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

Leyre López-González[1,2,3¥], Nerea Becerra-Tomás[1,2,4,5¥], Nancy Babio[1,2,3,4**], Miguel Ángel Martínez-González[4,6,7], Stephanie Nishi[1,2,4,8,9], Dolores Corella[4,10], Albert Goday[4,11], Dora Romaguera[4,12], Jesús Vioque[13,14], Ángel M. Alonso-Gómez[4,15], Julia Wärnberg[4,16], J. Alfredo Martínez[4,17,18], Luís Serra-Majem[4,19], Ramon Estruch[4,20], Rosa M. Bernal-López[4,21], José Lapetra[4,22], Xavier Pintó[4,23], Josep A. Tur[4,24], José López-Miranda[4,25], Aurora Bueno-Cavanillas[13,26], Miguel Delgado-Rodríguez[13,27], Pilar Matía-Martín[28], Lidia Daimiel[29], Vicente Martín Sánchez[4,30], Josep Vidal[31,32], Clotilde Vázquez[4,33], Emili Ros[4,34], Zenaida Vázquez-Ruiz[4,6], Francisco M Martín-Luján[35, 36], José V Sorlí[4,10], Olga Castañer[4,11], M Ángeles Zulet[4, 17]; Lucas Tojal-Sierra[4,15], Rosa Carabaño-Moral[37]; Josefa Román-Maciá[38]; Elena Rayó[12]; Antonio García-Ríos[4,25], Rosa Casas[4,20], Ana M. Gómez-Pérez[39], José M Santos-Lozano[4,22], M Pilar Buil[4,6,40], Eva M. Asensio[4,10], Camille Lassale[4,11], Itziar Abete[4,17]; Itziar Salaverria-Lete[4,15], M Carmen Sayón[6]; Helmut Schröder[11,13] and Jordi Salas-Salvadó[1,2,3,4*]; on behalf of the PREDIMED-Plus Investigators[#].

[#]A complete list of PREDIMED investigators is included as appendix.

[1]Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Unitat de Nutrició Humana, Reus, Spain.

[2]Institut d'Investigació Sanitària Pere Virgili (IISPV), Reus, Spain.

[3]University Hospital of Sant Joan de Reus, Nutrition Unit, Reus, Spain.

[4]Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain.

[5] Department of Epidemiology and Biostatistics, School of Public Health,Imperial College of London, London, United Kingdom.

[6]University of Navarra, Department of Preventive Medicine and Public Health, University of Navarra, Navarra, Spain.

[7]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA.

[8]Toronto 3D (Diet, Digestive tract and Disease) Knowledge Synthesis and Clinical Trials Unit, Toronto, ON, Canada.

[9]Clinical Nutrition and Risk Factor Modification Centre, St. Michael´s Hospital, Unity Health Toronto, ON, Canadá.

[10]Department of Preventive Medicine, University of Valencia, Valencia, Spain.

[11]Cardiovascular Risk and Nutrition research group (CARIN), Hospital del Mar Research Institute (IMIM), Barcelona, Spain.

[12]Health Research Institute of the Balearic Islands (IdISBa).

[13]CIBER de Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain.

[14]Alicante Institute for Health and Biomedical Research (ISABIAL-UMH). Alicante, Spain.

[15]Bioaraba Health Research Institute; Osakidetza Basque Health Service, Araba University Hospital; University of the Basque Country UPV/EHU; Vitoria-Gasteiz, Spain.

[16]Epi-PHAAN Research Group, School of Health Science, Universidad de Malaga, Instituto de Investigación Biomédica de Málