0.53[c] | 16.87±0.05[c] | 16.83±0.07 | 0.004 | 16.17±0.04[c,d] | 17.11±0.05[c,e] | 17.71±0.07[d,e] | <0.001 |
| Total fat | 39.67±0.14 | 39.56±0.12 | 39.45±0.17 | 0.610 | 40.04±0.12[c,d] | 39.20±0.14[c] | 39.07±0.19[d] | <0.001 |
| MUFA | 20.45±0.10 | 20.58±0.09 | 20.58±0.12 | 0.546 | 20.75±0.08[c,d] | 20.35±0.10[c] | 20.33±0.13[d] | 0.002 |
| PUFA | 6.74±0.04[d] | 6.85±0.04 | 6.92±0.05[d] | 0.012 | 6.79±0.03 | 6.83±0.04 | 9.91±0.05 | 0.167 |
| SFA | 10.17±0.04[c,d] | 9.85±0.04[c] | 9.78±0.05[d] | <0.001 | 10.22±0.04[c,d] | 9.78±0.04[c,e] | 9.57±0.06[d,e] | <0.001 |
| Trans-FA | 0.24±0.01[c,d] | 0.21±0.01[c] | 0.21±0.01[d] | <0.001 | 0.24±0.01[c,d] | 0.21±0.01[c,e] | 0.20±0.01[d,e] | <0.001 |
| Carbohydrates | 40.04±0.14[c,d] | 40.68±0.13[c] | 41.16±0.18[d] | <0.001 | 40.42±0.12 | 40.75±0.14 | 40.59±0.20 | 0.216 |
| **Protein g/kg/d** | 1.13±0.01[c,d] | 1.16±0.01[c] | 1.17±0.01[d] | <0.001 | 1.10±0.01[c,d] | 1.18±0.0[c,e] | 1.22±0.01[d,e] | <0.001 |
| **Fiber g/day** | 23.98±0.16[c,d] | 26.62±0.14[c,e] | 28.56±0.19[d,e] | <0.001 | 23.40±0.13[c,d] | 27.38±0.15[c,e] | 30.82±0.21[d,e] | <0.001 |
| **Micronutrients** | | | | | | | | |
| Vitamin A (µg/d) | 1028.13±12.87[c,d] | 1124.69±11.78[c,e] | 1188.16±16.04[d,e] | <0.001 | 963.27±10.91[c,d] | 1172.07±12.59[c,e] | 1343.86±17.58[d,e] | <0.001 |
| Vitamin B1 (mg/d) | 1.58±0.01[c,d] | 1.66±0.01[c,e] | 1,69±0.01[d,e] | <0.001 | 1.55±0.01[c,d] | 1.68±0.01[c,e] | 1.79±0.01[d,e] | <0.001 |
| Vitamin B2 (mg/d) | 1.96±0.01[c,d] | 2.03±0.01[c] | 2.05±0.01[d] | <0.001 | 1.90±0.01[c,d] | 2.05±0.01[c,e] | 2.22±0.02[d,e] | <0.001 |
| Vitamin B3 (mg/d) | 39.76±0.16[c,d] | 41.06±0.15[c] | 41.50±0.20[d] | <0.001 | 38.64±0.13[c,d] | 41.59±0.15[c,e] | 44.34±0.22[d,e] | <0.001 |
| Vitamin B6 (mg/d) | 2.20±0.01[c,d] | 2.37±0.01[c,e] | 2.47±0.01[d,e] | <0.001 | 2.16±0.01[c,d] | 2.41±0.01[c,e] | 2.63±0.01[d,e] | <0.001 |
| Vitamin B9 (µg/d) | | | | | 310.83±1.39[c,d] | 369.31±1.61[c,e] | 423.01±2.25[d,e] | <0.001 |
| Vitamin B12 (µg/d) | 9.61±0.09[c,d] | 10.09±0.08[c] | 10.17±0.11[d] | <0.001 | 9.39±0.08[c,d] | 10.19±0.09[c,e] | 10.88±0.12[d,e] | <0.001 |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Vitamin C (mg/d) | 178.96±1.63[c,d] | 207.15±1.49[c,e] | 229.23±2.03[d,e] | <0.001 | 172.56±1.35[c,d] | 214.88±1.55[c,e] | 254.79±2.17[d,e] | <0.001 |
| Vitamin D (µg/d) | 5.81±0.07[c,d] | 6.31±0.06[c] | 6.56±0.09[d] | <0.001 | 5.74±0.06[c,d] | 6.38±0.07[c,e] | 6.99±0.10[d,e] | <0.001 |
| Vitamin E (mg/d) | 10.17±0.07[c,d] | 10.67±0.06[c,e] | 11.15±0.08[d,e] | <0.001 | 10.08±0.06[c,d] | 10.79±0.07[c,e] | 11.61±0.09[d,e] | <0.001 |
| Vitamin K (µg/d) | 304.53±3.86[c,d] | 331.59±3.53[c,e] | 353.43±4.81[d,e] | <0.001 | 247.45±3.03[c,d] | 364.90±3.50[c,e] | 460.66±4.88[d,e] | <0.001 |
| Calcium (mg/d) | 1019.50±6.10[c,d] | 1042.01±5.56[c] | 1043.60±7.59[d] | 0.010 | 1000.15±5.25[c,d] | 1048±0.8±6.06[c,e] | 1096.92±8.46[d,e] | <0.001 |
| Phosphorus (mg/d) | 1730.00±6.20[c,d] | 1769.3±5.67[c] | 1786.84±7.73[d] | <0.001 | 1684.9±5.22[c,d] | 1794.95±6.02[c,e] | 1884.31±8.41[d,e] | <0.001 |
| Magnesium (mg/d) | 402.71±1.61[c,d] | 425.09±1.47[c,e] | 439.34±2.01[d,e] | <0.001 | 395.69±1.34[c,d] | 430.51±2.16[c,e] | 465.51±2.16[d,e] | <0.001 |
| Iron (mg/d) | 15.92±0.05[c,d] | 16.59±0.05[c,e] | 17.07±0.07[d,e] | <0.001 | 15.38±0.04[c,d] | 16.95±0.05[c,e] | 18.35±0.07[d,e] | <0.001 |
| Potassium (mg/d) | 4230.51±16.96[c,d] | 4547.58±15.52[c,e] | 4724.74±21.13[d,e] | <0.001 | 4105.44±13.50[c,d] | 4651.47±15.58[c,e] | 5098.73±21.75[d,e] | <0.001 |
| Iodine (µg/d) | 287.09±3.17 | 289.09±2.90[e] | 276.71±3.95[e] | 0.035 | 274.13±2.75[c,d] | 294.53±3.17[c] | 297.90±4.42[d] | <0.001 |
| Selenium (µg/d) | 116.11±0.50[c] | 117.80±0.46[c] | 117.15±0.62 | 0.045 | 113.39±0.43[c,d] | 119.08±0.50[c,e] | 122.68±0.69[d,e] | <0.001 |
| Zinc (mg/d) | 13.10±0.04 | 13.21±0.04 | 13.21±0.06 | 0.084 | 12.83±0.04[c,d] | 12.36±0.04[c,e] | 13.73±0.06[d,e] | <0.001 |
| **Polyphenols** | | | | | | | | |
| Total polyphenols (mg/d) | 811.39±5.68[c,d] | 858.41±5.19[c] | 875.65±7.07[d] | <0.001 | 809.98±4.90[c,d] | 860.06±5.65[c,e] | 910.64±7.89[d,e] | <0.001 |
| Flavonoids (mg/d) | 460.99±4.70[c,d] | 497.54±4.30[c,e] | 525.04±5.85[d,e] | <0.001 | 463.86±4.06[c,d] | 501.43±4.69[c,e] | 540.69±6.55[d,e] | <0.001 |
| Phenolic acids (mg/d) | 277.91±2.63 | 285.39±2.40[e] | 274.89±3.27[e] | 0.017 | 271.38±2.27[c,d] | 285.65±2.63[c] | 293.32±3.66[d] | <0.001 |
| **MedDiet adherence** [f] | 8.11±0.05[c,d] | 8.70±0.05[c] | 8.82±0.07[d] | <0.001 | 7.98±0.05[c,d] | 8.83±0.05[c,e] | 9.30±0.07[d,e] | <0.001 |

Data are presented as adjusted means ± Standard Error.
[a]P-value <0.05 for differences among tertiles of fruit variety and vegetable variety was calculated by ANCOVA with Bonferroni´s post-hoc test, and adjusted for sex (male/female), age (in years) and energy intake(kcal/day).
[b]Total energy intake was only adjusted for sex and age.
Differences (p-value <0.05) between [c]Tertile 1 vs. Tertile 2, [d]Tertile 1 vs. Tertile 3, [e]Tertile 2 vs. Tertile 3 were indicated.
[f]MedDiet adherence: adherence to Mediterranean using the 17 item questionnaire adherence with scale 0-17 points.
Abbreviations: MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; T, tertile; TE, total energy intake; Trans-FA, trans fatty acid.

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**Supplementary Table 4. Food groups consumption, mean energy, nutrients and polyphenols intake, and adherence to energy-restricted Mediterranean diet according to tertiles of quantity in fruit and vegetable consumption**

| | Quantity in fruit and vegetable consumption | | | |
|---|---|---|---|---|
| | T1 (Lowest) (n=2216) 394.97±98.72 | T2 (n=2216) 629.99±60.32 | T3 (Highest) (n=2215) 960.34±219.11 | P-value[a] |
| **Food groups, g/day** | | | | |
| Fruits | 191.68±3.02[c,d] | 334.25±2.96[c,e] | 551.83±3.03[d,e] | <0.001 |
| Vegetables | 210.08±2.21[c,d] | 296.23±2.16[c,e] | 401.23±2.21[d,e] | <0.001 |
| Legumes | 18.31±0.24[c,d] | 20.63±0.23[c,e] | 23.18±0.24[d,e] | <0.001 |
| Cereals | 158.29±1.41[c,d] | 152.35±1.38[c,e] | 140.18±1.42[d,e] | <0.001 |
| Dairy products | 342.38±4.19 | 346.83±4.10 | 349.24±4.20 | 0.516 |
| Meat | 150.79±1.15[d] | 147.67±1.13 | 144.56±1.15[d] | <0.001 |
| Olive oil | 40.72±0.35[d] | 40.97±0.34[e] | 37.94±0.35[d,e] | <0.001 |
| Fish | 92.12±1.00[c,d] | 102.85±0.97[c,e] | 111.69±1.00[d,e] | <0.001 |
| Nuts | 12.33±0.36[c,d] | 15.03±0.35[c,e] | 17.39±0.36[d,e] | <0.001 |
| Cookies and pastries | 30.24±0.59[c,d] | 26.28±0.58[c,e] | 23.98±0.59[d,e] | <0.001 |
| **Total energy, kcal/day[b]** | 2216.47±11.00[c,d] | 2343.16±10.91[c,e] | 2536.18±10.97[d,e] | <0.001 |
| **Alcohol, g/day** | 13.71±0.28[c,d] | 10.57±0.28[c,e] | 8.81±0.28[d,e] | <0.001 |
| **Macronutrient %TE** | | | | |
| Protein | 16.26±0.05[c,d] | 16.78±0.05[c,e] | 17.28±0.05[d,e] | <0.001 |
| Total fat | 40.87±0.14[c,d] | 39.98±0.14[c,e] | 37.86±0.14[d,e] | <0.001 |
| MUFA | 21.13±0.10[d] | 20.85±0.10[e] | 19.62±0.10[d,e] | <0.001 |
| PUFA | 6.84±0.04 | 6.90±0.04[e] | 6.73±0.04[e] | 0.011 |
| SFA | 10.57±0.04[c,d] | 9.96±0.04[c,e] | 9.31±0.04[d,e] | <0.001 |
| Trans-FA | 0.25±0.01[c,d] | 0.22±0.01[c,e] | 0.19±0.01[d,e] | <0.001 |
| Carbohydrate | 39.01±0.14[c,d] | 40.32±0.14[c,e] | 42.35±0.14[d,e] | <0.001 |
| **Protein g/kg/day** | 1.11±0.01[c,d] | 1.15±0.01[c,e] | 1.20±0.01[d,e] | <0.001 |
| **Fiber g/day** | 20.81±0.14[c, d] | 25.68±0.13[c, e] | 31.91±0.14[d, e] | <0.001 |
| **Micronutrients** | | | | |
| Vitamin A (µg/d) | 923.88±13.11[c,d] | 1089.65±12.83[c,e] | 1301.42±13.13[d,e] | <0.001 |
| Vitamin B1 (mg/d) | 1.49±0.01[c,d] | 1.62±0.01[c,e] | 1.80±0.01 [d,e] | <0.001 |
| Vitamin B2 (mg/d) | 1.88±0.01[c,d] | 1.99±0.01[c,e] | 2.17±0.01[d,e] | <0.001 |
| Vitamin B3 (mg/d) | 38.46±0.16[c,d] | 40.50±0.16[c,e] | 43.14±0.16[d,e] | <0.001 |
| Vitamin B6 (mg/d) | 2.04±0.01[c,d] | 2.31±0.01[c,e] | 2.65±0.01[d,e] | <0.001 |
| Vitamin B9 (µg/d) | 285.27±1.49[c,d] | 344.30±1.46[c,e] | 425.04±1.49[d,e] | <0.001 |

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| | | | | |
|---|---|---|---|---|
| Vitamin B12 (µg/d) | 9.56±0.09[c,d] | 9.91±0.09[c,e] | 10.33±0.09[d,e] | <0.001 |
| Vitamin C (mg/d) | 136.23±1.29[c,d] | 194.84±1.26[c,e] | 275.56±1.29[d,e] | <0.001 |
| Vitamin D (µg/d) | 5.56±0.07[c,d] | 6.18±0.07[c,e] | 6.83±0.07[d,e] | <0.001 |
| Vitamin E (mg/d) | 9.75±0.07[c,d] | 10.63±0.07[c,e] | 11.42±0.07[d,e] | <0.001 |
| Vitamin K (µg/d) | 242.68±3.77[c,d] | 323.29±3.69[,e] | 415.03±3.78[d,e] | <0.001 |
| Calcium (mg/d) | 977.69±6.28[c,d] | 1026.59±6.15[c,e] | 1098.88±6.29[d,e] | <0.001 |
| Phosphorus (mg/d) | 1669.39±6.29[c,d] | 1751.54±6.16[c,e] | 1857.21±6.30[d,e] | <0.001 |
| Magnesium (mg/d) | 380.62±1.55[c,d] | 416.15±1.52[c,e] | 464.48±1.55[d,e] | <0.001 |
| Iron (mg/d) | 15.06±0.05[c,d] | 16.29±0.05[c,e] | 18.04±0.05[d,e] | <0.001 |
| Potassium (mg/d) | 3819.65±13.87[c,d] | 4409.74±13.58[c,e] | 5197.74±13.89[d,e] | <0.001 |
| Iodine (µg/d) | 278.27±3.31[d] | 283.98±3.24 | 294.38±3.32[d] | 0.003 |
| Selenium (µg/d) | 114.24±0.52[c,d] | 117.45±0.51[c,e] | 119.47±0.52[d,e] | <0.001 |
| Zinc (mg/d) | 12.79±0.05[c,d] | 13.16±0.05[c,e] | 13.58±0.05[d,e] | <0.001 |
| **Polyphenols** | | | | |
| Total polyphenols (mg/d) | 746.48±5.63[c,d] | 820.24±5.51[c,e] | 970.32±5.63[d,e] | <0.001 |
| Flavonoids (mg/d) | 404.94±4.63[c,d] | 466.81±4.53[c,e] | 600.91±4.63[d,e] | <0.001 |
| Phenolic acids (mg/d) | 267.67±2.73[c,d] | 279.08±2.68[c,e] | 294.23±2.74[d,e] | <0.001 |
| **MedDiet adherence** [f] | 7.60±0.05[c,d] | 8.59±0.05[c,e] | 9.35±0.06[d,e] | <0.001 |

Data are presented as adjusted means ± Standard Error.
[a]P-value <0.005 for differences among categories of quantity in fruit and vegetable consumption was calculated by ANCOVA with Bonferroni's post-hoc test, and adjusted for sex (male/female), age (in years) and energy intake (kcal/day).
[b]Total energy intake was only adjusted for sex and age.
Differences (p-value <0.05) between [c]Tertile 1 vs. Tertile 2, [d]Tertile 1 vs. Tertile 3, [e]Tertile 2 vs. Tertile 3 were indicated.
Abbreviations: MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; T, tertile; TE, total energy intake; Trans-FA, trans fatty acid.

**Supplementary Table 5. Food groups consumption, mean energy, nutrients and polyphenols intake, and adherence to energy-restricted Mediterranean diet according to tertiles of fruit quantity and vegetable quantity**

| | Quantity in fruit consumption | | | | Quantity in vegetable consumption | | | |
|---|---|---|---|---|---|---|---|---|
| | T1 (Lowest) (n=2216) 164.02±63.32 | T2 (n=2217) 329.42±44.57 | T3 (Highest) (n=2214) 584.45±180.57 | *p*-value[a] | T1 (Lowest) (n=2361) 5.23±1.91 | T2 (n=2761) 8.56±0.50 | T3 (Highest) (n=1525) 10.00±0.00 | *p*-value[a] |
| **Food groups, g/day** | | | | | | | | |
| Fruits | 168.52±2.43[c,d] | 329.80±2.39[c,e] | 579.57±2.43[d,e] | <0.001 | 312.44±4.18[c,d] | 353.88±4.14[c,e] | 411.38±4.19[d,e] | <0.001 |
| Vegetables | 268.67±2.66[c,d] | 302.28±2.62[c,e] | 336.58±2.67[d,e] | <0.001 | 176.09±1.38[c,d] | 285.34±1.37[c,e] | 446.12±1.39[d,e] | <0.001 |
| Legumes | 19.06±0.24[c,d] | 20.83±0.23[c,e] | 22.23±0.24[d,e] | <0.001 | 18.39±0.23[c,d] | 20.40±0.23[c,e] | 23.33±0.23[d,e] | <0.001 |
| Cereals | 158.45±1.41[c,d] | 151.30±1.38[c,e] | 141.06±1.41[d,e] | <0.001 | 154.79±1.40[d] | 151.24±1.39[e] | 144.80±1.41[d,e] | <0.001 |
| Dairy products | 341.53±4.17 | 343.75±4.10 | 353.18±4.17 | 0.117 | 347.52±4.14 | 341.68±4.10 | 349.26±4.15 | 0.393 |
| Meat | 153.19±1.14[c,d] | 148.32±1.13[c,e] | 141.51±1.14[d,e] | <0.001 | 144.91±1.14[d] | 147.65±1.13 | 150.47±1.14[d] | 0.003 |
| Olive oil | 40.32±0.35[d] | 41.02±0.34[e] | 38.28±0.35[d,e] | <0.001 | 40.19±0.35[d] | 41.19±0.34[e] | 38.25±0.35[d,e] | <0.001 |
| Fish | 98.67±1.00[c,d] | 102.38±0.99[c] | 105.61±1.00[d] | <0.001 | 88.92±0.97[c,d] | 102.52±0.96[c,e] | 115.21±0.98[d,e] | <0.001 |
| Nuts | 13.02±0.36[c,d] | 14.88±0.35[c,e] | 16.85±0.36[d,e] | <0.001 | 12.79±0.35[c,d] | 14.58±0.35[c,e] | 17.37±0.35[d,e] | <0.001 |
| Cookies and pastries | 28.82±0.59[c,d] | 25.86±0.58[c] | 25.82±0.59[d] | <0.001 | 30.95±0.58[c,d] | 26.74±0.58[c,e] | 22.81±0.59[d,e] | <0.001 |
| **Total energy[b], kcal/day** | 2242.81±11.08[c,d] | 2340.07±11.01[c,e] | 2513.00±11.04[d,e] | <0.001 | 2266.43±11.13[c,d] | 2345.16±11.09[c,e] | 2484.19±11.14[d,e] | <0.001 |
| **Alcohol, g/day** | 13.53±0.28[c,d] | 10.63±0.28[c,e] | 8.92±0.28[d,e] | <0.001 | 12.32±0.28[c,d] | 11.27±0.28[c,e] | 9.50±0.28[d,e] | <0.001 |
| **Macronutrient %TE** | | | | | | | | |
| Protein | 16.67±0.05[d] | 16.78±0.05 | 16.88±0.05[d] | 0.035 | 15.96±0.05[c,d] | 16.70±0.05[c,e] | 17.67±0.05[d,e] | <0.001 |
| Total fat | 40.82±0.14[c,d] | 40.02±0.14[c,e] | 37.87±0.14[d,e] | <0.001 | 40.13±0.14[d] | 39.91±0.14[e] | 38.67±0.14[d,e] | <0.001 |
| MUFA | 21.06±0.10[d] | 20.88±0.10[e] | 19.65±0.01[d,e] | <0.001 | 20.79±0.10[d] | 20.74±0.10[e] | 20.07±0.10[d,e] | <0.001 |
| PUFA | 6.91±0.04[d] | 6.87±0.04[e] | 6.70±0.04[d,e] | <0.001 | 6.74±0.04[c] | 6.88±0.04[c] | 6.86±0.04 | 0.030 |
| SFA | 10.53±0.04[c,d] | 9.98±0.04[c,e] | 9.33±0.04[d,e] | <0.001 | 10.33±0.04[c,d] | 9.96±0.04[c,e] | 9.55±0.04[d,e] | <0.001 |
| Trans-FA | 0.25±0.01[c,d] | 0.22±0.01[c,e] | 0.20±0.01[d,e] | <0.001 | 0.24±0.01[c,d] | 0.22±0.01[c,e] | 0.20±0.01[d,e] | <0.001 |
| Carbohydrates | 38.72±0.14[c,d] | 40.24±0.14[c,e] | 42.73±0.14[d,e] | <0.001 | 40.44±0.15[d] | 40.26±0.14[e] | 40.99±0.15[d,e] | 0.001 |
| **Protein g/kg/day** | 1.14±0.01[d] | 1.15±0.01 | 1.16±0.01[d] | <0.001 | 1.09±0.01[c,d] | 1.14±0.01[c,e] | 1.22±0.01[d,e] | <0.001 |
| **Fiber g/day** | 21.73±0.15[c,d] | 25.92±0.14[c,e] | 30.75±0.15[d,e] | <0.001 | 22.11±0.15[c,d] | 25.57±0.15[c,e] | 30.72±0.15[d,e] | <0.001 |
| **Micronutrients** | | | | | | | | |
| Vitamin A (µg/d) | 1019.88±13.34[c,d] | 1126.83±13.15[c] | 1168.20±13.37[d] | <0.001 | 867.93±12.58[c,d] | 1067.52±12.46[c,e] | 1379.54±12.62[d,e] | <0.001 |
| Vitamin B1 (mg/d) | 1.52±0.01[c,d] | 1.63±0.01[c,e] | 1.76±0.01[d,e] | <0.001 | 1.51±0.01[c,d] | 1.62±0.01[c,e] | 1.79±0.01[d,e] | <0.001 |
| Vitamin B2 (mg/d) | 1.91±0.01[c,d] | 2.02±0.01[c,e] | 2.10±0.01[d,e] | <0.001 | 1.88±0.01[c,d] | 1.99±0.01[c,e] | 2.17±0.01[d,e] | <0.001 |
| Vitamin B3 (mg/d) | 39.80±0.17[c,d] | 40.68±0.16[c,e] | 41.63±0.17[d,e] | <0.001 | 37.67±0.16[c,d] | 40.38±0.15[c,e] | 44.06±0.16[d,e] | <0.001 |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Vitamin B6 (mg/d) | 2.13±0.01[c,d] | 2.32±0.01[c,e] | 2.55±0.01[d,e] | <0.001 | 2.07±0.01[c,d] | 2.30±0.01[c,e] | 2.63±0.01[d,e] | <0.001 |
| Vitamin B9 (µg/d) | 303.90±1.70[c,d] | 347.18±1.67[c,e] | 403.56±1.70[d,e] | <0.001 | 292.57±1.53[c,d] | 342.36±1.52[c,e] | 419.68±1.54[d,e] | <0.001 |
| Vitamin B12 (µg/d) | 9.85±0.09 | 10.07±0.09 | 9.89±0.09 | 0.180 | 9.22±0.09[c,d] | 9.91±0.09[c,e] | 10.67±0.09[d,e] | <0.001 |
| Vitamin C (mg/d) | 149.14±1.45[c,d] | 196.62±1.43[c,e] | 260.90±1.46[d,e] | <0.001 | 152.26±1.45[c,d] | 193.64±1.44[c,e] | 260.73±1.46[d,e] | <0.001 |
| Vitamin D (µg/d) | 5.91±0.07[c,d] | 6.18±0.07[c,e] | 6.47±0.07[d,e] | <0.001 | 5.44±0.07[c,d] | 6.16±0.07[c,e] | 6.96±0.07[d,e] | <0.001 |
| Vitamin E (mg/d) | 10.18±0.07[c,d] | 10.67±0.07[c,e] | 10.96±0.07[d,e] | <0.001 | 9.62±0.07[c,d] | 10.37±0.07[c,e] | 11.81±0.07[d,e] | <0.001 |
| Vitamin K (µg/d) | 291.78±3.98[c,d] | 329.45±3.92[c,e] | 359.77±3.98[d,e] | <0.001 | 217.29±3.46[c,d] | 313.79±3.42[c,e] | 449.95±3.47[d,e] | <0.001 |
| Calcium (mg/d) | 996.06±6.28[c,d] | 1024.21±6.19[c,e] | 1082.91±6.29[d,e] | <0.001 | 988.61±6.20[c,d] | 1013.68±6.15[c,e] | 1100.88±6.23[d,e] | <0.001 |
| Phosphorus (mg/d) | 1707.89±6.39[c,d] | 1756.71±6.29[c,e] | 1813.55±6.39[d,e] | <0.001 | 1660.25±6.12[c,d] | 1738.43±6.06[c,e] | 1879.47±6.14[d,e] | <0.001 |
| Magnesium (mg/d) | 390.91±1.62[c,d] | 418.50±1.59[c,e] | 451.85±1.62[d,e] | <0.001 | 384.97±1.55[c,d] | 414.21±1.53[c,e] | 462.08±1.55[d,e] | <0.001 |
| Iron (mg/d) | 15.63±0.05[c,d] | 16.38±0.05[c,e] | 17.38±0.05[d,e] | <0.001 | 14.91±0.05[c,d] | 16.26±0.05[c,e] | 18.21±0.05[d,e] | <0.001 |
| Potassium (mg/d) | 3992.42±15.82[c,d] | 4424.31±15.58[c,e] | 5010.57±15.83[d,e] | <0.001 | 3911.85±14.64[c,d] | 4398.18±14.50[c,e] | 5117.06±14.69[d,e] | <0.001 |
| Iodine (µg/d) | 282.59±3.29 | 281.82±3.24 | 292.22±3.30 | 0.047 | 277.53±3.27[d] | 280.69±3.24[e] | 298.41±3.28[d,e] | <0.001 |
| Selenium (µg/d) | 117.09±0.52 | 117.44±0.51 | 116.62±0.52 | 0.546 | 111.88±0.51[c,d] | 116.97±0.50[c,e] | 122.31±0.51[d,e] | <0.001 |
| Zinc (mg/d) | 13.00±0.05[c,d] | 13.18±0.05[c,e] | 13.36±0.05[d,e] | <0.001 | 12.67±0.04[c,d] | 13.11±0.04[c,e] | 13.75±0.05[d,e] | <0.001 |
| **Polyphenols** | | | | | | | | |
| Total polyphenols (mg/d) | 734.97±5.56[c,d] | 830.68±5.47[c,e] | 971.47±5.56[d,e] | <0.001 | 797.58±5.82[c,d] | 843.95±5.76[c,e] | 895.48±5.84[d,e] | <0.001 |
| Flavonoids (mg/d) | 390.43±4.53[c,d] | 475.33±4.46[c,e] | 606.95±4.54[d,e] | <0.001 | 455.25±4.83[c,d] | 490.68±4.79[c,e] | 526.69±4.85[d,e] | <0.001 |
| Phenolic acids (mg/d) | 270.46±2.72[d] | 278.59±2.68[e] | 291.93±2.72[d,e] | <0.001 | 270.32±2.70[d] | 279.14±2.68[e] | 291.52±2.71[d,e] | <0.001 |
| **MedDiet adherence** [f] | 7.84±0.06[c,d] | 8.52±0.05[c,e] | 9.19±0.06[d,e] | <0.001 | 7.76±0.05[c,d] | 8.56±0.05[c,e] | 9.24±0.05[d,e] | <0.001 |

Data are presented as adjusted means ± Standard Error.
[a]P-value <0.05 for differences among tertiles of fruit quantity and vegetable quantity was calculated by ANCOVA with Bonferroni´s post-hoc test, and adjusted for sex (male/female), age (in years) and energy intake (Kcal/day).
[b]Total energy intake was only adjusted for sex and age.
Differences (p-value <0.05) between [c]Tertile 1 vs. Tertile 2, [d]Tertile 1 vs. Tertile 3, [e]Tertile 2 vs. Tertile 3 were indicated.
[f]MedDiet adherence: adherence to Mediterranean using the 17 item questionnaire adherence with scale 0-17 points.
Abbreviations: MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; T, tertile; TE, total energy intake; Trans-FA, trans fatty acid.

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**Supplementary Table 6. Lifestyle factors according to tertiles of fruit variety and vegetable variety.**

| | Variety in fruit consumption | | | | Variety in vegetable consumption | | | |
|---|---|---|---|---|---|---|---|---|
| | T1 (Lowest) | T2 | T3 (Highest) | *p*-value[a] | T1 (Lowest) | T2 | T3 (Highest) | *p*-value[a] |
| **Smoking habit, % (n)** | | | | <0.001 | | | | <0.001 |
| Current smoker | 362 (15.40) | 311 (11.30) | 150 (9.89) | | 453 (14.57) | 258 (11.19) | 112 (9.31) | |
| Former smoker | 1101 (46.85) | 1172 (42.59) | 603 (39.75) | | 1363 (43.83) | 1027 (44.54) | 486 (40.40) | |
| Never smoked | 887 (37.74) | 1269 (46.11) | 764 (50.36) | | 1294 (41.61) | 1021 (44.28) | 605 (50.29) | |
| **Physical activity** | | | | | | | | |
| Light PA, min/day | 26.84±0.70 | 27.05±0.64 | 28.02±0.87 | 0.549 | 26.64±0.61[c] | 26.58±0.70[d] | 29.82±0.98[c,d] | 0.012 |
| Moderate PA, min/day | 25.62±0.89[b] | 29.19±0.82[b] | 26.38±1.10 | 0.008 | 23.77±0.77[b,c] | 30.21±0.89[b] | 30.76±1.24[c] | <0.001 |
| Vigorous PA, min/day | 12.26±0.47[c] | 12.55±0.43[d] | 14.33±0.58[c,d] | 0.014 | 11.73±0.41[b,c] | 13.60±0.47[b] | 14.35±0.65[c] | <0.001 |
| **WHO Exercise Recommendations % (n)** | | | | 0.624 | | | | 0.001 |
| No | 1690 (71.58) | 1962 (71.06) | 1105 (72.46) | | 2302 (73.59) | 1597 (69.01) | 858 (71.20) | |
| Yes | 671 (28.42) | 799 (28.94) | 420 (27.54) | | 826 (26.41) | 717 (30.99) | 347 (28.80) | |
| **Sedentary behavior** | | | | | | | | |
| Sedentary time, h/d | 5.97±0.04[c] | 5.97±0.04[d] | 6.22±0.05[c,d] | <0.001 | 6.11±0.03[b] | 5.95±0.04[b] | 5.95±0.06 | 0.005 |
| TV-viewing time, h/d | 3.30±0.03 | 3.24±0.03 | 3.30±0.04 | 0.340 | 3.35±0.03[b,c] | 3.21±0.03[b] | 3.18±0.05[c] | <0.001 |
| **Sleeping (hours/day)** | 7.07±0.02 | 7.05±0.02 | 7.00±0.03 | 0.207 | 7.06±0.02 | 7.04±0.03 | 7.00±0.03 | 0.404 |

Data are presented as adjusted means ± Standard Error and n (%) for continuous and categorical variables, respectively.
In the analysis, there were missing data for smoking habit in 28 participants (0.42%),
[a]P-value <0.05 are for differences among tertiles of fruit variety and vegetable variety was calculated by Chi-square and ANCOVA with Bonferroni´s post-hoc test, and adjusted for sex (male/female) and age (in years), as appropriate.
Differences (p-value <0.005) between [b]Tertile 1 vs. Tertile 2, [c]Tertile 1 vs. Tertile 3, [d]Tertile 2 vs. Tertile 3 were indicated in continuous variables.
Abbreviations: PA, physical activity; T, tertile; WHO, World Health Organization

**Supplementary Table 7. Lifestyle factors according to categories of fruit and vegetable quantity**

| | T1 (Lowest) | T2 | T3 (Highest) | *p*-value[a] |
|---|---|---|---|---|
| **Smoking habit, % (n)** | | | | 0.001 |
| Current smoker | 354 (16.07) | 240 (10.88) | 229 (10.36) | |
| Former smoker | 1020 (46.30) | 953 (43.20) | 903 (40.86) | |
| Never smoked | 829 (37.63) | 1013 (45.92) | 1078 (48.78) | |
| **Physical activity** | | | | |
| Light PA, min/day | 26.66±0.73 | 27.18±0.72 | 27.75±0.72 | 0.569 |
| Moderate PA, min/day | 23.10±0.92[b,c] | 26.43±0.91[b,d] | 32.30±0.92[c,d] | <0.001 |
| Vigorous PA, min/day | 11.14±0.48[b,c] | 12.93±0.48[b] | 14.49±0.48[c] | <0.001 |
| **WHO Exercise Recommendations % (n)** | | | | 0.015 |
| No | 1619 (73.06) | 1602 (72.29) | 1536 (69.35) | |
| Yes | 597 (26.94) | 614 (27.71) | 679 (30.65) | |
| **Sedentary behaviour** | | | | |
| Sedentary time, h/d | 6.17±0.04[b,c] | 5.94±0.04[b] | 5.97±0.04[c] | <0.001 |
| TV-viewing time, h/d | 3.35±0.04[b] | 3.21±0.04[b] | 3.25±0.04 | 0.021 |
| **Sleeping (hours/day)** | 7.08±0.03 | 7.03±0.03 | 7.01±0.03 | 0.121 |

Data are presented as adjusted means ± Standard Error and n (%) for continuous and categorical variables, respectively.
In the analysis, there were missing data for smoking habit in 28 participants (0.42%),
[a]P-value <0.05 for differences among tertiles of quantity and variety in vegetable consumption was calculated by Chi-square and ANCOVA with Bonferroni´s post-hoc test, and adjusted for sex (male/female) and age (in years), as appropriate.
Differences (p-value <0.05) between [b]Tertile 1 vs. Tertile 2, [c]Tertile 1 vs Tertile 3, [d]Tertile 2 vs. Tertile 3 were indicated in continuous variables.
Abbreviations: PA, physical activity; T, tertile; WHO, World Health Organization.

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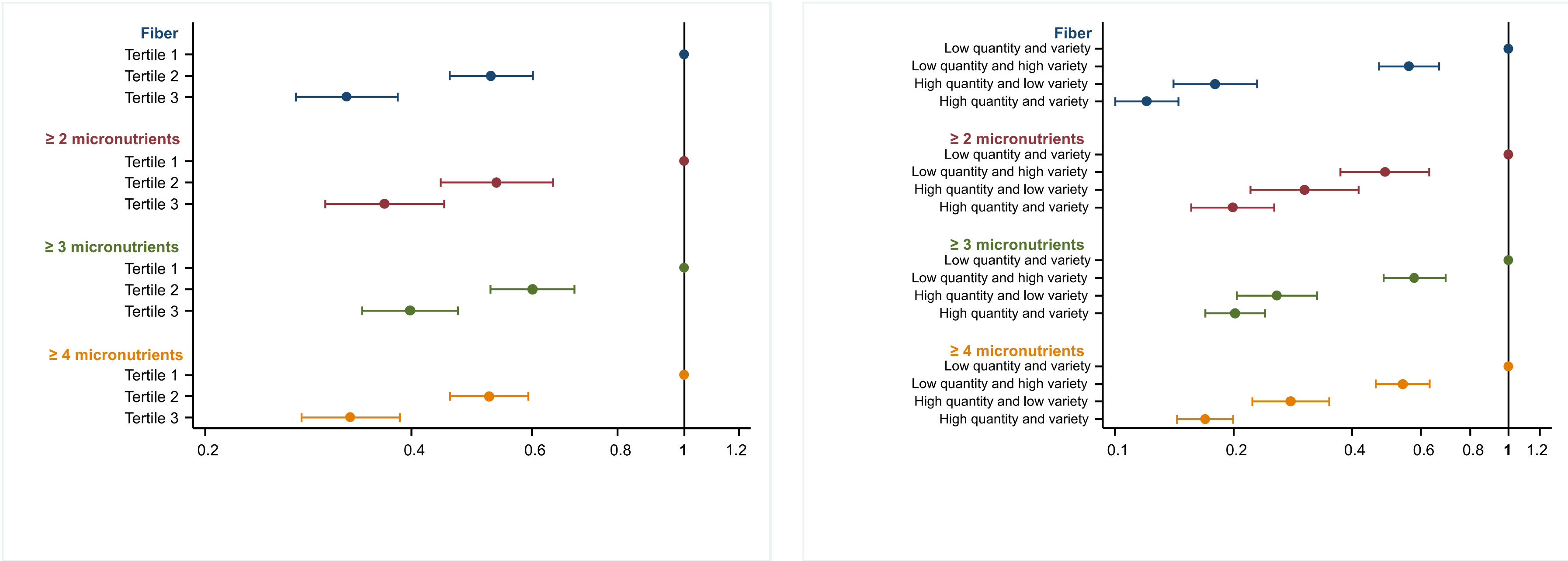
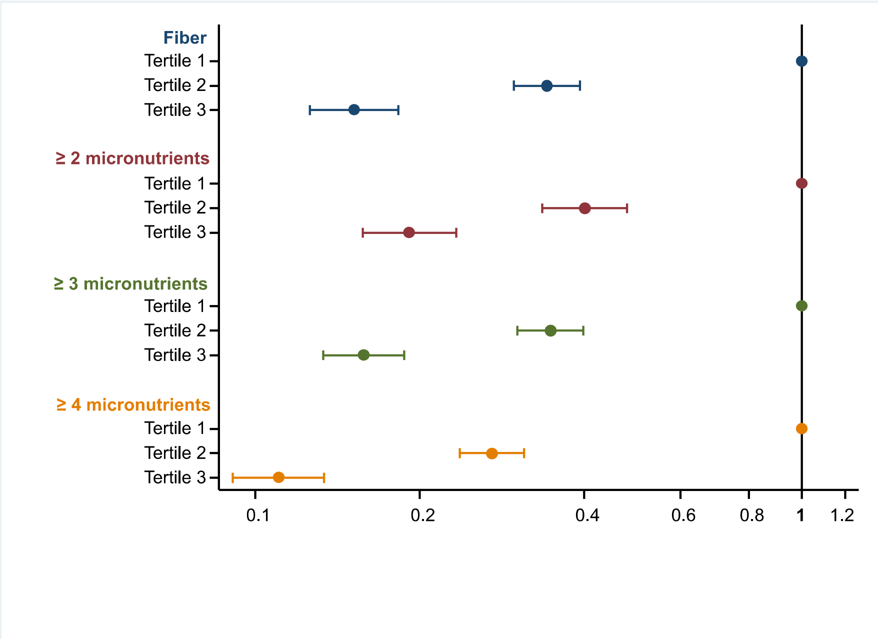
**Supplementary Table 8. Lifestyle factors according to tertiles of fruit quantity and vegetable quantity.**

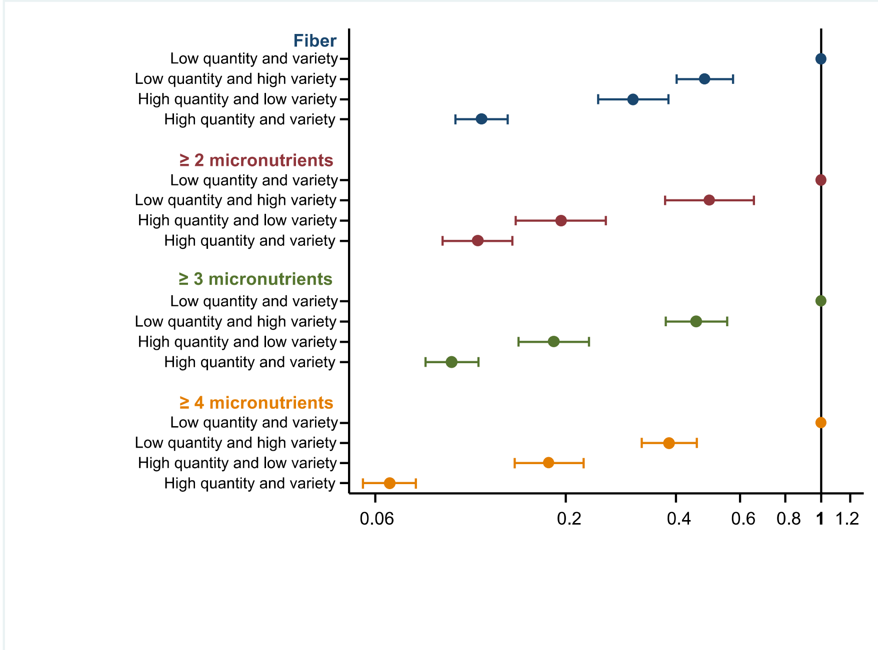
| | Quantity in fruit consumption | | | | Quantity in vegetable consumption | | | |
|---|---|---|---|---|---|---|---|---|
| | T1 (Lowest) | T2 | T3 (Highest) | *p*-value[a] | T1 (Lowest) | T2 | T3 (Highest) | *p*-value[a] |
| **Smoking habit, % (n)** | | | | <0.001 | | | | <0.001 |
| Current smoker | 370 (16.80) | 220 (9.95) | 233 (10.57) | | 309 (14.02) | 254 (11.51) | 260 (11.78) | |
| Former smoker | 1011 (45.89) | 963 (43.55) | 902 (40.91) | | 987 (44.78) | 993 (44.99) | 896 (40.58) | |
| Never smoked | 822 (37.31) | 1028 (46.49) | 1070 (48.53) | | 908 (41.20) | 960 (43.50) | 1052 (47.64) | |
| **Physical activity** | | | | | | | | |
| Light PA, min/day | 27.45±0.72 | 27.34±0.72 | 26.80±0.72 | 0.790 | 25.02±0.72[b,c] | 28.00±0.72[b] | 28.57±0.72[c] | 0.001 |
| Moderate PA, min/day | 23.70±0.92[b,c] | 27.38±0.91[b,d] | 30.75±0.92[c,d] | <0.001 | 23.17±0.91[b,c] | 27.13±0.91[b,d] | 31.54±0.92[c,d] | <0.001 |
| Vigorous PA, min/day | 11.00±0.48[b,c] | 14.06±0.48[b] | 13.51±0.48[c] | <0.001 | 11.24±0.48[b,c] | 12.96±0.48[b] | 14.36±0.48[c] | <0.001 |
| **WHO Exercise Recommendations % (n)** | | | | 0.109 | | | | <0.001 |
| No | 1605 (72.43) | 1604 (72.35) | 1548 (69.92) | | 1659 (74.86) | 1549 (69.90) | 1549 (69.93) | |
| Yes | 611 (27.57) | 613 (27.65) | 666 (30.08) | | 557 (25.14) | 667 (30.10) | 666 (30.07) | |
| **Sedentary behavior** | | | | | | | | |
| Sedentary time, h/d | 6.12±0.04[b] | 5.97±0.04[b] | 6.00±0.04 | 0.032 | 6.11±0.04 | 5.98±0.04 | 5.99±0.04 | 0.038 |
| TV-viewing time, h/d | 3.29±0.04 | 3.27±0.04 | 3.25±0.04 | 0.688 | 3.34±0.04 | 3.23±0.04 | 3.24±0.04 | 0.056 |
| **Sleeping (hours/day)** | 7.07±0.03 | 7.03±0.03 | 7.02±0.03 | 0.321 | 7.06±0.03 | 7.05±0.03 | 7.02±0.03 | 0.478 |

Data are presented as adjusted means ± Standard Error and n (%) for continuous and categorical variables, respectively.
In the analysis, there were missing data for smoking habit in 28 participants (0.42%),
[a]P-value <0.05 are for differences among tertiles of fruit quantity and vegetable quantity was calculated by Chi-square and ANCOVA with Bonferroni´s post-hoc test, and adjusted for sex (male/female) and age (in years), as appropriate.
Differences (p-value <0.005) between [b]Tertile 1 vs. Tertile 2, [c]Tertile 1 vs. Tertile 3, [d]Tertile 2 vs. Tertile 3 were indicated in continuous variables.
Abbreviations: PA, physical activity; T, tertile; WHO, World Health Organization

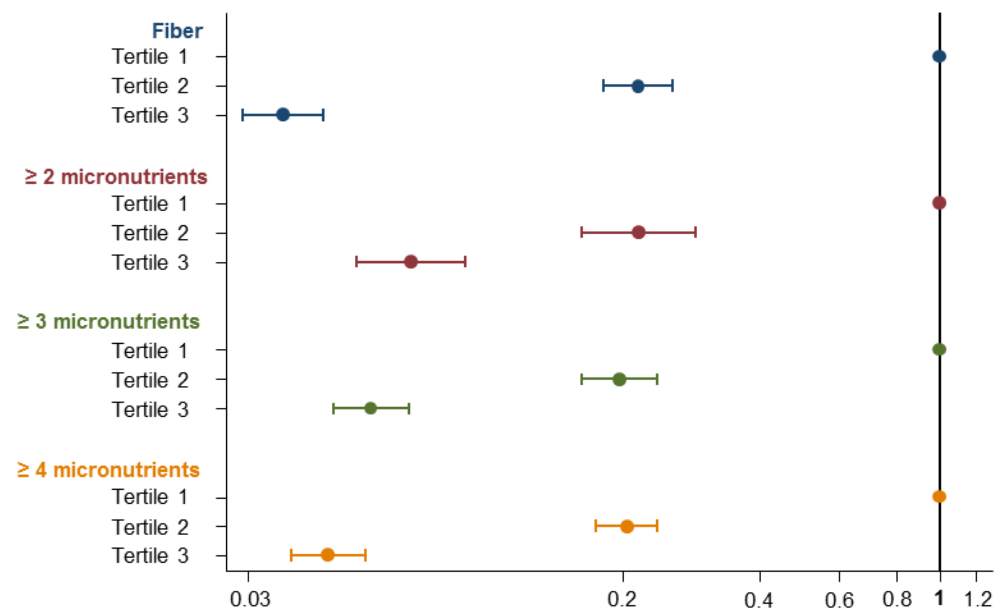



**Supplementary figure 1.** OR and 95% CI for inadequate intake of fiber, two or more, three or more and four or more micronutrients by tertiles of fruit variety and QV categories of fruit consumption.

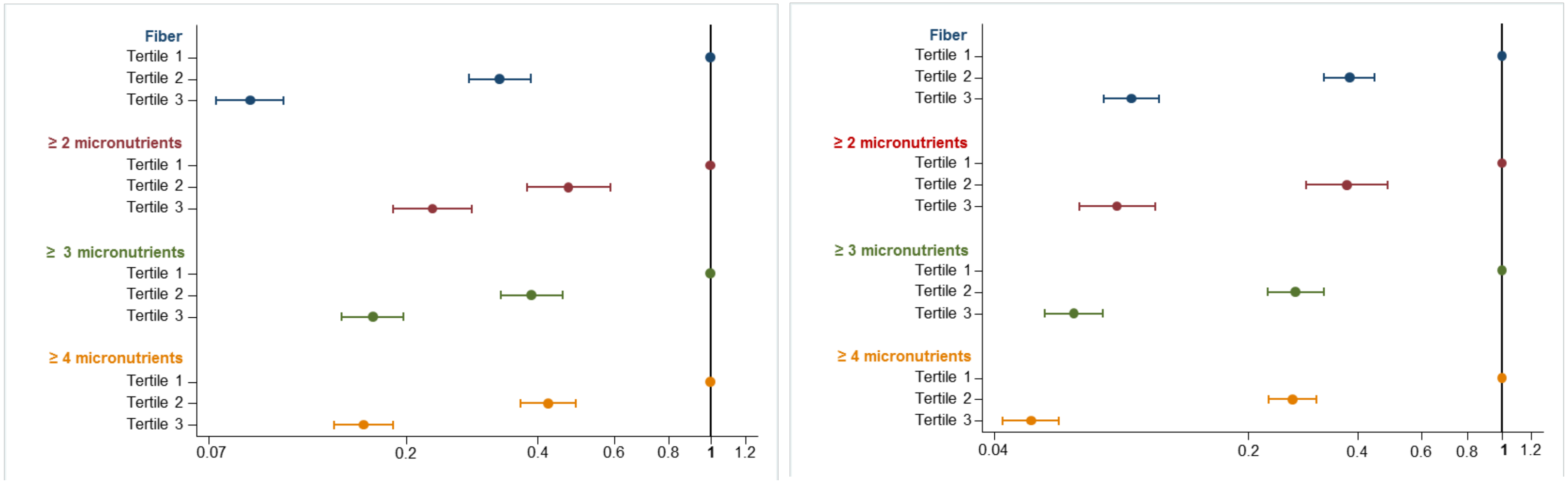



**Supplementary figure 2.** OR and 95% CI for inadequate intake of fiber, two or more, three or more and four or more micronutrients by tertiles of vegetable variety and QV categories of vegetable consumption.




**Supplementary Figure 3.** OR and 95% CI for inadequate intake of fiber and 2 or more, 3 or more and 4 or more micronutrients by tertiles of fruit and vegetable quantity.




**Supplementary Figure 4.** OR and 95% CI for inadequate intake of fiber and 2 or more, 3 or more and 4 or more micronutrients by tertiles of fruit quantity (left) and vegetable quantity (right).

## 5.2 One-year changes in fruit and vegetable variety and cardiometabolic risk factors changes in an elderly Mediterranean population at high cardiovascular risk

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**Key teaching points**

- Few epidemiological studies have analyzed the association between variety in FV consumption and cardiometabolic risk factors.

- Previous results from the PREDIMED-plus Reus center showed a decrease in some cardiometabolic risk factors with the consumption of certain fruit groups (red-purple-white fruits) at baseline.

- The present study has gone further and has longitudinally analyzed the association between one-year changes in variety of FV consumption and concurrent changes in several cardiometabolic risk factors in elderly subjects at high CVD risk.

- A one-year data longitudinal analysis with 6647 elderly subjects at high CVD risk was conducted in the framework of the PREDIMED-plus study.

- An increase in fruit and/or vegetable variety was associated with a concurrent decrease in some cardiometabolic risk factors (fasting blood glucose, body weight and WC) after one year of follow-up.

- Besides, changes in FV intake that led to higher quantity and variety of fruits and/or vegetables were associated with downward changes in some cardiometabolic risk factors (fasting blood glucose, body weight and WC) after one year of follow-up.

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ARTICLE

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Nutrition and health (including climate and ecological aspects)

# One-year changes in fruit and vegetable variety intake and cardiometabolic risk factors changes in a middle-aged Mediterranean population at high cardiovascular risk



**BACKGROUND AND AIMS:** Previous studies have shown beneficial associations between fruit and vegetable (FV) consumption and cardiometabolic risk factors. However, variety in FV, which may play an important role on cardiovascular health due to the different nutrient and phytochemical content among the different groups and subgroups of FV has been poorly investigated. We longitudinally investigated associations between 1-year changes in variety and quantity of FV and concurrent changes in cardiometabolic risk factors in elderly subjects with overweight/obesity and metabolic syndrome.
**METHODS:** a one-year data longitudinal analysis of 6647 PREDIMED-plus study participants (48% women) was conducted. Data were collected at baseline, six months and 1-year of follow-up. Variety and quantity of FV were estimated using a food frequency questionnaire and continuous scores for variety were created based on items/month of FV. Linear mixed-models adjusted for potential confounders were performed to estimate associations (β-coefficients and 95% confidence interval) between 1-year changes in FV variety and/or quantity and concurrent changes in cardiometabolic risk factors.
**RESULTS:** Two points increment in the FV variety score over one year was associated with a concurrent decrease in glucose (−0.33 mg/dL (0.58, −0.07)), body weight (−0.07 kg (−0.13, −0.02)) and waist circumference (WC) (−0.08 cm (−0.16, −10.01)). An increment of 100 g/d of FV over one year was associated with a concurrent decrease in triglycerides (−0.50 mg/dL (−0.93, −0.08)), glucose (−0.21 mg/dL (−0.32, −0.11)), body weight (−0.11 kg (−0.15, −0.07)) and WC (−0.10 cm (−0.14, −0.06)) over 1-year. Changes in FV consumption which led to higher quantity and variety over one year were associated with downward changes in glucose (−1.26 mg/dL (−2.09, −0.43)), body weight (−0.40 kg (−0.58, −0.23)) and WC (−0.50 cm (−0.73, −0.28)).
**CONCLUSION:** Greater variety, in combination with higher quantity of FV was significantly associated with a decrease in several cardiometabolic risk factors among elderly subjects at high cardiovascular risk.

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## INTRODUCTION

A regular consumption of fruits and vegetables (FV) has been widely recommended for the prevention of several non-communicable diseases such as cardiovascular disease (CVD), different types of cancer or type 2 diabetes [1]. However, although the World Health Organization (WHO) placed low FV consumption (below 400 g or five portions per day) among the top ten risk factors for global mortality [2, 3], a high percentage of the population do not meet these recommendations in the majority of countries [4].

FV are low energy-density foods because of its high water and dietary fiber and low caloric content. Besides, they are good sources of minerals (mainly potassium and magnesium), vitamins (C, E and K, beta-carotene and folates) and phytochemicals, especially phenolic compounds.

FV consumption has been associated with an improvement of some cardiometabolic risk factors and human health possibly due to the synergistic effect of its components, including the antioxidant and anti-inflammatory properties of the phenolic compounds [5, 6]. In this sense, some systematic reviews and meta-analyses of either cross-sectional studies [7] or randomized controlled clinical trials (RCTs) [8, 9] revealed that increasing FV consumption significantly reduced triglycerides (TG) and diastolic blood pressure (DBP), and to a lesser extent total and LDL cholesterol. Nevertheless, no discernible effect on body weight was observed by increasing FV consumption in other two systematic reviews and meta-analyses of RCTs [10, 11].

To note, these previous associations or effects have been mainly investigated only considering the total amount of FV consumed per day, without taking into consideration its variety. A wide variety of FV should be consumed daily, given that phytochemicals differ widely in composition and ratio among the different groups and subgroups of FV [6] being possible multiple interactions with potential health benefits. In this sense, a longitudinal study conducted in a representative population of Tehran revealed that colors of FV subgroups had different

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associations with some cardiometabolic risk factors after 3 years of follow-up. While a higher intake of red and purple FV was associated with a reduction in body weight and abdominal fat, a greater consumption of yellow, white and green vegetables was inversely related with lipid parameters [12]. Furthermore, in a cross-sectional study conducted in the frame of the PREDIMED-Plus study, we reported different associations between subgroups of fruits based on their color and cardiometabolic risk factors, which highlights that not all fruit varieties are similarly associated with CVD risk [13].

Importantly, to the best of our knowledge, no previous studies have assessed the jointly associations considering FV quantity and variety and different cardiometabolic risk factors.

Therefore, in view of the above, the main objective of the present study was to longitudinally assess associations between changes in variety and quantity of FV with concurrent changes in cardiometabolic risk factors in elderly subjects at high cardiovascular risk after one year of follow-up. The associations between cardiometabolic risk factors and joint categories of FV variety and quantity were also investigated.

## MATERIALS AND METHODS

### Design and study population

The present study is a longitudinal analysis of 1-year data from the ongoing PREDIMED-plus study, a 6-year parallel group, multicenter, randomized controlled clinical trial for the primary prevention of CVD. This trial was conducted in Spain with the aim of evaluating the effect of an intensive weight loss intervention (based on an energy-restricted Mediterranean Diet (MedDiet), physical activity promotion and behavioral support) on CVD morbimortality compared to an usual care intervention only advising participants to adhere to an unrestricted caloric MedDiet. Details of the study protocol are available at https://www.predimedplus.com [14].

This trial was registered in 2014 at the International Standard Randomized Controlled Clinical Trial (ISCRT; http://www.isrctn.com/ISRCTN89898870). The pilot study evaluating the effects of the PREDIMED-Plus lifestyle interventions on body weight and CVD risk factors has been published elsewhere [15].

Participants were recruited between October 2013 to December 2016 by 23 Spanish centers from the National Health System, like universities, research institutes hospitals and primary health care centers [14]. Randomized participants were women aged between 60–75 years and men between 55–75 years, with a BMI between 27–40 kg/m$^2$, who meet at least three Metabolic Syndrome (MetS) criteria defined according to the updated harmonized criteria of the AHA/National Heart, Lung and Blood Institute and the International Diabetes Federation [16]. Participants with history of CVD or active cancer were excluded from the study. Other exclusion criteria for the trial have been reported elsewhere.

In this report, data of all the PREDIMED-plus randomized participants was longitudinally analyzed from baseline to 1-year of follow-up as if it was an observational prospective cohort study. We excluded 36 participants who did not complete the Food Frequency Questionnaire (FFQ) at baseline, as well as 191 participants who had energy intake values beyond the specified limits (500–3500 Kcal/day in women and 800–4000 kcal/day in men) at baseline [17]. Therefore, a total final sample of 6647 participants was included in the analyses.

### Fruit and vegetable consumption assessment

To determine dietary intake, trained dietitians administered at baseline, six months and one-year of follow-up a 143-item semiquantitative FFQ, resulting from the adaptation of a previous validated one in Spain [18]. The FFQ includes 13 and 17 items about fruit and vegetable intake, respectively. In each item, a typical portion size was defined as well as nine potential categories of fruit and vegetable frequency intake that varied from never or almost never to more than six times per day.

The present study has been focused on solid and raw fruits and vegetables which are frequently consumed in Spain, so fruit and vegetable juices were excluded. Besides, as dried fruits refer to more than one type of fruit, they were also excluded because it was impossible to find out what type of raw fruit would be equivalent. Finally, potatoes and mushrooms were not considered vegetables because its nutritional composition differs from that of vegetables [19]. Therefore, ten items of fruits such as oranges, bananas, apples, strawberries, cherries, melon, watermelon, kiwis, grapes and peaches and eleven of vegetables including chards, cabbage, lettuce, tomatoes, carrots, green beans, courgette, peppers, asparagus, onions and garlics were finally incorporated to our study.

Variety of fruit and vegetable intake was measured as the sum of the total number of unique items consumed, regardless of quantity, which corresponds to the at least 1–3 per month response category in the FFQ [19, 20]. After that, continuous scores for variety in items consumed per month of fruits (0–10), vegetables (0–11) and both (0–21) were created at baseline, six months and 1-year of follow-up. This scoring method is similar to those used for reducing the risk of several chronic diseases in other cohorts [21, 22].

Finally, to determine the amount of fruit and vegetable intake, information of the selected items of fruits and vegetables in the FFQ was transformed into grams per day, multiplying serving sizes by its frequency of consumption and dividing the result by the assessed period. Subsequently, continuous variables for fruit and vegetable quantity were created by summing up the total amount of fruits and vegetables in grams per day, at baseline, six months and 1-year of follow-up. In addition, continuous variables for fruit and vegetable quantity were created separately, also at baseline, six months and 1-year of follow-up.

### Cardiometabolic risk factors and other covariates assessment

For the present study, several cardiometabolic risk parameters such as triglycerides (TG), HDL-cholesterol (HDL-c), LDL-cholesterol (LDL-c), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma blood glucose, waist circumference (WC), body weight and BMI were determined at baseline, six months and one year of follow-up by trained staff and according to the PREDIMED-Plus study protocol. Body weight was measured twice with light clothes and no shoes using calibrated scales. WC was determined twice midway between the last rib and the iliac crest using an anthropometric tape. Blood pressure was measured three times after at least five min of rest with a validated semiautomatic oscillometer (Omron HEM-705CP, Netherlands). Mean values of the previous parameters were used in the present study.

Blood samples were collected after an overnight fast and TG, total HDL-c and blood glucose levels were determined using standard enzymatic methods by laboratory technicians. The Friedewald formula was used to estimate LDL-c in our participants when TG levels were below to 400 mg/dL [23].

With regard to covariate assessment, trained dietitians collected information in a face-to-face visit about, age, sex, marital status, employment status, living alone, educational level, smoking habit, physical activity, presence of hypercholesterolemia, hypertension and diabetes, BMI, MedDiet adherence, alcohol consumption and energy intake at baseline, six months and one year of follow-up.

Physical activity was assessed using the validated Regicor Short Physical Activity Questionnaire for adult population [24]. Adherence to an energy-reduced MedDiet was evaluated using a 17-item questionnaire, adapted from a previous validated one [25], which includes two items related to fruit and vegetable consumption respectively. Compliance with each item of the questionnaire was scored with one point while non-compliance with zero. A total score of 0 means no adherence whereas 17 points reflects maximum adherence. To control the analysis for the overall dietary pattern, we removed the items related to fruit and vegetable consumption from the MedDiet adherence questionnaire when our exposure was fruit and vegetable variety and/or quantity, so the total score varied from 0 to 15 points. Furthermore, the item related to fruit consumption was removed when the exposure was vegetable variety and/or quantity, and vice versa, ranging the total score from 0 to 16 points in both cases.

### Statistical analysis

All the statistical analyses were performed using the latest PREDIMED-plus study dataset generated in December 2020. Baseline characteristics of the participants were presented as mean±standard errors (SE) for continuous variables and numbers and percentages for categorical ones.

Prior to analysis, continuous variables for the increment of two points in the variety score of fruits, vegetables and both were created at baseline, six months and 1-year of follow up, as well as continuous variables for the increment of 100 grams per day of fruits and/or vegetables. Besides, categorical variables for the combination of quantity and variety (QV) of fruits and/or vegetables were created at baseline, six months and 1-year of follow-up. The cut-off points for fruit and vegetable QV were population

**Table 1.** Baseline, 6-month and 1-year characteristics of the study population ($n = 6647$ participants)[a].

| | Baseline | 6-months | 1-year |
|---|---|---|---|
| Age, years | 64.99 ± 4.90 | – | – |
| Women, *n* (%) | 3218 (48.41) | – | – |
| BMI, kg/m$^2$ | 32.70 ± 3.42 | 31.83 ± 3.57 | 31.73 ± 3.65 |
| Educational level, *n* (%) | | | |
| Primary school | 3270 (49.20) | – | – |
| Secondary school | 1918 (28.86) | – | – |
| Tertiary school[b] | 1459 (21.95) | – | – |
| Marital status, (%) | | | |
| Married | 5080 (76.67) | – | – |
| Widowed | 688 (10.38) | – | – |
| Divorced/separated | 517 (7.80) | – | – |
| Others[c] | 341 (5.15) | – | – |
| Living alone, *n* (%) | 821 (12.36) | – | – |
| Employment status, *n* (%) | | | |
| Retired | 3720 (56.31) | – | – |
| Employed | 1360 (20.59) | – | – |
| Housekeeper | 982 (14.87) | – | – |
| Others[d] | 544 (8.23) | – | – |
| Smoking habit, *n* (%) | | | |
| Former smoker | 2876 (43.45) | 2367 (41.83) | 2235 (41.63) |
| Current smoker | 823 (12.43) | 671 (11.86) | 658 (12.26) |
| Non-smoker | 2920 (44.12) | 2621 (46.32) | 2476 (46.12) |
| Type 2 diabetes, (%) | 2047 (30.80) | – | – |
| Intervention group, (%) | 3300 (49.65) | – | – |
| Alcohol intake (g/d) | 11.03 ± 15.02 | 9.61 ± 13.26 | 9.91 ± 13.89 |
| Energy intake (kcal/d) | 2365.24 ± 551.47 | 2225.36 ± 471.58 | 2218.41 ± 473.22 |
| PA (min/day) | | | |
| Light | 27.20 ± 34.02 | 28.01 ± 33.38 | 29.13 ± 33.71 |
| Moderate | 27.28 ± 43.76 | 34.40 ± 48.23 | 34.59 ± 48.62 |
| Vigorous | 12.85 ± 22.77 | 15.01 ± 24.78 | 16.36 ± 28.08 |
| Variety (items/month) | | | |
| Fruits and vegetables | 15.44 ± 3.42 | 15.47 ± 3.32 | 15.84 ± 3.20 |
| Fruits | 7.71 ± 2.26 | 7.43 ± 2.32 | 7.77 ± 2.20 |
| Vegetables | 7.73 ± 2.04 | 8.04 ± 1.95 | 8.07 ± 1.93 |
| Quantity (g/day) | | | |
| Fruits and vegetables | 661.72 ± 272.26 | 729.66 ± 256.08 | 738.87 ± 252.85 |
| Fruits | 359.23 ± 206.78 | 394.19 ± 197.17 | 403.28 ± 189.63 |
| Vegetables | 302.50 ± 129.44 | 335.47 ± 127.60 | 335.59 ± 127.76 |
| Weight (kg) | 86.52 ± 12.97 | 84.59 ± 12.97 | 84.26 ± 13.13 |
| WC (cm) | | | |
| Women | 103.95 ± 9.21 | 101.80 ± 9.45 | 101.48 ± 9.66 |
| Men | 110.95 ± 8.77 | 108.37 ± 9.12 | 107.90 ± 9.37 |
| SBP (mm Hg) | 139.59 ± 16.91 | 137.27 ± 16.79 | 136.43 ± 16.60 |
| DBP (mm Hg) | 80.86 ± 9.93 | 79.34 ± 9.79 | 78.87 ± 9.82 |
| TG (mg/dL) | 151.96 ± 77.62 | 143.35 ± 73.24 | 143.86 ± 73.23 |
| HDL-c (mg/dL) | 48.10 ± 11.81 | 49.51 ± 12.17 | 49.51 ± 12.08 |
| LDL-c (mg/dL) | 118.96 ± 32.98 | 117.86 ± 32.75 | 116.46 ± 32.70 |
| Fasting blood glucose (mg/dL) | 113.58 ± 29.17 | 111.06 ± 28.85 | 110.94 ± 28.05 |

In the analysis, there were missing data at baseline for marital status in 21 participants (0.32%), for living alone in 7 participants (0.11%), for employment status in 41 participants (0.62%), for smoking habit in 28 participants (0.42%), for SBP and DBP in 43 participants (0.65%), for TG in 48 participants (0.72%), for HDL-c in 70 participants (1.05%), for LDL-c in 179 participants (2.69%) and for fasting blood glucose in 37 participants (0.56%). Also, there were missing data at 6-months of follow-up for light, moderate and vigorous PA in 477 participants (7.18%), for BMI, body weight in 291 participants (4.38%), for smoking habit in 988 participants (14.86%), for alcohol and energy intake, and for fruit, vegetable and fruit and vegetable quantity and variety in 816 participants (12.28%), for WC in 530 participants (7.97%), for SBP and DBP in 407 participants (6.12%), for TG in 746 participants (11.22%), for HDL-c in 765 participants (11.51%), for LDL-c in 834 participants (12.55%) and for fasting blood glucose in 714 participants (10.74%). Finally, there were missing data at 1-year of follow-up for light, moderate and vigorous PA in 527 participants (7.93%), for BMI and body weight in 304 participants (4.57%), for smoking habit in 1278 participants (19.23%), for alcohol and energy intake, and for fruit, vegetable and fruit and vegetable quantity and variety in 710 participants (10.68%), for WC in 604 participants (9.09%), for SBP and DBP in 395 participants (5.94%), for TG in 634 participants (9.54%), for HDL-c in 676 participants (10.17%), for LDL-c in 747 participants (11.24%) and for fasting blood glucose in 608 participants (9.15%).

*BMI* body mass index, *PA* physical activity, *TG* triglycerides, *HDL-c* HDL cholesterol, *LDL-c* LDL cholesterol, *SBP* Systolic blood pressure, *DBP* Diastolic blood pressure, *WC* waist circumference.

[a]Data are presented as *n* (%) for categorical variables and mean ±standard deviation for continuous variables.

[b]Includes senior technician and university degree.

[c]Includes single and religious.

[d]Includes incapacity, sick leaves, students and unemployed.

driven. For its determination, the average amount of fruit and/or vegetables consumed by our participants was determined at baseline, six months and 1-year of follow-up, as well as the average score for variety. A total amount of fruits and/or vegetables below the average amount was considered low quantity, while a total amount equal or upper the average amount was regarded as high quantity. The same approach was followed to determine low and high variety. Consequently, participants were divided into four different categories of fruit and vegetable QV, which are low quantity and variety, low quantity and high variety, high quantity and low variety and high quantity and variety, at baseline, six months and 1-year of follow-up.

Linear mixed-effects models (LMM) with specific random intercepts at recruitment center, family members and patient level, a random slope for each visit (baseline, six months and one year) and with an unstructured correlation matrix were performed to examine the concurrent changes in quantity and variety in FQ intake and cardiometabolic risk factors over the first year of follow-up. For that purpose, exposure variable was considered as both continuous (two-point increment in variety score or 100 g/day increment in quantity) and categorical (divided in 4 categories based on fruit and vegetable QV) being the low quantity and low variety the reference category.

LMM were adjusted for time varying covariates such as energy intake (in kcal/day), body mass index (in kg/m$^2$, except for body weight and WC), physical activity (in Mets·min/week), smoking habit (current/former/never), alcohol consumption (in g/day and adding the quadratic term), MedDiet adherence (0–16 or 0–15 points, as appropriate) and visit (baseline/six months/one year), as well as for baseline information of age (in years), sex (women/men), recruitment center (in quartiles by number of participants), educational level (primary/secondary/tertiary school), intervention group (yes/no), diabetes status (yes/no), hypertension (yes/no) and hypercholesterolemia (yes/no). Furthermore, associations between changes in variety or quantity of fruits and cardiometabolic risk factors were additionally adjusted for variety or quantity of vegetables (continuous), and vice versa.

Statistical interactions between categories of quantity and variety of FV and sex, (men/women), diabetes status at baseline (yes/no), smoking habit (yes/no) and the intervention group (yes/no) were examined by means of likelihood ratio tests, comparing the fully adjusted LMM with and without cross-product terms. For those statistically significant interactions (sex and diabetes status) stratified analysis were conducted. All statistical analyses were conducted using the software Stata 14 (StataCorp, Texas, USA) and the level of significance was set as $p < 0.05$ for bilateral contrast.

## RESULTS

Baseline information of the PREDIMED-plus study participants is shown in Table 1. The mean age of the study participants was 65 years, most of them were married and retired, and women comprised the 51.6% of our population. With regard to lifestyle, the majority of participants were former or non-smoker and spent more time doing light and moderate physical activity rather than vigorous. Participants consumed more quantity of fruits than vegetables and its average score for variety was similar in fruits and vegetables.

Six-month and one-year information on diet, physical activity and cardiometabolic risk factors is also shown in Table 1. Related to diet, an increased in fruit and/or vegetable consumption, in terms of quantity and variety, was observed in our study participants after one-year of follow-up, as well as a decreased in alcohol and energy intake. With regard to lifestyle, the amount of time spent doing physical activity was increased after one-year of follow-up. Finally, a decrease in the majority of the cardiometabolic risk factors (except for HDL-c) was observed after one-year of follow-up.

Table 2 shows β-coefficients and 95% CI for cardiometabolic changes over 1-year according to an increment of two points in the variety score or an increment of 100 grams/day of fruits and vegetables. An increment of two points in the variety score for FV over 1-year was significantly associated with a decrease in fasting blood glucose (−0.32 mg/dL (−0.13, −0.02), $P = 0.017$), body weight (−0.07 kg (−0.13, −0.02), $P = 0.007$) and WC (−0.09 cm (−0.16, −0.01), $P = 0.019$).

Besides, an increase of 100 grams/day of FV over 1-year was associated with a significant decrease in TG levels (−0.52 mg/dL (−0.95, −0.09), $P = 0.017$), fasting blood glucose (−0.21 mg/dL (−0.31, −0.10), $P < 0.001$), body weight (−0.11 kg (−0.15, −0.07), $P = <0.001$) and WC (−0.10 cm (−0.14, −0.06), $P < 0.001$).

Results remained substantially the same when the previous associations were assessed considering only vegetable consumption (Supplementary Table 1). An increment of two points in the variety score for vegetables over 1-year was significantly associated with a decrease in fasting blood glucose (−0.40 mg/dL (−0.72, −0.08), $P = 0.014$), body weight (−0.16 kg (−0.24, −0.08), $P < 0.001$) and WC (−0.17 cm (−0.27, −0.06), $P < 0.001$). Furthermore, an increase of 100 grams per day of vegetables over 1-year was associated with a significant decrease in fasting blood glucose (−0.63 mg/dL (−0.84, −0.42), $P < 0.001$), body weight (−0.19 kg (−0.24, −0.14), $P < 0.001$) and WC (−0.15 cm (−0.22, −0.08), $P < 0.001$).

Considering only fruit consumption, no associations were found between an increment of 2 points in the variety score for fruits and cardiometabolic changes over 1-year. However, an increment of 100 grams per day of fruits over 1-year was significantly associated with a decrease in TG (−0.59 mg/dL (−1.14, −0.04), $P = 0.036$) body weight (−0.07 kg (−0.12, −0.02), $P = 0.005$) and WC (−0.08 cm (−0.14, −0.02), $P = 0.007$) (Supplementary table 2).

Beta-coefficients and 95% CI for cardiometabolic changes over 1-year according to changes in QV categories of FV are represented in Fig. 1. Changes in FV consumption which led to higher quantity and variety over 1-year were associated with downward changes in fasting blood glucose (−1.26 mg/dL (−2.09, −0.43), $P = 0.004$), body weight (−0.40 kg (−0.58, −0.23), $P < 0.001$) and WC (−0.50 cm (−0.73,−0.28), $P < 0.001$) when compared to 1-year changes towards lower variety and quantity of FV. Likewise, changes in FV consumption that led to higher quantity and lower variety over 1-year were also associated with significant downward changes in body weight (−0.27 kg (−0.43, −0.10), $P = 0.001$) and WC (−0.37 cm (−0.59, −0.16), $P < 0.001$).

Moreover, changes in vegetable consumption which led to higher quantity and variety over 1-year were significantly associated with downward changes in fasting blood glucose (−1.24 mg/dL (−1.90, −0.57), $P < 0.001$), body weight (−0.40 kg (−0.58, −0.21), $P < 0.001$) and WC (−0.36 cm (−0.55, −0.17), $P < 0.001$), and upward changes in LDL-cholesterol (0.90 mg/dL (0.09, 1.72, $P = 0.030$), when compared with the reference category (1-year changes towards low quantity and variety in vegetable consumption). Likewise, changes in vegetable consumption that led to higher quantity and lower variety over 1-year were associated with downward changes in SBP (−0.97 mm Hg (−1.51, −0.43), $P < 0.001$), fasting blood glucose (−1.38 mg/dL (−2.23, −0.53), $P = 0.001$), body weight (−0.23 kg (−0.43, −0.04), $P = 0.020$) and WC (−0.27 cm (−0.52–0.02), $P = 0.032$) **(**Supplementary Fig. 1).

However, no significant associations were found between 1-year changes in the combination of fruit quantity and variety and concurrent changes in cardiometabolic risk factors. (Supplementary Fig. 2).

Significant interactions were found between changes in the combination of FV quantity and variety and sex for WC ($P$ for interaction = 0.007). As well, significant interactions were found between changes in the combination of FV quantity and variety and diabetes prevalence for body weight ($P$ for interaction = 0.024) and fasting blood glucose ($P$ for interaction = 0.019). In the stratified analysis, considering these variables, the association between changes that led to higher quantity and variety in FV consumption and concurrent changes in WC over 1-year remained significant in both sexes (β = −0.66 cm and $P < 0.001$ for men and β = −0.34 and $P = 0.029$ cm for women). However, the association between changes towards higher quantity and lower variety and concurrent changes in WC over 1-year remained significant only in

**Table 2.** Cardiometabolic changes over 1 year according to an increase of two points in the fruit and vegetable variety score or the increase of 100 grams per day in fruit and vegetable intake[a].

| Cardiometabolic risk factors[b] | Variety in FV (per each 2 pts increment in the variety score) | | Quantity in FV (per each 100 g/d increment) | |
|---|---|---|---|---|
| | β-coefficient (95% CI) | *p* value | β-coefficient (95% CI) | *p* value |
| TG (mg/dL) | 0.28 (−0.42, 0.97) | 0.435 | −0.52 (−0.95, −0.09) | 0.017 |
| HDL-c (mg/dL) | 0.03 (−0.07, 0.13) | 0.551 | −0.03 (−0.10, 0.04) | 0.438 |
| LDL-c (mg/dL) | 0.18 (−0.02, 0.37) | 0.075 | 0.07 (−0.08, 0.22) | 0.336 |
| SBP (mm Hg) | 0.18 (0.01, 0.34) | 0.038 | −0.01 (−0.14, 0.13) | 0.923 |
| DBP (mm Hg) | 0.07 (−0.01, 0.14) | 0.068 | 0.01 (−0.08, 0.09) | 0.950 |
| Fasting glucose (mg/dL) | −0.32 (−0.58, −0.06) | 0.017 | −0.21 (−0.31, −0.10) | <0.001 |
| Weight (kg) | −0.07 (−0.13, −0.02) | 0.007 | −0.11 (−0.15, −0.07) | <0.001 |
| WC (cm) | −0.09 (−0.16, −0.01) | 0.019 | −0.10 (−0.14, −0.06) | <0.001 |

*TG* triglycerides, *HDL-c* HDL cholesterol, *LDL-c* LDL cholesterol, *SBP* Systolic blood pressure, *DBP* Diastolic blood pressure, *WC* waist circumference.
[a]Results are β-coefficients (95% CI) from the LMM adjusted for baseline covariates such as age (in years), sex (male/female), intervention group (yes/no), recruitment center (in quartiles by number of participants), educational level (primary/secondary/tertiary school) and presence of diabetes (yes/no), hypertension (yes/no) and hypercholesterolemia (yes/no), and for changes from baseline in smoking habit (current/former/never), marital status (married/widowed/divorced/other), living alone (yes/no), employment status (retired/employed/housekeeper/others), energy intake (in kcal/d), alcohol consumption (g/day and adding the quadratic term), physical activity (Mets.min/week), body mass index (except for body weight and WC) and 15-point screener of Mediterranean diet adherence without considering the contribution of fruits and vegetables (continuous).
[b]A total of 6538 participants were analyzed for TG changes, 6524 participants for HDL-c changes, 6502 participants for LDL-c changes, 6546 participants for SBP changes, 6546 participants for DBP changes, 6542 participants for blood glucose changes, 6559 participants for weight changes and 6559 participants for WC changes.

men (β = −0.62 cm and $P = 0.001$). Furthermore, the association between changes which led to higher quantity and variety in FV consumption and concurrent changes in fasting blood glucose over 1-year remained significant in diabetic (β = −2.39 mg/dL and $P = 0.043$) and non-diabetic participants (β = −0.72 mg/dL and $P = 0.011$). Finally, the association between changes towards higher quantity and variety in FV consumption and concurrent changes in body weight over 1-year remained significant in diabetic (β = −0.51 cm and $P < 0.001$) and non-diabetic participants (β = −0.36 kg and $P = 0.001$). However, the association between changes that led to higher quantity and lower variety in FV consumption and concurrent changes in body weight over 1-year remained significant only in non-diabetic participants (β = −0.39 cm and $P < 0.001$).

Although no significant interactions were found between changes in the combination of FV quantity and variety and the intervention group for cardiometabolic risk factors, β-coefficients and 95% CI for cardiometabolic changes over 1-year stratified by the intervention group were also shown according to the combination of FV variety and quantity (Supplementary Fig. 3), fruit variety and quantity (Supplementary Fig. 4), and vegetable variety and quantity (Supplementary Fig. 5). Overall, the associations observed in all the participants were in the same direction for each of the intervention groups analyzed separately.

## DISCUSSION

To the best of our knowledge, this is the first study which have longitudinally assessed associations between not only quantity, but also variety (expressed as a continuous score) in FV consumption, combined or separately, with several cardiometabolic risk factors in elderly subjects at high cardiovascular risk. The results showed a decrease in some cardiometabolic risk factors by increasing 2 points in the variety score or 100 g/day of FV consumption over one year. Furthermore, changes in FV consumption, which led to higher variety and quantity over one year, were associated with downward changes in some cardiometabolic risk factors.

To date, the association between FV consumption and cardiometabolic risk factors have been widely investigated in epidemiological studies considering mostly the total amount of FV consumed per day [7–11], although there is controversy around the results. In the present study, a significant decrease in TG, fasting blood glucose, body weight, and WC by an increment of 100 g/day of FV was reported. This is in line with a meta-analysis of prospective studies that revealed an inverse association between increasing FV intake body weight at 4 years of follow-up [26]. Besides, a meta-analysis of cross-sectional studies reported that greater intakes of fruits were inversely associated with TG levels [7]. Finally, a cross-sectional study in urban south Indians determined that higher intakes of FV were inversely associated with WC [27]. However, two systematic reviews and meta-analysis of RCTs showed no discernible effect on body weight by increasing FV consumption [10, 11]. This lack of association might be due to the short period of follow-up observed in the majority of the included studies in both meta-analysis [10, 11], which is less than 10 and 12 weeks, respectively.

However, only few studies have investigated the association between FV variety (expressed as continuous scores or color groups) and cardiometabolic risk factors. Our results of decreases in fasting blood glucose, body weight and WC by increasing 2 points in the variety score over one year are in line with previous longitudinal and cross-sectional [12, 13] studies. In fact, a previous cross-sectional analysis conducted by of our research group reported that higher intakes of red-purple and white fruits were associated with lower baseline levels of fasting blood glucose and WC, respectively, in the PREDIMED-Plus study [13]. Moreover, Mirmiran et al reported inverse associations between higher intakes of red and purple FV and changes in body weight and WC in men, and changes in fasting blood glucose and body weight in women, at 3 years of follow-up in a representative population of Tehran [12]. Nevertheless, Lamb et al. did not find longitudinal associations between changes in FV variety, combined and separately, with cardiometabolic risk factors at 1 and 5 years of follow-up in middle-aged participants with type 2 diabetes [28]. Study differences in terms of population, FV variety score and follow-up period may explain differences in results found by these investigations.

On the other hand, we surprisingly reported a significant positive association between an increase in FV variety over one

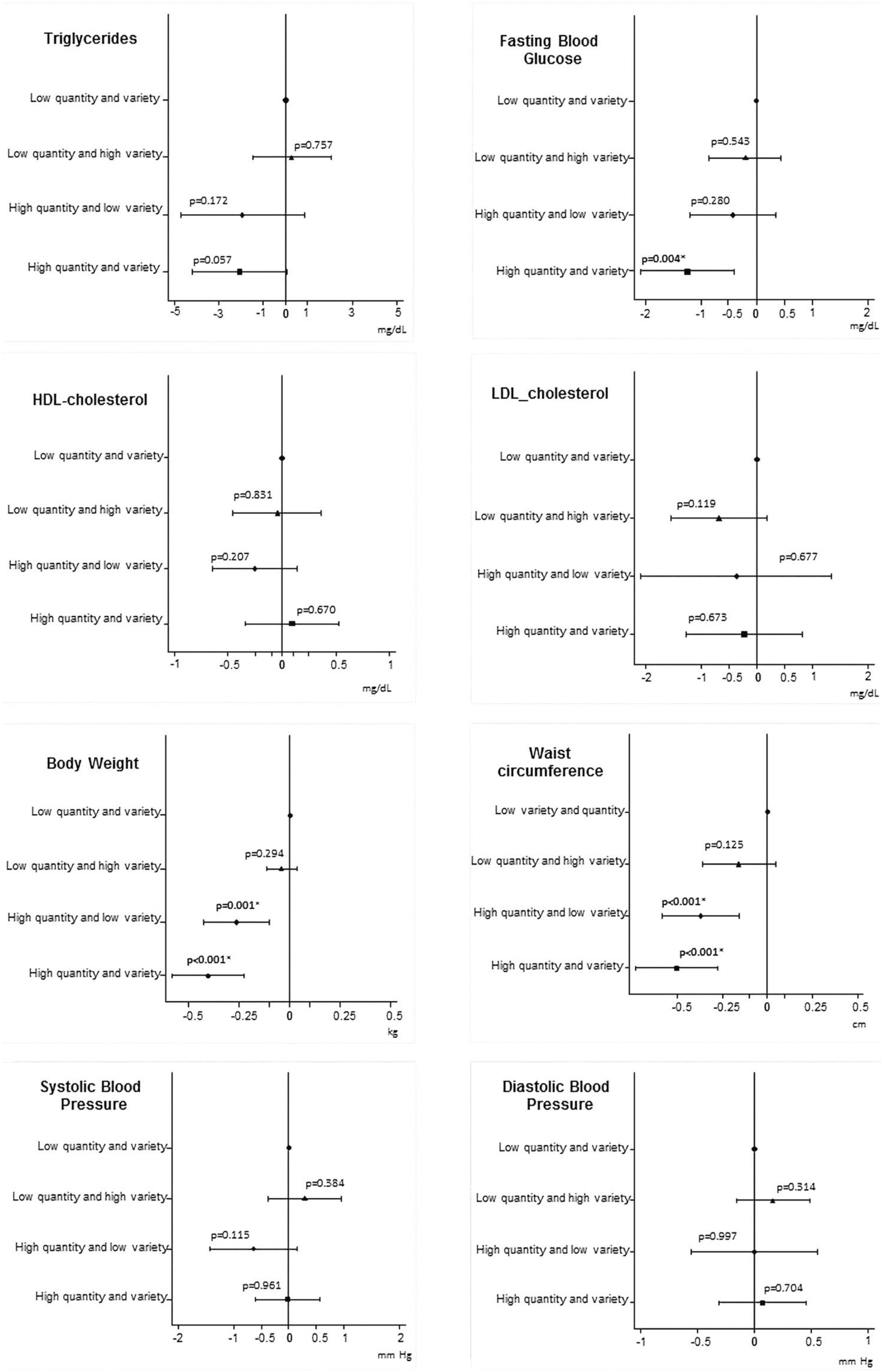


**Fig. 1 Beta-coefficients and 95% CI for cardiometabolic changes by 1-year changes in categories of fruit and vegetable quantity and variety.** LMM were adjusted for baseline covariates such as age (in years), sex (male/female), intervention group (yes/no), recruitment center (in quartiles by number of participants), educational level (primary/secondary/tertiary school), and presence of diabetes (yes/no), hypertension (yes/no) and hypercholesterolemia (yes/no), and for changes from baseline in smoking habit (current/former/never), marital status (married/widowed/divorced/other), living alone (yes/no), employment status (retired/employed/housekeeper/others), energy intake (in kcal/d), alcohol consumption (g/day and adding the quadratic term), physical activity (Mets.min/week), body mass index (except for body weight and WC) and 15-point screener of Mediterranean diet adherence without considering the contribution of fruits and vegetables (continuous). A total of 6538 participants were analyzed for triglyceride changes, 6524 participants for HDL-cholesterol changes, 6502 participants for LDL-cholesterol changes, 6546 participants for systolic blood pressure changes, 6546 participants for diastolic blood pressure changes, 6542 participants for fasting blood glucose changes, 6559 participants for body weight changes and 6559 participants for waist circumferences changes. Adjusted *P*-values were obtained by the Benjamini–Hochberg method to decrease the false discovery rate (FDR).

year and SBP. In line with our findings, some epidemiological studies which have assessed different groups and subgroups of FV instead of a FV variety score, have demonstrated that not all FV subtypes were associated to blood pressure levels or hypertension risk in the same direction. For instance, citrus consumption was associated with higher levels of blood pressure in Western participants from the INTERMAP Study [29]. Besides, some vegetables such as cruciferous, green beans, onions and Brussel sprouts have been significantly associated with an increased risk of developing hypertension [30, 31]. Although there is no clear explanation for these findings, previous studies have suggested that the different cooking techniques for some vegetables and the addition of salt might counteract the beneficial effects of FV on blood pressure [30, 32]. Nonetheless, further longitudinal studies are needed to confirm our results related to blood pressure, as well as to clarify its potential associated mechanisms, especially in elderly individuals at high cardiovascular risk.

Finally, the present study also reported that changes in FV intake which led to higher quantity and variety were significantly associated with downward changes in fasting blood glucose, body weight and WC. Besides, changes in FV consumption towards higher quantity and lower variety were significantly associated with downward changes in body weight and WC in our study. To the best of our knowledge, this is the first study assessing associations between FV consumption and some cardiometabolic risk factors considering jointly FV variety and quantity.

The benefits of FV on cardiovascular health may be related to the potential interactions between its different constituents [6]. FV are good sources of dietary fiber, vitamins C and E, potassium, and phytochemicals (especially beta-carotene and polyphenols), which may act synergistically to promote normal body function [1, 33]. Specifically, previous studies have reported higher intakes of vitamins, minerals, dietary fiber and phytochemicals related to greater variety of FV intake [34, 35]. Phytochemicals contributes to cardiovascular health by reducing oxidative stress, inhibiting LDL oxidation and platelet aggregation, lowering blood pressure and inflammation and modulating the synthesis and absorption of cholesterol, due to its antioxidant and anti-inflammatory activities [36–38]. Besides, dietary fiber attenuates postprandial levels of blood glucose and lipoproteins, reduces circulating cholesterol levels, promotes satiation, alters secretion of gut hormones and decreases absorption of macronutrients [39, 40]. Plant-based dietary fiber and flavonoids may mediate these beneficial effects partly through their interaction with gut microbiota [41]. In particular, both may synergistically enhance the growth of beneficial bacteria within the gut, while inhibiting the growth of certain pathogenic bacteria [42].

The present study has some strengths. Firstly, the present investigation used repeated measures for all exposures and outcomes over 1 year of follow-up and all analyses were adjusted for several potential confounders. Secondly, this is the first time that the association between FV consumption and some cardiometabolic risk factors has been analyzed in a joint analysis considering both quantity and variety of FV. Thirdly, it is important to mention that the assessment of these previous associations in an elderly Mediterranean population at high cardiovascular risk is another added value for the present investigation as it is the first time that these associations have been longitudinally reported for this population.

However, the present study has some limitations. Firstly, although FFQ have been widely used in epidemiological studies [43], recall bias cannot be ruled out because it depends on participant´s memory. Besides, the use of FFQ may difficult the real FV intake assessment accurately. Secondly, the sample consisted in a Mediterranean population at high cardiovascular risk, and therefore results cannot be extrapolated to the general population or other populations. Thirdly, the observational nature of the study design does not allow establishing a cause-effect relationship and are not exempt to some bias associated with residual or unmeasured confounding. Fourthly, our follow-up period was relatively short (only one year) [44]. Therefore, further well-conducted longitudinal studies with a longer follow-up are needed to confirm our results. Finally, the results of the present study derived from a lifestyle interventional study, whose main objective was weight loss. Therefore, our results for weight changes could have been influenced by the intervention. However, our analyses were adjusted for the intervention group, and furthermore, no interaction was found between the intervention group and our exposure variable (FV quantity and/or variety) for weight changes. Moreover, when the analyses were stratified by the intervention group (intervention and control), the results were in the same line with those for the entire cohort.

## CONCLUSION

In conclusion, the current study demonstrated that greater variety, in combination with higher quantity of FV was significantly associated with a decrease in several cardiometabolic risk factors among middle-aged subjects at high cardiovascular risk, suggesting that not only quantity but also variety in these food groups may play an important role on CVD risk reduction. Therefore, as previous studies have reported higher intakes of vitamins, minerals, dietary fiber and phytochemicals related to greater variety of FV intake, a wide range of FV should be consumed daily for the proper body function.

## DATA AVAILABILITY

The datasets generated and analyzed during the current study are not expected to be made available outside the core research group, as neither participants' consent forms nor ethics approval included permission for open access. However, the researchers will follow a controlled data-sharing collaboration model, as in the informed consent participants agreed with a controlled collaboration with other investigators for research related to the project's aims. Therefore, investigators who are interested in this study can contact the PREDIMED Steering Committee by sending a request letter to predimed_scommittee@googlegroups.com. A data-sharing agreement indicating the characteristics of the collaboration and data management will be completed for the proposals that are approved by the Steering Committee.

## REFERENCES

1. Slavin JL, Lloyd B. Health benefits of fruits and vegetables. Adv Nutr. 2012;3:506–16. https://doi.org/10.3945/an.112.002154.
2. World Health Organization. Global Strategy on Diet, Physical Activity and Health. Promoting fruit and vegetable consumption around the world. https://www.who.int/dietphysicalactivity/fruit/en/index2.html. Accessed 21 April 2021.
3. Micha R, Khatibzadeh S, Shi P, Andrews KG, Engell RE, Mozaffarian D. Global, regional and national consumption of major food groups in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys worldwide. BMJ Open. 2015;5:e008705 https://doi.org/10.1136/bmjopen-2015-008705.
4. Wang X, Ouyang Y, Liu J, Zhu M, Zhao G, Bao W, et al. Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. BMJ. 2014;349:1–14. https://doi.org/10.1136/bmj.g4490.
5. Rodriguez-Casado A. The health potential of fruits and vegetables phytochemicals: notable examples. Crit Rev Food Sci Nutr. 2016;56:1097–107. https://doi.org/10.1080/10408398.2012.755149.
6. Liu RH. Health-promoting components of fruits and vegetables in the diet. Adv Nutr. 2013;4:384S–392S. https://doi.org/10.3945/an.112.003517.
7. Kodama S, Horikawa C, Fujihara K, Ishii D, Hatta M, Takeda Y, et al. Relationship between intake of fruit separately from vegetables and triglycerides - a meta-analysis. Clin Nutr. 2018;27:53–8. https://doi.org/10.1016/j.clnesp.2018.07.001.
8. Toh DWK, Koh ES, Kim JE. Incorporating healthy dietary changes in addition to an increase in fruit and vegetable intake further improves the status of cardiovascular disease risk factors: a systematic review, meta-regression, and meta-analysis of randomized controlled trials. Nutr Rev. 2020;78:532–45. https://doi.org/10.1093/nutrit/nuz104.

9. Shin JY, Kim JY, Kang HT, Han KH, Shim JY. Effect of fruits and vegetables on metabolic syndrome: a systematic review and meta-analysis of randomized controlled trials. Int J Food Sci Nutr. 2015;66:416–25. https://doi.org/10.3109/09637486.2015.1025716.
10. Kaiser KA, Brown AW, Brown MMB, Shikany JM, Mattes RD, Allison DB. Increased fruit and vegetable intake has no discernible effect on weight loss: a systematic review and meta-analysis. Am J Clin Nutr. 2014;100:567–76. https://doi.org/10.3945/ajcn.114.090548.
11. Mytton OT, Nnoaham K, Eyles H, Scarborough P, Ni Mhurchu C. Systematic review and meta-analysis of the effect of increased vegetable and fruit consumption on body weight and energy intake. BMC Public Health. 2014;14:1–11. https://doi.org/10.1186/1471-2458-14-886.
12. Mirmiran P, Bahadoran Z, Moslehi N, Bastan S, Azizi F. Colors of fruits and vegetables and 3-year changes of cardiometabolic risk factors in adults: Tehran lipid and glucose study. Eur J Clin Nutr. 2015;69:1215–9. https://doi.org/10.1038/ejcn.2015.49.
13. Becerra-Tomás N, Paz-Graniel I, Tresserra-Rimbau A, Martínez-González MÁ, Barrubés L, Corella D, et al. Fruit consumption and cardiometabolic risk in the PREDIMED-plus study: a cross-sectional analysis. Nutr Metab Cardiovasc Dis. 2021;31:1702–13. https://doi.org/10.1016/j.numecd.2021.02.007.
14. Martínez-González MA, Buil-Cosiales P, Corella D, Bulló M, Fitó M, Vioque J, et al. Cohort profile: design and methods of the PREDIMED-Plus randomized trial. Int J Epidemiol. 2019;48:387–388o. https://doi.org/10.1093/ije/dyy225.
15. Salas-Salvadó J, Díaz-López A, Ruiz-Canela M, Basora J, Fitó M, Corella D, et al. Effect of a lifestyle intervention program with energy-restricted Mediterranean diet and exercise on weight loss and cardiovascular risk factors: one-year results of the PREDIMED-Plus trial. Diabetes Care. 2019;42:777–88. https://doi.org/10.2337/dc18-0836.
16. Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; National heart, lung, and blood institute; American heart association; World heart federation; International. Circulation. 2009;120:1640–5. https://doi.org/10.1161/CIRCULATIONAHA.109.192644.
17. Forman M. Nutritional epidemiology. Am J Clin Nutr. 1999;69:1020 https://doi.org/10.1093/ajcn/69.5.1020.
18. Fernández-Ballart JD, Piñol JL, Zazpe I, Corella D, Carrasco P, Toledo E, et al. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br J Nutr. 2010;103:1808–16. https://doi.org/10.1017/S0007114509993837.
19. Oude Griep LM, Verschuren WMM, Kromhout D, Ocké MC, Geleijnse JM. Variety in fruit and vegetable consumption and 10-year incidence of CHD and stroke. Public Health Nutr. 2012;15:2280–6. https://doi.org/10.1017/S1368980012000912.
20. Conklin AI, Forouhi NG, Suhrcke M, Surtees P, Wareham NJ, Monsivais P. Variety more than quantity of fruit and vegetable intake varies by socioeconomic status and financial hardship. Findings from older adults in the EPIC cohort. Appetite. 2014;83:248–55. https://doi.org/10.1016/j.appet.2014.08.038.
21. Cooper AJ, Khaw KT, Sharp SJ, Wareham NJ, Lentjes MAH, Forouhi NG, et al. A prospective study of the association between quantity and variety of fruit and vegetable intake and incident type 2 diabetes. Diabetes Care. 2012;35:1293–1300. https://doi.org/10.2337/dc11-2388.
22. Jeurnink SM, Büchner FL, Bueno-De-Mesquita HB, Siersema PD, Boshuizen HC, Numans ME, et al. Variety in vegetable and fruit consumption and the risk of gastric and esophageal cancer in the European prospective investigation into cancer and nutrition. Int J Cancer. 2012;131:E963–E973. https://doi.org/10.1002/ijc.27517.
23. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499–502.
24. Molina L, Sarmiento M, Peñafiel J, Donaire D, Garcia-Aymerich J, Gomez M, et al. Validation of the Regicor short physical activity questionnaire for the adult population. PLoS One. 2017;12:1e14 https://doi.org/10.1371/journal.pone.0168148.
25. Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota leisure time physical activity questionnaire in Spanish men. Am J Epidemiol. 1994;139:1197–209. https://doi.org/10.1093/oxfordjournals.aje.a116966.
26. Schröder H, Fitó M, Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, et al. A Short screener is valid for assessing mediterranean diet adherence among older spanish men and women. J Nutr. 2011;141:1140–5. https://doi.org/10.3945/jn.110.135566.
27. Bertoia ML, Mukamal KJ, Cahill LE, Hou T. Changes in intake of fruits and vegetables and weight change in United States men and women followed for up to 24 years: analysis from three prospective cohort studies. PLOS Med. 2015;12:e1001878 https://doi.org/10.1371/journal.pmed.1001878.
28. Radhika G, Sudha V, Mohan Sathya R, Ganesan A, Mohan V. Association of fruit and vegetable intake with cardiovascular risk factors in urban south Indians. Br J Nutr. 2008;99:398–405. https://doi.org/10.1017/S0007114507803965.
29. Lamb MJE, Griffin SJ, Sharp SJ, Cooper AJM. Fruit and vegetable intake and cardiovascular risk factors in people with newly diagnosed type 2 diabetes. Eur J Clin Nutr. 2017;71:115–21. https://doi.org/10.1038/ejcn.2016.180.
30. Griep LMO, Stamler J, Chan Q, Van Horn L, Steffen LM, Miura K, et al. Association of raw fruit and fruit juice consumption with blood pressure: The INTERMAP study. Am J Clin Nutr. 2013;97:1083–91. https://doi.org/10.3945/ajcn.112.046300.
31. Borgi L, Muraki I, Satija A, Willett WC, Rimm EB, Forman JP. Fruit and vegetable consumption and the incidence of hypertension in three prospective cohort studies. Hypertension. 2016;67:288–93. https://doi.org/10.1039/c5fo01096a.
32. Wang L, Manson JE, Gaziano JM, Buring JE, Sesso HD. Fruit and vegetable intake and the risk of hypertension in middle-aged and older women. Am J Hypertens. 2012;25:180–9. https://doi.org/10.1038/ajh.2011.186.
33. Stefler D, Malyutina S, Nikitin Y, Nikitenko T, Rodriguez-Artalejo F, Peasey A, et al. Fruit, vegetable intake and blood pressure trajectories in older age. J Hum Hypertens. 2019;33:671–8. https://doi.org/10.1038/s41371-019-0189-8.
34. Randall E, Nichaman MZ, Contant CFJ. Diet diversity and nutrient intake. J Am Diet Assoc. 1985;85:830–6.
35. Foote JA, Murphy SP, Wilkens LR, Basiotis PP, Carlson A. Dietary variety increases the probability of nutrient adequacy among adults. J Nutr. 2004;134:1779–85. https://doi.org/10.1093/jn/134.7.1779.
36. López-González L, Becerra-Tomás N, Babio N, Martínez-González MÁ, Díaz-López A, Corella D, et al. Variety in fruits and vegetables, diet quality and lifestyle in an older adult mediterranean population. Clin Nutr. 2021;40:1510–8. https://doi.org/10.1016/j.clnu.2021.02.024.
37. Gorzynik-Debicka M, Przychodzen P, Cappello F, Kuban-Jankowska A, Gammazza AM, Knap N, et al. Potential health benefits of olive oil and plant polyphenols. Int J Mol Sci. 2018;19:686 https://doi.org/10.3390/IJMS19030686.
38. Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP, et al. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules. 2015;20:21138–56. https://doi.org/10.3390/molecules201219753.
39. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr. 2003;78:517S–520S. https://doi.org/10.1093/AJCN/78.3.517S.
40. Slavin JL. Dietary fiber and body weight. Nutrition. 2005;21:411–8. https://doi.org/10.1016/j.nut.2004.08.018.
41. Alissa EM, Ferns GA. Dietary fruits and vegetables and cardiovascular diseases risk. Crit Rev Food Sci Nutr. 2017;57:1950–62. https://doi.org/10.1080/10408398.2015.1040487.
42. Klinder A, Shen Q, Heppel S, Lovegrove JA. Impact of increasing fruit and vegetables and flavonoid intake on the hum gut microbiota. Food Funct. 2016;7:1788–96. https://doi.org/10.1039/c5fo01096a.
43. Wan MLY, Ling KH, El-Nezami H, Wang MF. Influence of functional food components on gut health. Crit Rev Food Sci Nutr. 2019;59:1927–36. https://doi.org/10.1080/10408398.2018.1433629.
44. Shim JS, Oh K, Kim HC. Dietary assessment methods in epidemiologic studies. Epidemiol Health. 2014;36:e2014009 https://doi.org/10.4178/epih/e2014009.

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All the principal PREDIMED-plus investigators contributed to study concept and design and to data extraction from the participants. LL-G, NB-T, NB and JS-S performed the statistical analyses. LL-G, NB-T, NB and JS-S drafted the manuscript. All authors reviewed the manuscript for important intellectual content and approved the final version to be published.

## COMPETING INTERESTS

JS-S serves on the board of (and receives grant support through his institution from) the International Nut and Dried Fruit Council and the Eroski Foundation. He also serves on the Executive Committee of the Instituto Danone, Spain, and on the Scientifc Committee of the Danone International Institute. He has received research support from the Patrimonio Comunal Olivarero, Spain, and Borges S.A., Spain. He receives consulting fees or travel expenses from Eroski Foundation, the Instituto Danone, Spain, Abbot Laboratories, and Mundipharma. ER has received research funding through his institution from the California Walnut Commission, Folsom, CA, USA; was a paid member of its Health Research Advisory Group; and is a non-paid member of its Scientifc Advisory Council. SKN is a volunteer member of the non-for-profit organization called Plant-Based Canada. RE reports grants from Cerveza y Salud, Spain and Fundación Dieta Mediterranea, Spain. Also personal fees for given lectures from Brewers of Europe, Belgium; Fundación Cerveza y Salud, Spain; Pernaud-Ricard, Mexico; Instituto Cervantes, Alburquerque, USA; Instituto Cervantes, Milano, Italy; Instituto Cervantes, Tokio, Japan; Lilly Laboratories, Spain and Wine and Culinary International Forum, Spain and non-financial support to organize a National Congress on Nutrition. Also feeding trials with product from Grand Fountain and Uriach Laboratories, Spain. LL-G, NB-T, NB, MAM-G, DC, AG, DR, JV, AMA-G, JW, JAM, LS-M, FT, JL, XP, JAT, JL-M, AB-C, MD-R, PM-M, LD, VM-S, JV, CV, ZV-R, FM-L, JV-S, OC, MA-Z, LT-S, RC-M, JR-M, ER, AG-R, RC, JS-L, PB, EM-A, CL, IA, IS-L, MC-S and HS have nothing to declare.

## ETHICS APPROVAL

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 les 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 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 del Instituto Madrileño de Estudios Avanzados, CEIC del Hospital Clínico San Carlos, CCEI de la Investigación Biomédica de Andalucía and CCEIC de León. The code of the Ethical Committe aproval of the Cordinated Center (CEIC del Hospital Universitari Sant Joan de Reus) is 13-07-25/7proj2 (approval date: 30/07/2013).

## ADDITIONAL INFORMATION

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**Supplemental table 1. Cardiometabolic changes over one year according to an increase of two points in the vegetable variety score and the increase of 100 g per day in vegetable intake[1].**

| | **Variety in vegetables** (per each 2 pts of increment in the variety score) | | **Quantity in vegetables** (per each 100 g/d of increment) | |
|---|---|---|---|---|
| **Cardiometabolic risk factors[2]** | β-coefficient (95%CI) | p-value | β-coefficient (95%CI) | p-value |
| TG (mg/dL) | -0.53 (-1.36, 0.29) | 0.206 | -0.39 (-1.51, 0.74) | 0.502 |
| HDL-c (mg/dL) | 0.06 (-0.07, 0.19) | 0.398 | -0.08 (-0.22, 0.05) | 0.231 |
| LDL-c (mg/dL) | 0.17 (-0.22, 0.57) | 0.392 | 0.18 (-0.15, 0.50) | 0.286 |
| SBP (mm Hg) | 0.23 (-0.02, 0.48) | 0.072 | -0.16 (-0.40, 0.07) | 0.174 |
| DBP (mm Hg) | 0.15 (-0.02, 0.31) | 0.081 | -0.07 (-0.23, 0.09) | 0.391 |
| Fasting blood glucose (mg/dL) | -0.40 (-0.72, 0.08) | 0.014 | -0.63 (-0.84, -0.42) | <0.001 |
| Weight (kg) | -0.16 (-0.24, -0.08) | <0.001 | -0.19 (-0.24 -0.14) | <0.001 |
| WC (cm) | -0.17 (-0.27, -0.06) | <0.002 | -0.15 (-0.22, -0.08) | <0.001 |

Abbreviations: TG, triglycerides; HDL-c, HDL cholesterol, LDL-c, LDL cholesterol, SBP, Systolic blood pressure; DBP, Diastolic blood pressure; WC, waist circumference.

[1]Results are β-coefficients (95% CI) from the LMM adjusted for baseline covariates such as age (in years), sex (male/female), intervention group (yes/no), recruitment center (in quartiles by number of participants), educational level (primary/secondary/tertiary school) and presence of diabetes (yes/no), hypertension (yes/no) and hypercholesterolemia (yes/no), and for changes from baseline in smoking habit (current/former/never), marital status (Married/Widowed/Divorced/other), living alone (yes/no), employment status (retired/employed/housekeeper/others), energy intake (in kcal/d), alcohol consumption (g/day and adding the quadratic term), physical activity (Mets.min/week), body mass index (except for body weight and WC) and 16-point screener of Mediterranean diet adherence without considering the contribution of vegetables (continuous).
LMM with variety in vegetables as exposure were additionally adjusted for fruit variety (continuous). LMM with quantity in vegetables were additionally adjusted for fruit quantity (continuous).

[2]A total of X participants were analyzed for TG changes, X participants for HDL-c changes, X participants for LDL-c changes, X participants for SBP changes, X participants for DBP changes, X participants for blood glucose changes, X participants for weight changes and X participants for WC changes

SUPPLEMENTARY ONLINE MATERIAL 2

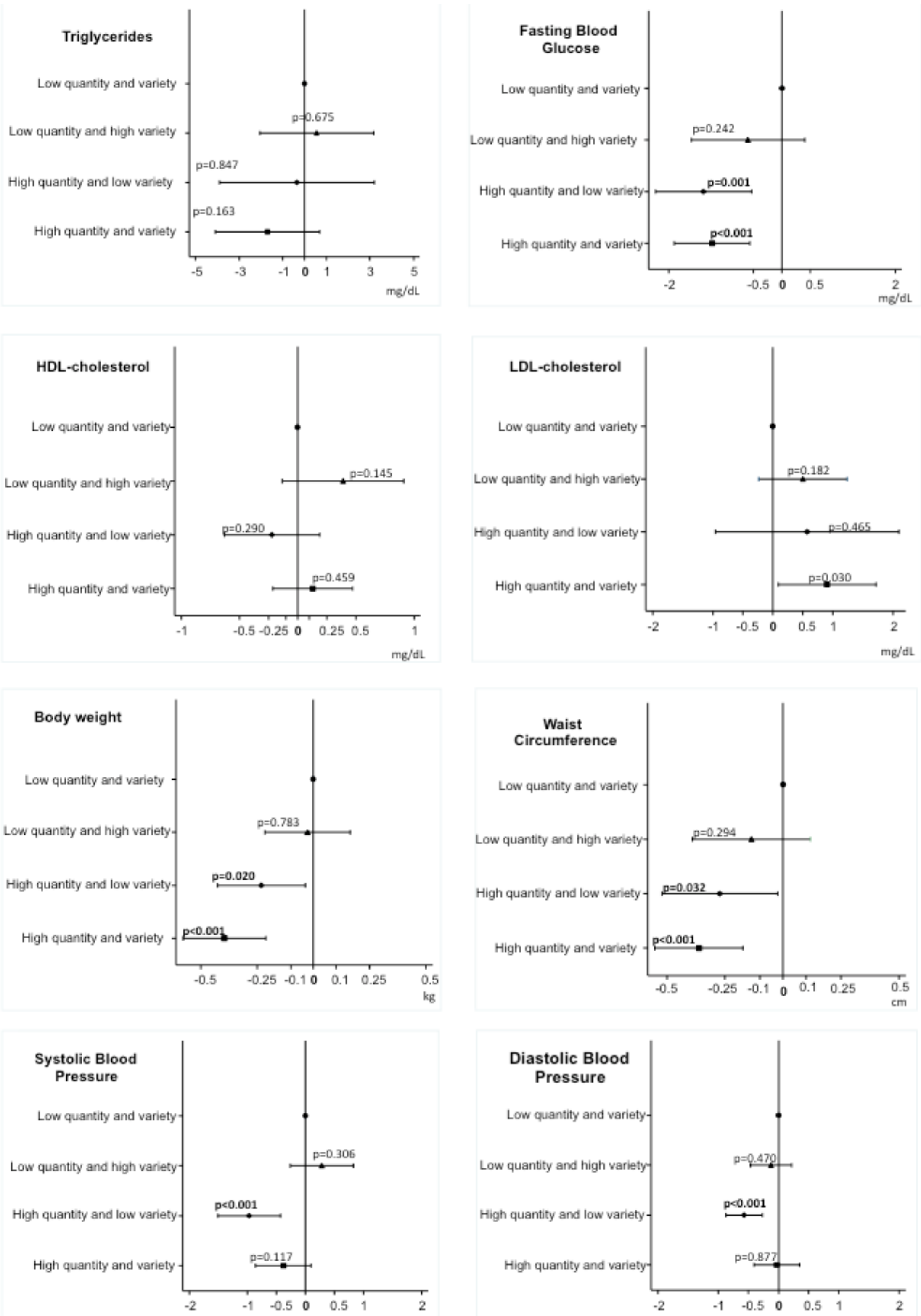
**Supplemental table 2. Cardiometabolic changes over one year according to an increase of two points in the fruit variety score and the increase of 100 g per day in fruit intake[1].**

| | **Variety in fruits** (per each 2 pts of increment in the variety score) | | **Quantity in fruits** (per each 100 g/d of increment) | |
|---|---|---|---|---|
| **Cardiometabolic risk factors[2]** | β-coefficient (95%CI) | p-value | β-coefficient (95%CI) | p-value |
| TG (mg/dL) | 1.06 (0.17, 1.94) | 0.019 | -0.59 (-1.14, -0.04) | 0.036 |
| HDL-c (mg/dL) | 0.01 (-0.16, 0.17) | 0.948 | -0.01 (-0.08, 0.07) | 0.964 |
| LDL-c (mg/dL) | 0.20 (-0.20, 0.59) | 0.335 | 0.03 (-0.19, 0.25) | 0.761 |
| SBP (mm Hg) | 0.13 (-0.15, 0.41) | 0.362 | 0.07 (-0.11, 0.25) | 0.462 |
| DBP (mm Hg) | -0.01 (-0.13, 0.12) | 0.969 | 0.04 (-0.06, 0.14) | 0.465 |
| Fasting blood glucose (mg/dL) | -0.22 (-0.57, 0.12) | 0.210 | -0.01 (-0.14, 0.12) | 0.902 |
| Weight (kg) | 0.01 (-0.07, 0.08) | 0.833 | -0.07 (-0.12, -0.02) | 0.005 |
| WC (cm) | -0.01 (-0.12, 0.11) | 0.960 | -0.08 (-0.14, -0.02) | 0.007 |

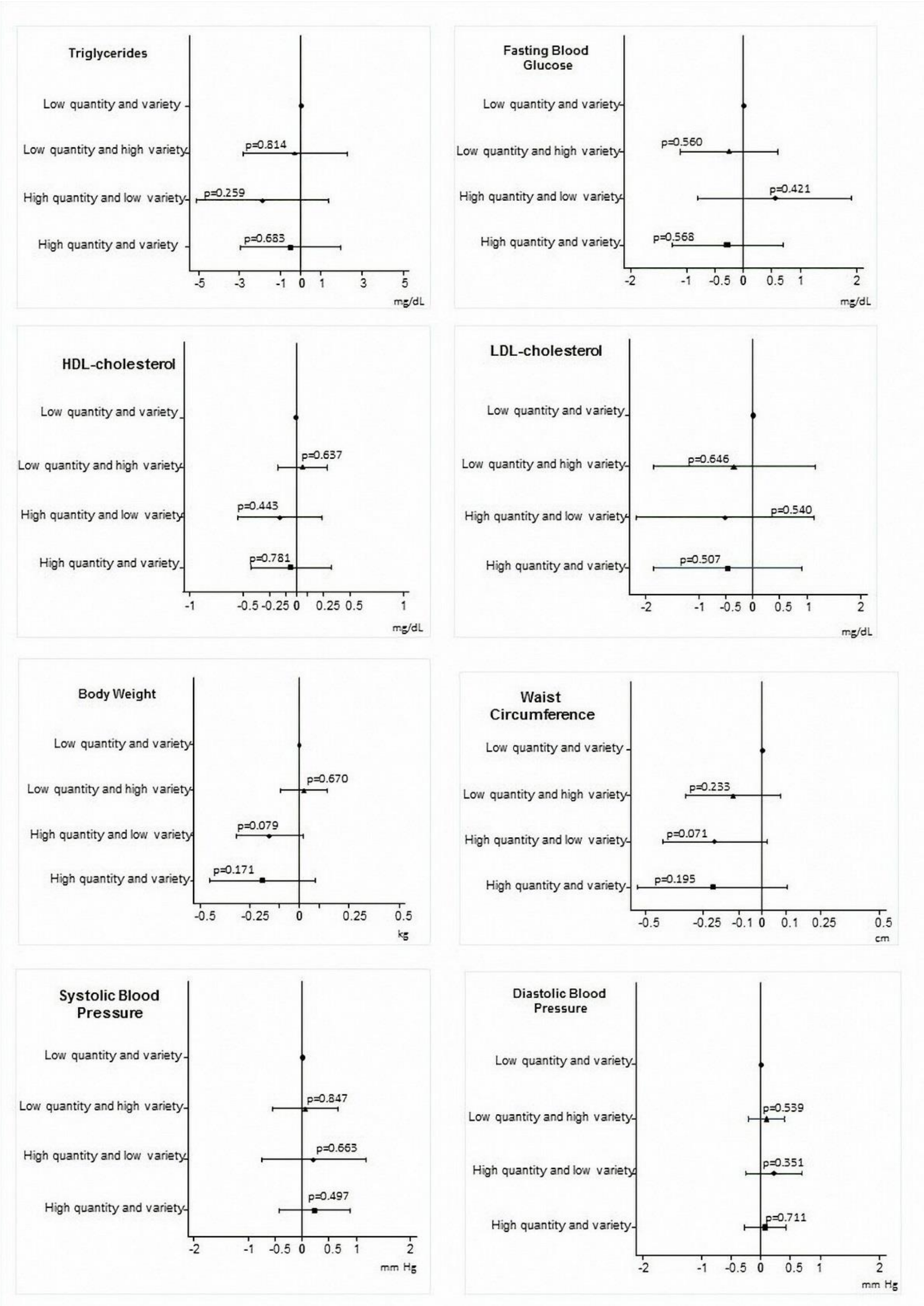
Abbreviations: TG, triglycerides; HDL-c, HDL cholesterol, LDL-c, LDL cholesterol, SBP, Systolic blood pressure; DBP, Diastolic blood pressure; WC, waist circumference.

[1]Results are β-coefficients (95% CI) from the LMM adjusted for baseline covariates such as age (in years), sex (male/female), intervention group (yes/no), recruitment center (in quartiles by number of participants), educational level (primary/secondary/tertiary school) and presence of diabetes (yes/no), hypertension (yes/no) and hypercholesterolemia (yes/no), and for changes from baseline in smoking habit (current/former/never), marital status (Married/Widowed/Divorced/other), living alone (yes/no), employment status (retired/employed/housekeeper/others), energy intake (in kcal/d), alcohol consumption (g/day and adding the quadratic term), physical activity (Mets.min/week), body mass index (except for body weight and WC) and 16-point screener of Mediterranean diet adherence without considering the contribution of fruits (continuous). LMM with variety in fruits as exposure were additionally adjusted for vegetable variety (continuous). LMM with quantity in fruits were additionally adjusted for vegetable quantity (continuous).

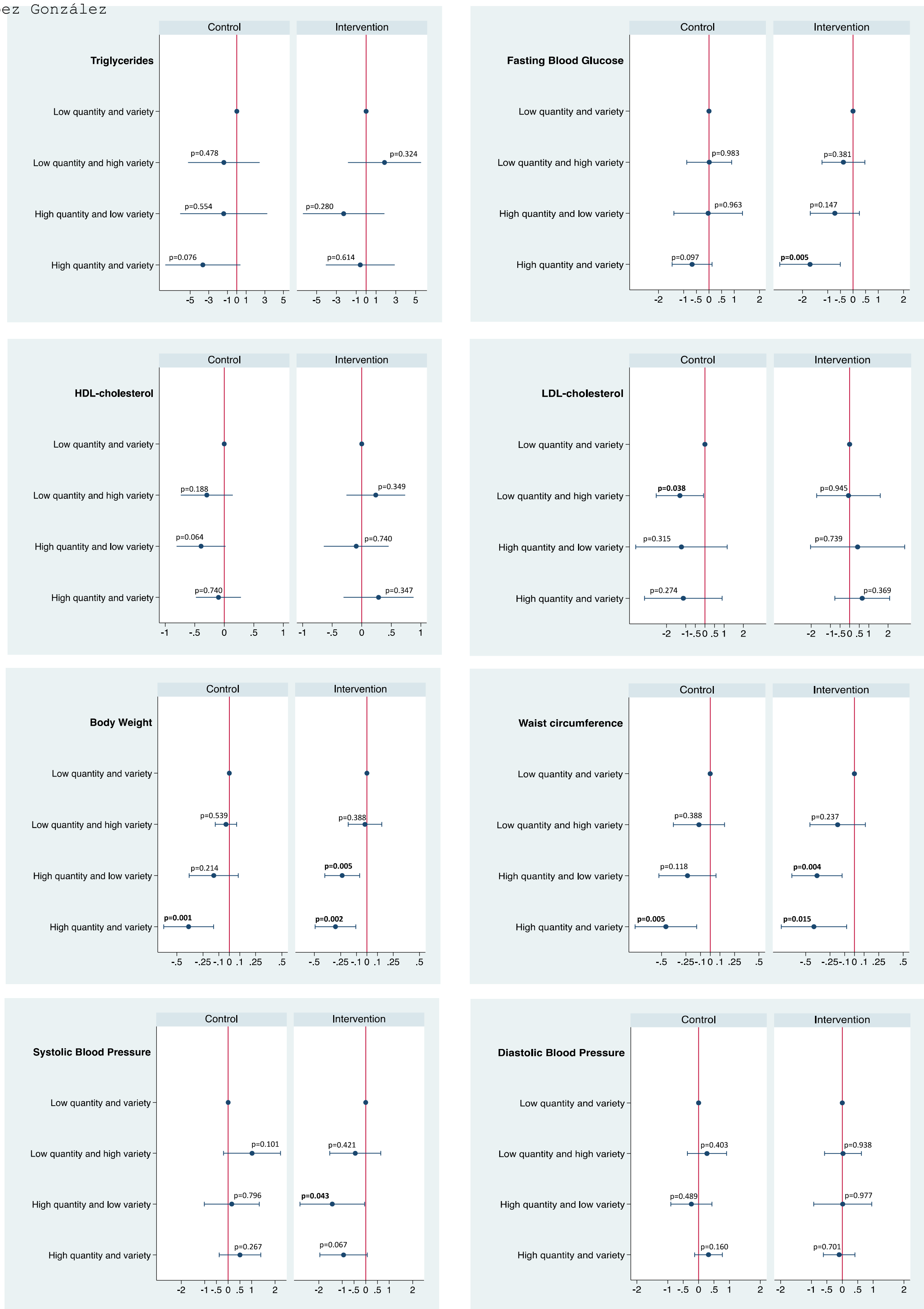
[2]A total of 6538 participants were analyzed for TG changes, 6524 participants for HDL-c changes, 6502 participants for LDL-c changes, 6546 participants for SBP changes, 6546 participants for DBP changes, 6542 participants for blood glucose changes, 6559 participants for weight changes and 6559 participants for WC changes.



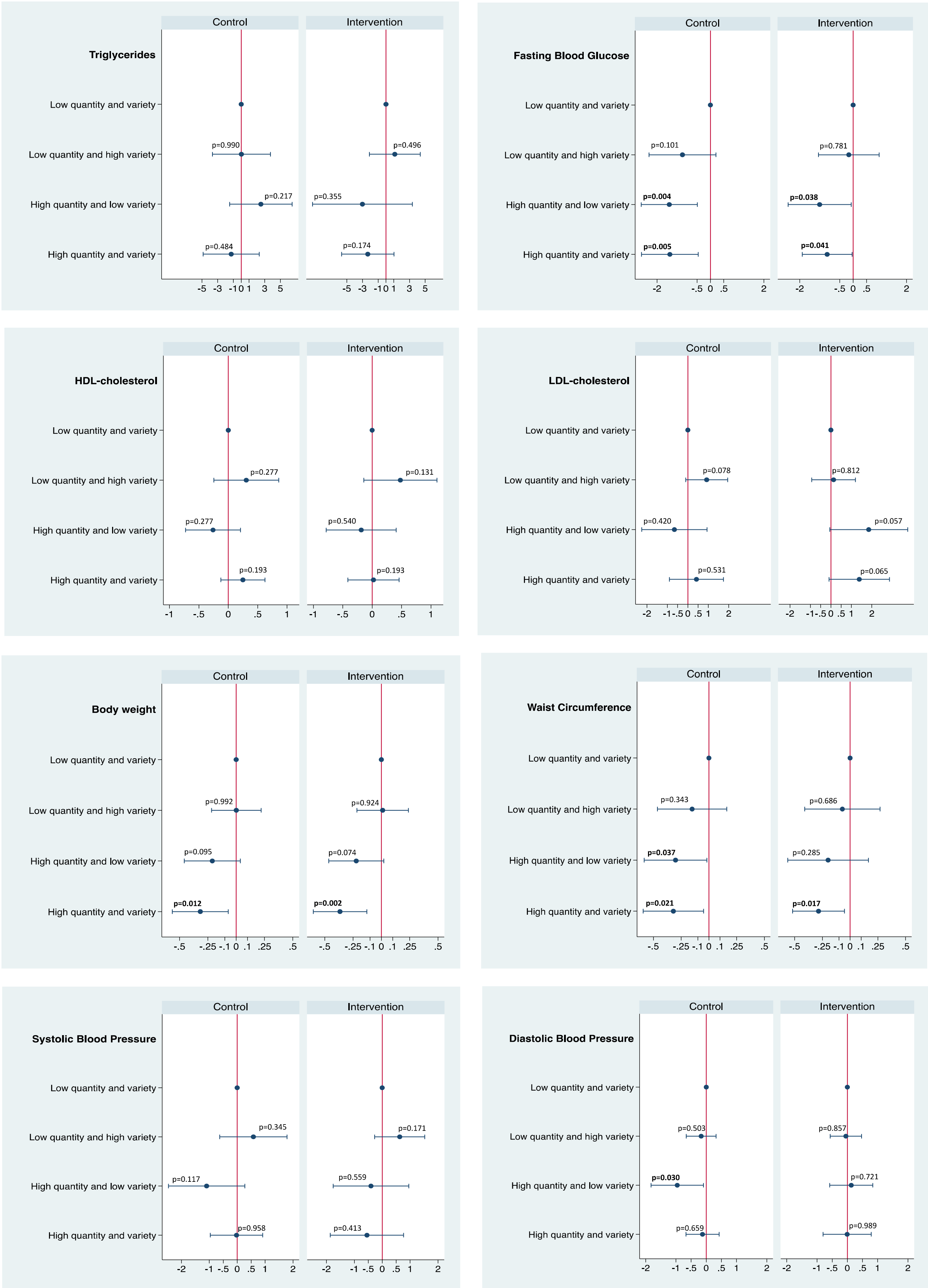

**Supplemental Figure 1. Beta-coefficients and 95% CI for cardiometabolic changes by categories of vegetable quantity and variety over one-year.** LMM were adjusted for baseline covariates such as age (years), sex (male/female), intervention group (yes/no), recruitment center (quartiles by number of participants), educational level(primary/secondary/tertiary school) and presence of diabetes (yes/no), hypertension (yes/no) and hypercholesterolemia (yes/no), and for changes from baseline in smoking habit (current/former/never), marital status (Married/Widowed/Divorced/other), living alone (yes/no), employment status (retired/employed/housekeeper/others), energy intake (in kcal/d), alcohol consumption (g/day and adding the quadratic term), physical activity (Mets.min/week), body mass index (except for body weight and WC) and 16-point screener of Mediterranean diet adherence without considering the contribution of vegetables (continuous). A total of 6538 participants were analyzed for triglycerides changes, 6524 participants for HDL-cholesterol changes, 6502 participants for LDL-cholesterol changes, 6546 participants for Systolic blood pressure changes, 6546 participants for Diastolic blood pressure changes, 6542 participants for fasting blood glucose changes, 6559 participants for body weight changes and 6559 participants for waist circumference changes.



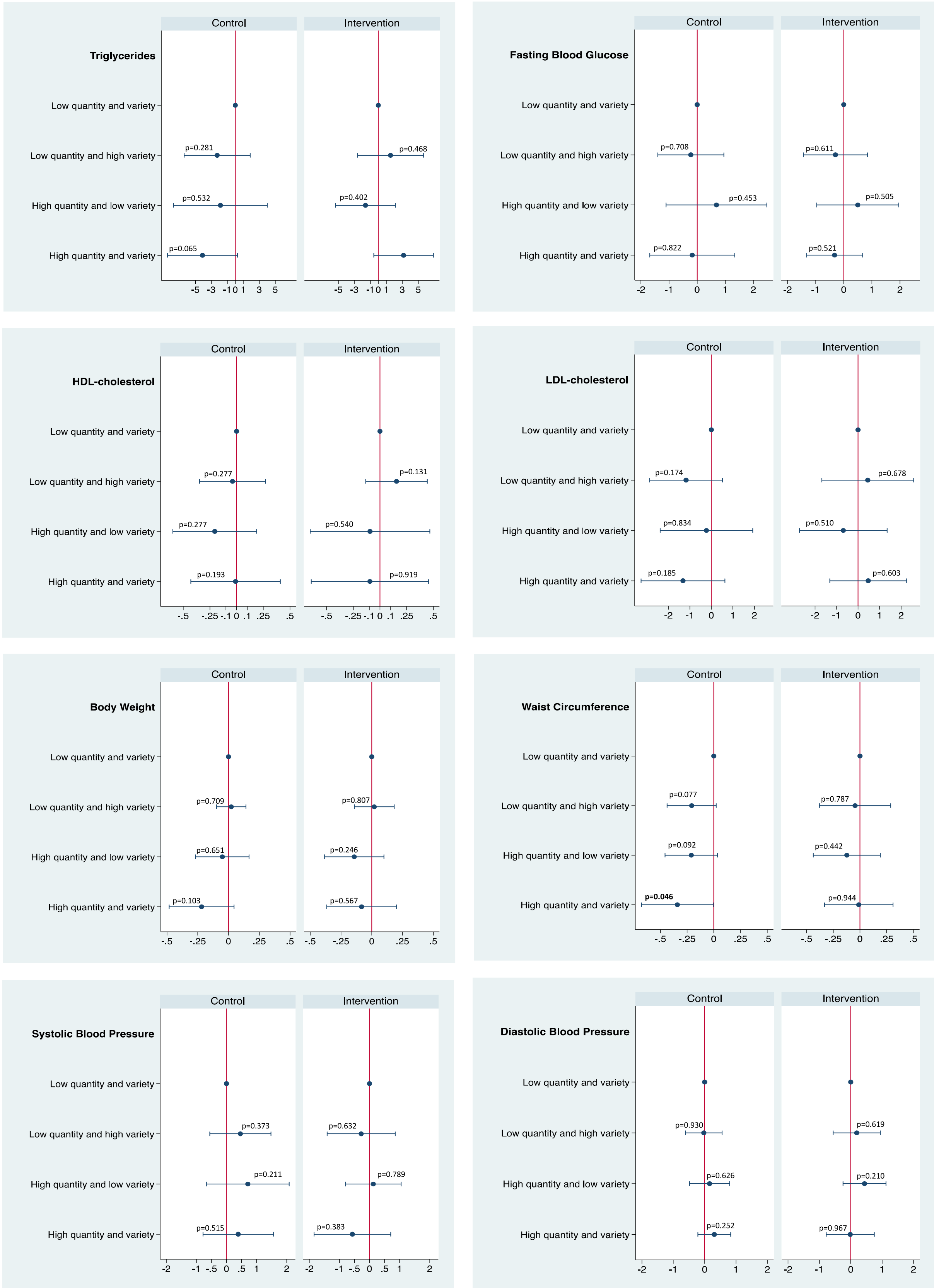

**Supplemental Figure 2. Beta-coefficients and 95% CI for cardiometabolic changes by categories of fruit quantity and variety over one-year.** LMM were adjusted for baseline covariates such as age (in years), sex (male/female), intervention group (yes/no), recruitment center (in quartiles by number of participants), educational level (primary/secondary/tertiary school) and presence of diabetes (yes/no), hypertension (yes/no) and hypercholesterolemia (yes/no), and for changes from baseline in smoking habit (current/former/never), marital status (Married/Widowed/Divorced/other), living alone (yes/no), employment status (retired/employed/housekeeper/others), energy intake (in kcal/d), alcohol consumption (g/day and adding the quadratic term), physical activity (Mets.min/week), body mass index (except for body weight and WC) and 16-point screener of Mediterranean diet adherence without considering the contribution of fruits (continuous). A total of 6538 participants were analyzed for triglycerides changes, 6524 participants for HDL-cholesterol changes, 6502 participants for LDL-cholesterol changes, 6546 participants for Systolic blood pressure changes, 6546 participants for Diastolic blood pressure changes, 6542 participants for fasting blood glucose changes, 6559 participants for body weight changes and 6559 participants for waist circumference changes.




**Supplemental Figure 3. Beta-coefficients and 95% CI for cardiometabolic changes by categories of FV quantity and variety over one-year, stratified by the intervention group.** LMM were adjusted for baseline covariates such as age (years), sex (male/female), intervention group (yes/no), recruitment center (quartiles by number of participants), educational level (primary/secondary/tertiary school) and presence of diabetes (yes/no), hypertension (yes/no) and hypercholesterolemia (yes/no), and for changes from baseline in smoking habit (current/former/never), marital status (Married/Widowed/Divorced/other), living alone (yes/no), employment status (retired/employed/housekeeper/others), energy intake (in kcal/d), alcohol consumption (g/day and adding the quadratic term), PA (Mets.min/week), body mass index (except for body weight and WC) and 16-point screener of Mediterranean diet adherence without considering the contribution of vegetables (continuous). A total of 6538 participants were analyzed for TG changes (3333-control and 3-intervention), 6524 participants for HDL-c changes (3287-control and 3237-intervention), 6502 participants for LDL-c changes (3275-control and 3277-intervention), 6546 participants for SBP changes (3292-control and 3244-intervention), 6546 participants for DBP changes (3292-control and 3244-intervention), 6542 participants for fasting blood glucose changes, (3294-control and 3248-intervention) 6559 participants for body weight changes (3304-control and 3255-intervention) and 6559 participants for WC changes (3304-control and 3255-intervention).




**Supplemental Figure 4. Beta-coefficients and 95% CI for cardiometabolic changes by categories of vegetable quantity and variety over one-year, stratified by the intervention group.** LMM were adjusted for baseline covariates such as age (years), sex (male/female), intervention group (yes/no), recruitment center (quartiles by number of participants), educational level (primary/secondary/tertiary school) and presence of diabetes (yes/no), hypertension (yes/no) and hypercholesterolemia (yes/no), and for changes from baseline in smoking habit (current/former/never), marital status (Married/Widowed/Divorced/other), living alone (yes/no), employment status (retired/employed/housekeeper/others), energy intake (in kcal/d), alcohol consumption (g/day and adding the quadratic term), PA (Mets.min/week), BMI (except for body weight and WC) and 16-point screener of Mediterranean diet adherence without considering the contribution of vegetables (continuous). A total of 6538 participants were analyzed for TG changes (3290-control and 3248-intervention), 6524 participants for HDL-c changes (3287-control and 3237-intervention), 6502 participants for LDL-c changes (3275-control and 3227-intervention), 6546 participants for SBP changes (3292-control and 3244-intervention), 6546 participants for DBP changes (3292-control and 3244-intervention), 6542 participants for fasting blood glucose changes (3294-control and 3248-intervention), 6559 participants for body weight changes (3304-control and 3225-intervention) and 6559 participants for WC changes (3304-control and 3225-intervention).




**Supplemental Figure 5. Beta-coefficients and 95% CI for cardiometabolic changes by categories of fruit quantity and variety over one-year, stratified by the intervention group.** LMM were adjusted for baseline covariates such as age (years), sex (male/female), intervention group (yes/no), recruitment center (quartiles by number of participants), educational level (primary/secondary/tertiary school) and presence of diabetes (yes/no), hypertension (yes/no) and hypercholesterolemia (yes/no), and for changes from baseline in smoking habit (current/former/never), marital status (Married/Widowed/Divorced/other), living alone (yes/no), employment status (retired/employed/housekeeper/others), energy intake (in kcal/d), alcohol consumption (g/day and adding the quadratic term), PA (Mets.min/week), BMI (except for body weight and WC) and 16-point screener of Mediterranean diet adherence without considering the contribution of vegetables (continuous). A total of 6538 participants were analyzed for TG changes (3290-control and 3248-intervention), 6524 participants for HDL-c changes (3287-control and 3237-intervention), 6502 participants for LDL-c changes (3275-control and 3227-intervention), 6546 participants for SBP changes (3292-control and 3244-intervention), 6546 participants for DBP changes (3292-control and 3244-intervention), 6542 participants for fasting blood glucose changes, (3294-control and 3248-intervention) 6559 participants for body weight changes (3304-control and 3255-intervention) and 6559 participants for WC changes (3304-control and 3255-intervention).

# 6. DISCUSSION

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The present doctoral thesis has focused on evaluating the association between variety in FV consumption and different parameters, including diet quality, lifestyle and several cardiometabolic risk factors in an elderly Mediterranean population at high CVD risk.

The results derived from the two studies conducted in the framework of this doctoral thesis provide new evidences to better understand the role played not only by variety in FV consumption, but also by the combination of quantity and variety in FV intake, on diet quality and cardiovascular health.

Each of the two studies previously presented in this doctoral thesis includes a discussion section. However, it is essential to further comment other important or general considerations related to them that have not been previously mentioned, as well as indicating the potential strengths and limitations derived from this dissertation.

## 6.1 Global discussion

The present doctoral thesis conducted in elderly Mediterranean individuals at high CVD risk revealed: 1) a beneficial cross-sectional association between greater variety in fruit and/or vegetable intake in relation to diet quality and lifestyle; and a positive cross-sectional association between higher variety in fruit and/or vegetable consumption (also in combination with higher quantity of fruits and/or vegetables) and the risk of inadequate intakes of dietary fiber and micronutrients; 2) a positive longitudinal association between greater variety in fruit and/or vegetable intake (also in combination with higher amounts of fruits and/or vegetables) and the improvement of several cardiometabolic risk factors, including body weight, WC, TG or fasting blood glucose.

It is well known that a high consumption of FV has positive effects on cardiovascular health, and human health in general. In addition, it contributes to improving diet quality and preventing nutritional deficiencies in the general population. This is mainly due to

the amounts of essential micronutrients, dietary fiber and bioactive compounds they contribute to the diet[111].
However, recent and novel evidences have suggested that variety in FV intake may also protect against different chronic diseases, such as CVD, and nutritional deficiencies, as FV vary widely in nutritional and phytochemical content among their different color groups and subgroups[111].

Some studies have indicated that not all groups and subgroups of FV exert the same health properties, suggesting that these beneficial effects depend mainly on the content and proportion of micronutrients (vitamins and minerals), dietary fiber and phytochemicals of the fruit or vegetable under consideration, and the interactions between them (synergistic effects)[112–114].

The FFQ used in the PREDIMED-plus study has made it possible to obtain consumption data for the different types and subtypes of FV in our participants, which has in turn facilitated the creation of variety scores for FV in combination and separately[115]. As a result, the relationship between a varied consumption of FV and different outcomes of interest has been possible to analyze.

In this sense, our cross-sectional study revealed that a varied FV intake was directly associated with better diet quality and nutrient adequacy in elderly subjects at high CVD risk. These results are supported by the findings of other epidemiological studies which have previously assessed the role of a varied FV intake on nutrient adequacy and diet quality in different populations such as healthy adults, preschoolers or women [26,27,116]. However, given the limited evidence, more studies are necessary to confirm our findings.

Findings from the present study also showed that a varied FV consumption was directly associated with a healthier lifestyle in elderly participants at high CVD risk. However, although there are epidemiological studies that have shown that FV consumption was associated with a healthier lifestyle, characterized by being physically active and non-smoker[29,117,118], these studies have only considered the total amount of FV consumed.

Therefore, many studies in different populations are needed to evaluate the association between lifestyle and a varied consumption of FV, in order to confirm and extrapolate our findings.

To conclude with the findings derived from the first publication of this thesis, an inverse association was found between variety in fruit and/or vegetable consumption (also in combination with higher amounts of fruits and/or vegetables) and the risk of having inadequate intakes of dietary fiber and micronutrients. To the best of our knowledge, this is the first epidemiological study evaluating the role of FV consumption on diet quality and dietary intake, considering both quantity and variety of the FV consumed. Hence, further studies conducted in diverge populations and considering a cross-classification of quantity and variety in FV consumption are needed to provide new insight into this field.

Findings from the second publication of this doctoral thesis revealed that variety of fruits and/or vegetables consumed over one year was inversely associated with a decrease in certain cardiometabolic risk factors, including fasting blood glucose, body weight and WC. However, there is controversy surrounding these results. For instance, although there are epidemiological studies[74,97] that have reported similar associations, both cross-sectionally and longitudinally, they have assessed FV variety classifying FV by color, instead of using FV variety scores, as it has been done in our studies. Besides, there are studies[78] that have not reported longitudinal associations between changes in FV variety and cardiometabolic risk factors, although they have used variety scores to measure FV variety. Disparity between these results may be due to the different approaches used to assess FV variety, different characteristics of study populations or study design: cross-sectional or longitudinal studies or the different follow-up periods for the prospective studies.

Furthermore, in this longitudinal study we also reported an inverse association between the total amount of fruits and/or vegetables consumed over one-year and the decrease in certain cardiometabolic risk factors, such as TG, fasting blood glucose, body weight and WC. In this sense, although there are epidemiological studies[65,79,104] whose results are in line with our findings, there is controversy surrounding body weight, as two systematic reviews with meta-analysis[68,82] of randomized controlled clinical trials did not report a decrease in body weight after increasing the total amount of FV consumed. This lack of association observed could be due to the short period of follow-up used in the majority of the included studies in these meta-analyses. As a consequence, further studies with a long follow-up and clinical trials are needed to confirm and extrapolate our results, especially in the case of body weight.

Finally, findings from this prospective study also showed that variety of fruits and/or vegetables consumed over one year (also in combination with the total amounts of fruits and/or vegetables consumed over one year) was inversely associated with a decrease in some cardiometabolic risk factors, including fasting blood glucose, body weight and WC. However, further studies in populations of different origins, ages and health status are required, since no epidemiological study has evaluated the role of FV consumption on cardiometabolic risk factors, considering both the quantity and variety of FV consumed are warranted.

## 6.2 Strengths and limitations

Several limitations are derived from the present doctoral thesis and deserved to be highlighted. Firstly, the observational design of the two studies conducted in the present thesis does not allow us to make inferences about causality, so only associations between the different exposures and outcomes of interest have been claimed.

Secondly, since the participants of the PREDIMED-plus study are elderly Mediterranean subjects, our findings cannot be extrapolated to the general population. As a consequence, further studies in other populations from other regions and of different age groups are necessary to confirm our results.

Thirdly, we used a FFQ to estimate the habitual dietary intake of our participants, which is the most widely, used method for dietary data collection in epidemiological studies.

Nevertheless, the FFQ is a tool susceptible for measurement errors, since patients have difficulties in determining the exact amount of the different foods they have consumed during the previous year. Besides, it is common that participants tend to overestimate the intake of healthy foods, such as FV, while underestimating the consumption of high-calorie foods.

Fourthly, the follow-up period of the longitudinal study is relatively short to observe whether the improvement in certain cardiometabolic risk factors is maintained over time. In this sense, a longer follow-up period could have suggested an improvement in other cardiometabolic risk factors that were not affected at 1-year of follow-up.

The present work has also some strengths that need to be mentioned. To begin with, all statistical analyses were adjusted for a large number of potential confounders to avoid the validity of our results being affected by the influence of confounding factors related to lifestyle, dietary intake or health status.

Besides, the use of FFQs to determine dietary intake in our participants allow us to determine the specific consumption of the different types and subtypes of FV, and thus create different variety scores for fruits and/or vegetables.

Furthermore, the use of the DRIs for American population, instead of the different diet quality indexes, gives another perspective when assessing the quality of people´s diets.

Finally, studying the associations between FV consumption and diet quality, lifestyle and cardiometabolic risk, considering both quantity and variety in FV consumption, provides more reliable information on the influence of both food groups on human diet and health.

# 7. CONCLUSIONS

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The conclusions derived from the two analyses conducted in the framework of the PREDIMED-plus study in an elderly Mediterranean population at high CVD risk, are presented as a response of each hypothesis specified at the beginning of the present doctoral thesis:

**Hypothesis 1**: A greater variety in FV consumption is associated with better diet quality and lifestyle.

- A greater variety in fruit and/or vegetable consumption was significantly associated with better diet quality and a healthy lifestyle in an elderly Mediterranean population at high CVD risk.

- A higher variety in FV consumption, also in combination with greater intakes of total FV, was significantly associated with a lower risk of having inadequate intakes of dietary fiber and micronutrients in an elderly Mediterranean population at high CVD risk.

**Hypothesis 2**: A greater variety in FV consumption is associated with an improvement in several cardiometabolic risk factors.

- A greater variety in FV intake was significantly associated with a decrease in several cardiometabolic risk factors, such as fasting plasma glucose, body weight and WC, among elderly Mediterranean subjects at high CVD risk.

- A higher consumption of FV intake was significantly associated with a decrease in several cardiometabolic risk factors, such as TG, fasting plasma glucose, body weight and WC, among elderly Mediterranean subjects at high CVD risk.

- A greater variety, in combination with higher intakes of total FV, was significantly associated with a decrease in several cardiometabolic risk factors, such as fasting plasma glucose, body weight and WC, among elderly Mediterranean subjects at high CVD risk.

# 8. GLOBAL AND FUTURE INSIGHTS

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Chronic diseases such as CVD, T2D and cancer can be prevented with lifestyle changes, including the increase of FV daily consumption. Findings derived from the present thesis add further scientific evidence to understand the role that a varied consumption of FV, in combination with higher amounts of FV, plays on nutrient adequacy, diet quality and cardiometabolic risk factors in an elderly population with MetS. This new scientific evidence may help to design new dietary guidelines, or improving the existing ones, with the aim of preventing important nutrient deficiencies and chronic diseases such as CVD or T2D.

Based on our results, we could take into consideration several important aspects of future research on variety in FV consumption, nutrient adequacy, diet quality and cardiovascular health:

- Given that our findings were found in a Mediterranean population, it would be necessary to conduct similar prospective studies in other populations from different geographical areas and metabolic status conditions to confirm our results and extrapolate them to the general population.

- Furthermore, long-term randomized controlled clinical trials are also warranted to evaluate the potential benefits of a varied consumption of FV on CVD and T2D in adult and elderly populations from different geographical areas and with different health status.

- Moreover, future investigations are needed to clarify the potential mechanisms implicated in the associations between a varied consumption of FV, in combination with higher quantities of FV, and CVD, T2D and other related-illnesses. In addition to focusing on the biological properties exerted by the main nutrients and bioactive compounds present in FV, these investigations should be based on the study of the genetic contributions in the above associations. It is essential to pay special attention to nutrient-gene interactions with the aim of better understanding the relationship between specific genetic *loci*, a varied FV consumption and the incidence and/or risk of diet-related illnesses[119].

- In addition, given that the implication of gut microbiota in human health has received special attention in recent years, it would be essential to conduct studies aimed at determining the interactions between diet (in this case the varied consumption of FV in combination with higher quantities of FV), gut microbiota and its metabolites, and their role on human health. Understanding the implications of diet-microbiota interactions in human health is required for the design of new preventive and therapeutic measures that allow us to deal with chronic diet-related illnesses.

- Finally, to address the limitations of the FFQ, such as overestimation of FV intake, metabolomics could be used as a tool for identifying the metabolic fingerprint associated to a varied consumption of FV.

# 9. REFERENCES

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1. Slavin, J. L. & Lloyd, B. Health benefits of fruits and vegetables. *Advances in Nutrition*. **3**, 506–516 (2012).

2. Lintas, C. Nutritional aspects of fruit and vegetable consumption. *https://agris.fao.org/agris-search/search.do?recordID=QC9240011* (2021).

3. Pennington, J. A. T. & Fisher, R. A. Classification of fruits and vegetables. *Journal of Food Composition and Analysis* **22S**, S23-S31 (2009).

4. Pennington, J. A. T. & Fisher, R. A. Food component profiles for fruit and vegetable subgroups. *Journal of Food Composition and Analysis* **23**, 411–418 (2010).

5. World Health Organization (WHO). Promoting fruit and vegetable consumption. https://www.euro.who.int/en/health-topics/disease-prevention/nutrition/activities/technical-support-to-member-states/promoting-fruit-and-vegetable-consumption (2022).

6. U.S. Department of Agriculture and U.S. Department of Health and Human Services. *Dietary Guidelines for Americans, 2020-2025. https://www.dietaryguidelines.gov/* (2020)

7. Martínez Hernández, A. J. *et al.* Informe del Comité Científico de la Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) de revisión y actualización de las Recomendaciones Dietéticas para la población española. *Revista del Comité Científico* **32**, 11-58 (2020).

8. Grupo Colaborativo de la Sociedad Española de Nutrición Comunitaria (SENC). Guías alimentarias para la población española (SENC, diciembre 2016): la nueva pirámide de la alimentación saludable. *Nutrición Hospitalaria* **33**, 1–48 (2016).

9. Micha, R. *et al.* Global, regional and national consumption of major food groups in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys worldwide on behalf of the Global Burden of Diseases Nutrition and Chronic Diseases Expert Group (NutriCoDE). *BMJ Open* **5**, e:008705 (2015).

10. U.S. Department of Agriculture & U.S. Department of Health and Human Services. *Dietary Guidelines for Americans 2015-2020* http://health.gov/dietaryguidelines/2015/guidelines/ (2015).

11. U.S. Department of Agriculture & U.S. Department of Health and Human Services. Scientific Report of the 2015 Dietary Guidelines Advisory Committee. https://health.gov/sites/default/files/2019-09/Scientific-Report-of-the-2015-Dietary-Guidelines-Advisory-Committee.pdf (2015).

12. Elmadfa, I. A comparative analysis of nutrition policies and plans of action in WHO European Member States. European Nutrition and Health Report. *Forum. Nutr* **62**, 12-13 (2006).

13. European Food Safety Authority (EFSA). Food consumption data. https://www.efsa.europa.eu/en/data-report/food-consumption-data (2022).

14. Erostat. A key to European statistics. European Comission. How much fruits and vegetables do you eat daily? https://ec.europa.eu/eurostat/web/products-eurostat-news/-/ddn-20220104-1 (2022).

15. Arroyo, P. *et al.* Informe de estado de situación sobre "Frutas y hortalizas: Nutrición y salud en la España del S.XXI" https://www.sennutricion.org/es/2018/04/26/informe-estado-de-situacin-sobre-frutas-y-hortalizas-nutricin-y-salud-en-la-espaa-del-s-xxi (2018).

16. Suarez, M., Rubio, V., Sanchidrián, J. & Robledo, R. Spanish National dietary survey in adults, elderly and pregnant women. https://www.efsa.europa.eu/publications (2016).

17. Agencia Española de Seguridad Alimentaria y Nutrición (AESAN). Encuesta ENALIA 2. Encuesta Nacional de Alimentación en población adulta, mayores y embarazadas.https://www.aesan.gob.es/AECOSAN/web/seguridad_alimentaria/subdetalle/enalia_2.htm#3 (2015).

18. Agencia Española de Consumo, Seguridad Alimentaria y Nutrición (AECOSAN). Resultados Encuesta ENALIA 2: Tablas de datos de consumo/día (18 a 74 años) https://www.aesan.gob.es/AECOSAN/docs/documentos/seguridad_alimentaria/evaluacion_riesgos/Edades_18_74.pdf (2015).

19. Ministerio de Sanidad, Gobierno de España. Porcentaje de personas de 15 y más años que consumen a diario fruta fresca, verduras, ensaladas y hortalizas, por sexo según la comunidad autónoma. https://www.sanidad.gob.es/ estadEstudios/sanidadDatos/tablas/tabla11.htm (2020).

20. Wirt, A. & Collins, C. E. Diet quality - What is it and does it matter? *Public Health Nutrition* **12**, 2473–2492 (2009).

21. Cano-Ibáñez, N. *et al.* Dietary diversity and nutritional adequacy among an older Spanish population with metabolic syndrome in the PREDIMED-plus study: A cross-sectional analysis. *Nutrients* **11**, 958 (2019).

22. Whybrow, S., Harrison, C. L. S., Mayer, C. & Stubbs, R. J. Effects of added fruits and vegetables on dietary intakes and body weight in Scottish adults. *British Journal of Nutrition* **95**, 496–503 (2006).

23. Freedman, M. R. & Fulgoni, V. L. Canned Vegetable and Fruit Consumption Is Associated with Changes in Nutrient Intake and Higher Diet Quality in Children and Adults: National Health and Nutrition Examination Survey 2001-2010. *Journal of the Academy of Nutrition and Dietetics* **116**, 940–948 (2016).

24. Angelino, D. *et al.* Fruit and vegetable consumption and health outcomes: an umbrella review of observational studies. *International Journal of Food Sciences and Nutrition* **0**, 1–16 (2019).

25. Fulton, S. L., McKinley, M. C., Young, I. S., Cardwell, C. R. & Woodside, J. The Effect of Increasing Fruit and Vegetable Consumption on Overall Diet: A Systematic Review and Meta-analysis. *Critical Reviews in Food Science and Nutrition* **56**, 802–816 (2016).

26. Foote, J. A., Murphy, S. P., Wilkens, L. R., Basiotis, P. P. & Carlson, A. Dietary variety increases the probability of nutrient adequacy among adults. *Journal of Nutrition* **134**, 1779–1785 (2004).

27. Keim, N. L., Forester, S. M., Lyly, M., Aaron, G. J. & Townsend, M. S. Vegetable variety is a key to improved diet quality in low-income women in California. *Journal of the Academy of Nutrition and Dietetics* **114**, 430–435 (2014).

28. Bernstein, M. A. *et al.* Higher dietary variety is associated with better nutritional status in frail elderly people. *Journal of the American Dietetic Association* **102**, 1096–1104 (2002).

29. Grosso, G. *et al.* Health risk factors associated with meat, fruit and vegetable consumption in cohort studies: A comprehensive meta-analysis. *PLoS ONE* **12**, 1–21 (2017).

30. Samtiya, M., Aluko, R. E., Dhewa, T. & Moreno-Rojas, J. M. Potential health benefits of plant food-derived bioactive components: An overview. *Foods* **10**, 839 (2021).

31. Liu, R. H. Health-promoting components of fruits and vegetables in the diet. *Advances in Nutrition* **4**, 384S-392S (2013).

32. Langi, P., Kiokias, S., Varzakas, T. & Proestos, C. Carotenoids: From plants to food and feed industries. *Methods in Molecular Biology* **1852**, 57–71 (2018).

33. Núñez-Córdoba, J. M. & Martínez-González, M. A. Antioxidant Vitamins and Cardiovascular Disease. *Current Topics in Medicinal Chemistry* **11**, 1861-1869 (2011).

34. Fabre, G. *et al.* Synergism of antioxidant action of vitamins E, C and quercetin is related to formation of molecular associations in biomembranes. *Chemical Communications* **51**, 7713–7716 (2015).

35. Miekus, N. *et al.* Health Benefits of Plant-Derived Sulfur Compounds, Glucosinolates, and Organosulfur Compounds. *Molecules* **25**, 3804 (2020).

36. Song, W. *et al.* Cellular antioxidant activity of common vegetables. *Journal of Agricultural and Food Chemistry* **58**, 6621–6629 (2010).

37. Manach, C., Scalbert, A., Morand, C., Rémésy, C. & Jiménez, L. Polyphenols: food sources and bioavailability. *Am J Clin Nutr* **79**, 727-47 (2004).

38. Panche, A. N., Diwan, A. D. & Chandra, S. R. Flavonoids: An overview. *Journal of Nutritional Science* **5**, e47 (2016).

39. Hollman, P. C. H. & Katan, M. B. Dietary Flavonoids: Intake, Health Effects and Bioavailability. *Food and Chemical Toxicology* **37**, 937-942 (1999).

40. Sosalagere, C., Adesegun Kehinde, B. & Sharma, P. Isolation and functionalities of bioactive peptides from fruits and vegetables: A reviews. *Food Chemistry* **366**, 130494 (2022).

41. Saleh, A. S. M., Zhang, Q. & Shen, Q. Recent Research in Antihypertensive Activity of Food Protein-derived Hydrolyzates and Peptides. *Critical Reviews in Food Science and Nutrition* **56**, 760–787 (2016).

42. García, M. C., Puchalska, P., Esteve, C. & Marina, M. L. Vegetable foods: A cheap source of proteins and peptides with antihypertensive, antioxidant, and other less occurrence bioactivities. *Talanta* **106**, 328–349 (2013).

43. Ribeiro, S. F. F. *et al.* Isolation and characterization of novel peptides from chilli pepper seeds: Antimicrobial activities against pathogenic yeasts. *Toxicon* **50**, 600–611 (2007).

44. Maestri, E., Marmiroli, M. & Marmiroli, N. Bioactive peptides in plant-derived foodstuffs. *Journal of Proteomics* vol. 147 140–155 (2016).

45. Howard, A. & Udenigwe, C. C. Mechanisms and prospects of food protein hydrolysates and peptide-induced hypolipidaemia. *Food and Function* **4**, 40–51 (2013).

46. Chang, V. H. S. *et al.* IbACP, a sixteen-amino-acid peptide isolated from *Ipomoea batatas* leaves, induces carcinoma cell apoptosis. *Peptides* **47**, 148–156 (2013).

47. Call, J. A., Eckhardt, G. & Camidge, R. Targeted manipulation of apoptosis in cancer treatment. *The Lancet Oncology* **9**, 1002-11 (2008).

48. Vanduchova, A., Anzenbacher, P. & Anzenbacherova, E. Isothiocyanate from Broccoli, Sulforaphane, and Its Properties. *Journal of Medicinal Food* **22**, 121–126 (2019).

49. Batiha, G. E. S. *et al.* Chemical constituents and pharmacological activities of garlic (*Allium sativum* L.): A review. *Nutrients*, **12**, 872 (2020).

50. European Commission, Health and Consumers Directorate-General. Guidance document for competent authorities for control of compliance with EU legistation on: Council Directive 90/496/EEC of 24 September 1990 on nutrition leballing of foodstuffs. https://ec.europa.eu/food/system/files/2016-10/labelling_nutrition-vitamins_minerals-guidance_tolerances_1212_en.pdf (2012).

51. Lattimer, J. M. & Haub, M. D. Effects of dietary fiber and its components on metabolic health. *Nutrients* **2**, 1266–1289 (2010).

52. Soliman, G. A. Dietary fiber, atherosclerosis, and cardiovascular disease. *Nutrients* **11**, 1155 (2019).

53. Surampudi, P., Enkhmaa, B., Anuurad, E. & Berglund, L. Lipid Lowering with Soluble Dietary Fiber. *Current Atherosclerosis Reports* **18**, 75 (2016).

54. Weickert, M. O. & Pfeiffer, A. F. H. Impact of dietary fiber consumption on insulin resistance and the prevention of type 2 diabetes. *Journal of Nutrition* **148**, 7–12 (2018).

55. Wan, M. L. Y., Ling, K. H., El-Nezami, H. & Wang, M. F. Influence of functional food components on gut health. *Critical Reviews in Food Science and Nutrition* **59**, 1927–1936 (2019).

56. Klinder, A. *et al.* Impact of increasing fruit and vegetables and flavonoid intake on the human gut microbiota. *Food and Function* **7**, 1788–1796 (2016).

57. Wan, M. L. Y., Ling, K. H., El-Nezami, H. & Wang, M. F. Influence of functional food components on gut health. *Critical Reviews in Food Science and Nutrition* **59**, 1927–1936 (2019).

58. Klinder, A. *et al*. Impact of increasing fruit and vegetables and flavonoid intake on the hum gut microbiota. *Food and Function* **7**, 1788–1796 (2016).

59. Evans, C. E. L. Dietary fibre and cardiovascular health: A review of current evidence and policy. *Proceedings of the Nutrition Society* **79**, 61–67 (2020).

60. Partula, V. *et al.* Associations between consumption of dietary fibers and the risk of cardiovascular diseases, cancers, type 2 diabetes, and mortality in the prospective NutriNet-Santé cohort. *The American Journal of Clinical Nutrition* **00**, 1-13 (2020).

61. Boeing, H. *et al.* Critical review: Vegetables and fruit in the prevention of chronic diseases. *European Journal of Nutrition* **51**, 637–663 (2012).

62. Randall, E., Nichaman, M. Z. & Contant, C. F. J. Diet diversity and nutrient intake. *Journal of American Dietetic Association* **85**, 830–836 (1985).

63. Oude Griep, L. M., Verschuren, W. M. M., Kromhout, D., Ocké, M. C. & Geleijnse, J. M. Variety in fruit and vegetable consumption and 10-year incidence of CHD and stroke. *Public Health Nutrition* **15**, 2280–2286 (2012).

64. Wang, X. *et al.* Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: Systematic review and dose-response meta-analysis of prospective cohort studies. *BMJ* **349**, 1–14 (2014).

65. Kodama, S. *et al.* Relationship between intake of fruit separately from vegetables and triglycerides - A meta-analysis. *Clinical Nutrition* **27**, 53–58 (2018).

66. Toh, D. W. K., Koh, E. S. & Kim, J. E. Incorporating healthy dietary changes in addition to an increase in fruit and vegetable intake further improves the status of cardiovascular disease risk factors: A systematic review, meta-regression, and meta-analysis of randomized controlled trials. *Nutrition Reviews* **78**, 532–545 (2020).

67. Shin, J. Y., Kim, J. Y., Kang, H. T., Han, K. H. & Shim, J. Y. Effect of fruits and vegetables on metabolic syndrome: A systematic review and meta-analysis of randomized controlled trials. *International Journal of Food Sciences and Nutrition* **66**, 416–425 (2015).

68. Mytton, O. T., Nnoaham, K., Eyles, H., Scarborough, P. & Ni Mhurchu, C. Systematic review and meta-analysis of the effect of increased vegetable and fruit consumption on body weight and energy intake. *BMC Public Health* **14**, 886 (2014).

69. Järvi, A., Karlström, B., Vessby, B. & Becker, W. Increased intake of fruits and vegetables in overweight subjects: Effects on body weight, body composition, metabolic risk factors and dietary intake. *British Journal of Nutrition* **115**, 1760–1768 (2016).

70. Schlesinger, S. *et al.* Food Groups and Risk of Overweight, Obesity, and Weight Gain: A Systematic Review and Dose-Response Meta-Analysis of Prospective Studies. *Advances in Nutrition* **10**, 205–218 (2019).

71. Lim, M. & Kim, J. Association between fruit and vegetable consumption and risk of metabolic syndrome determined using the Korean Genome and Epidemiology Study (KoGES). *European Journal of Nutrition* **59**, 1667–1678 (2020).

72. Schwingshackl, L. *et al.* Fruit and vegetable consumption and changes in anthropometric variables in adult populations: A systematic review and meta-analysis of prospective cohort studies. *PLoS ONE* **10**, e0140846 (2015).

73. Takahashi, K. *et al.* Effects of total and green vegetable intakes on glycated hemoglobin A1c and triglycerides in elderly patients with typeb2 diabetes mellitus: The Japanese Elderly Intervention Trial. *Geriatrics and Gerontology International* **12**, 50–58 (2012).

74. Becerra-Tomás, N. *et al.* Fruit consumption and cardiometabolic risk in the PREDIMED-plus study: A cross-sectional analysis. *Nutrition, Metabolism and Cardiovascular Diseases* **31**, 1702–1713 (2021).

75. Djoussé, L. *et al.* Fruit and vegetable consumption and LDL cholesterol: The National Heart, Lung, and Blood Institute Family Heart Study. *The American Journal of Clinical Nutrition* **79**, 213–217 (2004).

76. Radhika, G., Sudha, V., Mohan Sathya, R., Ganesan, A. & Mohan, V. Association of fruit and vegetable intake with cardiovascular risk factors in urban south Indians. *British Journal of Nutrition* **99**, 398–405 (2008).

77. Arnotti, K. & Bamber, M. Fruit and Vegetable Consumption and High-Density Lipoproteins in Overweight or Obese Individuals: A Meta-analysis. *Journal of Cardiovascular Nursing* **36**, 78–87 (2021).

78. Lamb, M. J. E., Griffin, S. J., Sharp, S. J. & Cooper, A. J. M. Fruit and vegetable intake and cardiovascular risk factors in people with newly diagnosed type 2 diabetes. *European Journal of Clinical Nutrition* **71**, 115–121 (2017).

79. Bertoia, M. L., Mukamal, K. J., Cahill, L. E. & Hou, T. Changes in Intake of Fruits and Vegetables and Weight Change in United States Men and Women Followed for Up to 24 Years: Analysis from Three Prospective Cohort Studies. *PLOS* Medicine **12**, e:1001878 (2015).

80. Sartorelli, D. S., Franco, L. J. & Cardoso, M. A. High intake of fruits and vegetables predicts weight loss in Brazilian overweight adults. *Nutrition Research* **28**, 233–238 (2008).

81. Buijsse, B. *et al.* Fruit and vegetable intakes and subsequent changes in body weight in European populations: Results from the project on Diet, Obesity, and Genes (DiOGenes). *The American Journal of Clinical Nutrition* **90**, 202–209 (2009).

82. Kaiser, K. A. *et al.* Increased fruit and vegetable intake has no discernible effect on weight loss: A systematic review and meta-analysis. *The American Journal of Clinical Nutrition* **100**, 567–576 (2014).

83. Balakumar, P., Maung-U, K. & Jagadeesh, G. Prevalence and prevention of cardiovascular disease and diabetes mellitus. *Pharmacological Research* **113**, 600–609 (2016).

84. Zurbau, A. *et al.* Relation of different fruit and vegetable sources with incident cardiovascular outcomes: A systematic review and meta-analysis of prospective cohort studies. *Journal of the American Heart Association* **9**, e017728 (2020).

85. Wang, L., Manson, J. E., Gaziano, J. M., Buring, J. E. & Sesso, H. D. Fruit and vegetable intake and the risk of hypertension in middle-aged and older women. *American Journal of Hypertension* **25**, 180–189 (2012).

86. Borgi, L. *et al.* Fruit and Vegetable Consumption and the Incidence of Hypertension in Three Prospective Cohort Studies. *Hypertension* **67**, 288–293 (2016).

87. Schwingshackl, L. *et al.* Food groups and risk of type 2 diabetes mellitus: a systematic review and meta-analysis of prospective studies. *European Journal of Epidemiology* **32**, 363–375 (2017).

88. Cooper, A. J. *et al.* A prospective study of the association between quantity and variety of fruit and vegetable intake and incident type 2 diabetes. *Diabetes Care* **35**, 1293–1300 (2012).

89. Wiseman, M. J. Nutrition and cancer: Prevention and survival. *British Journal of Nutrition* **122**, 481–487 (2019).

90. Margje, J. C. J. F. *et al.* Reports: Quantity and Variety of Fruit and Vegetable Consumption and Cancer Risk Quantity and Variety of Fruit and Vegetable Consumption and Cancer Risk. *Nutrition and Cancer* **48**, 142–148 (2009).

91. World Cancer Research Fund International. Third Expert Report-WCRF-AICR. https://www.wcrf.org/dietandcancer/wholegrains-vegetables-and-fruit/ (2018).

92. Bradbury, K. E., Appleby, P. N. & Key, T. J. Fruit, vegetable, and fiber intake in relation to cancer risk: Findings from the European Prospective Investigation into Cancer and Nutrition (EPIC). *The American Journal of Clinical Nutrition* **100**, 394S-8S (2014).

93. Leenders, M. *et al.* Subtypes of fruit and vegetables, variety in consumption and risk of colon and rectal cancer in the European Prospective Investigation into Cancer and Nutrition. *International Journal of Cancer* **137**, 2705–2714 (2015).

94. Vieira, A. R. *et al.* Fruits, vegetables and lung cancer risk: A systematic review and meta-analysis. *Annals of Oncology* **27**, 81–96 (2016).

95. Ferro, A. *et al.* Fruits and vegetables intake and gastric cancer risk: A pooled analysis within the Stomach cancer Pooling Project. *International Journal of Cancer* **147**, 3090–3101 (2020).

96. Griep, L. M. O. Fruit and vegetable consumption and the risk of cardiovascular diseases. 1–134 (Thesis manuscript, 2011).

97. Mirmiran, P., Bahadoran, Z., Moslehi, N., Bastan, S. & Azizi, F. Colors of fruits and vegetables and 3-year changes of cardiometabolic risk factors in adults: Tehran lipid and glucose study. *European Journal of Clinical Nutrition* **69**, 1215–1219 (2015).

98. Conklin, A. I. *et al.* Variety more than quantity of fruit and vegetable intake varies by socioeconomic status and financial hardship. Findings from older adults in the EPIC cohort. *Appetite* **83**, 248–255 (2014).

99. Jeurnink, S. M. *et al.* Variety in vegetable and fruit consumption and the risk of gastric and esophageal cancer in the European prospective investigation into cancer and nutrition. *International Journal of Cancer* **131**, (2012).

100. Alberti, K. G. M. M. *et al.* Harmonizing the metabolic syndrome. *Circulation* **120**, 1640–1645 (2009).

101. Fernández-Ballart, J. D. *et al.* Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. *British Journal of Nutrition* **103**, 1808–1816 (2010).

102. Moreiras, O., Carbajal, A. & Cabrera L, C. C. Tablas de composición de alimentos. https://www.sennutricion.org/es/2013/05/14/tablas-de-composicin-de-alimentos-moreiras-et-al (2013).

103. Mataix, J. *et al.* Tabla de composicion de alimentos. https://www.sennutricion.org/es/2013/05/11/tablas-de-composicin-de-alimentos-mataix-et-al (2009).

104. Schröder, H. *et al.* A Short screener is valid for assessing mediterranean diet adherence among older spanish men and women. *Journal of Nutrition* **141**, 1140–1145 (2011).

105. Elosua, R., Marrugat, J., Molina, L., Pons, S. & Pujol, E. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish Men. *American Journal of Epidemiology* **139**, 1197–1209 (1994).

106. Elosua, R. *et al.* Validation of the Minnesota leisure time physical activity questionnaire in Spanish women. *Medicine and Science in Sports and Exercise* **32**, 1431–1437 (2000).

107. Molina, L. *et al.* Validation of the regicor short physical activity questionnaire for the adult population. *PLoS ONE* **12**, 1–14 (2017).

108. Warburton, D. E. R., Jamnik, V. K., Bredin, S. S. D. & Gledhill, N. The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+). *Health & Fitness Journal* 4, 3-17 (2011).

109. Topolski, T. D. *et al.* The Rapid Assessment of Physical Activity (RAPA) Among Older Adults. *Preventing chronic disease* **3**, 1-8 (2006).

110. Martínez-González, M. A., López-Fontana, C., Varo, J. J., Sánchez-Villegas, A. & Martinez, J. A. Validation of the Spanish version of the physical activity questionnaire used in the Nurses' Health Study and the Health Professionals' Follow-up Study. *Public Health Nutrition* **8**, 920–927 (2005).

111. Wallace, T. C. *et al.* Fruits, vegetables, and health: A comprehensive narrative, umbrella review of the science and recommendations for enhanced public policy to improve intake. *Critical Reviews in Food Science and Nutrition* **60**, 2174–2211 (2020).

112. Eichholzer, M., Lüthy, J., Gutzwiller, F. & Stähelin, H. B. The role of folate, antioxidant vitamins and other constituents in fruit and vegetables in the prevention of cardiovascular disease: The epidemiological evidence. *International Journal for Vitamin and Nutrition Research* **71**, 5–17 (2001).

113. Liu, R.L. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *The American Journal of Clinical Nutrition* **78**, 517S-520S (2003).

114. Jacobs, D. R., Gross, M. D. & Tapsell, L. C. Food synergy: An operational concept for understanding nutrition. *The American Journal of Clinical Nutrition* **89**, 1543S-8S (2009).

115. López-González, L. *et al.* Variety in fruits and vegetables, diet quality and lifestyle in an older adult mediterranean population. *Clinical Nutrition* **40**, 1510–1518 (2021).

116. Ramsay, S. A., Shriver, L. H. & Taylor, C. A. Variety of fruit and vegetables is related to preschoolers' overall diet quality. *Preventive Medicine Reports* **5**, 112–117 (2017).

117. Popkin, B. M. & Udry, J. R. Community and International Nutrition. *Journal of Nutrition* **128**, 701–706 (1998).

118. Silva, D. A. S. & Silva, R. J. S. Association between physical activity level and consumption of fruit and vegetables among adolescents in northeast Brazil. *Revista Paulista de Pediatria* **33**, 167–173 (2015).

119. Alissa, E. M. & Ferns, G. A. Dietary fruits and vegetables and cardiovascular diseases risk. *Critical Reviews in Food Science and Nutrition* **57**, 1950–1962 (2017).

# 10. APPENDICES

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## 10.1 Scientific contributions

Publications derived from this doctoral thesis:

**López-González L**, Becerra-Tomás N, Babio N, Martínez-González MA, Díaz-López A, Corella C, Goday A, Romaguera D, Vioque J, Alonso-Gómez AM, Wärnberg J, Martínez JA, Serra-Majem L, Estruch R, Tinahones F, Lapetra J, Pintó X, Tur JA, López-Miranda J, Bueno-Cavanillas A, Delgado-Rodríguez M, Matía-Martín P, Daimiel L, Álvarez-Álvarez L, Vidal J, Vázquez C, Ros E, Vázquez-Ruiz Z, Canudas S, Fernández Carrión R, Castañer O, Zulet MA, Tojal Sierra L, Ajejas Bazán MJ, López García CM, Martín M, García-Ríos A, Casas R, Gómez-Pérez AM, Santos-Lozano JM, Goñi E, Guillem-Saiz P, Lassale C, Abete I, Salaverria Lete I, Eguaras S, Shroeder H, and Salas-Salvado J; PREDIMED-Plus Investigators. Variety in fruits and vegetables, diet quality and lifestyle in an elderly Mediterranean population. Clin Nutr. 2021; 40:1510-1518. PubMed PMID: 33743286.

**López-González L**, Becerra-Tomás N, Babio N, Martínez-González MA, Nishi SK, Corella D, Goday A, Romaguera D, Vioque J, Alonso-Gómez AM, Wärnberg J, Martínez JA, Serra-Majem L, Estruch R, Bernal-López RM, Lapetra J, Pintó X, Tur JA López-Miranda J, Bueno-Cavanillas A, Delgado-Rodríguez M, Matía-Martín P, Daimiel L, Martín Sánchez V, Vidal J, Vázquez C, Ros E,Vázquez-Ruiz Z, Martín-Luján FM, Sorlí JV, Castañer O, Zulet MA, Tojal-Sierra L, Carabaño-Moral R, Román-Macia J, Rayó E, García-Ríos A, Casas R, Gómez-Pérez AM, Santos-Lozano JM, Buil-Cosiales P, Asensio EM, Lassale C, Abete I, Salaverria-Lete I, Sayón-Orea C ,Schröder H, and Salas-Salvadó J; PREDIMED-Plus Investigators. Eur J Clin Nutr **(Accepted, in Print).**

Other publications:

Babio N, Becerra-Tomás N, Nishi SK, **López-González L**, Paz-Graniel I, García-Gavilán J, Schröder H, Martín-Calvo N, Salas-Salvadó J. Total dairy consumption in relation to overweight and obesity in children and adolescents: a systematic review and meta-analysis. Obes Rev. 2022;23Suppl1:e13400. PubMed PMID: 34881504.

## 10.2 Participation in international and national conferences

**Conference**: XI Symposium Ciber Fisiopatología de la Obesidad. Madrid, Spain. 26-29 October 2020. ONLINE.

**Authors**: López-González L, Becerra-Tomás N, Babio N, Martínez-González MA, Díaz-López A, Corella D, Fitó M, Martínez JA, Alonso-Gómez AM, WärnberG J, Vioque J, Romaguera D, López-Miranda J, Estruch R, Tinahones FJ, Lapetra J, Serra-Majem L, Bueno-Cavanillas A, Tur JA, Martín Sánchez V, Pintó X, Delgado-Rodríguez M, Matía-Martín P, Vidal J, Vázquez C, Daimiel L, Ros E, Canudas S and Salas-Salvadó J.

**Title**: Variety in fruits and vegetables, diet quality and lifestyle in an elderly Mediterranean population.

**Format**: Poster.

**Conference**: 38th International Symposium on Diabetes and Nutrition. Reus, Spain. 21-24 June 2021. ONLINE.

**Authors**: **López-González L**, Becerra-Tomás N, Babio N, Martínez-González MA, Mestres C, Corella D, Fitó M, Martínez JA, Alonso-Gómez AM, Wärnberg J, Vioque J, Romaguera D, López-Miranda J, Estruch R, Tinahones F, Lapetra J, Serra-Majem L, Bueno-Cavanillas A, Tur JA, Martín-Sánchez V, Pintó X, Delgado-Rodríguez M, Matía-Martín P, Vidal J, Vázquez C, Daimiel L, Ros E, Megias-Rangil I, Salas-Salvadó J.

**Title**: One-year changes in variety of fruit and vegetable intake and cardiometabolic risk factors in an elderly Mediterranean population at high cardiovascular risk.

**Format**: Poster.

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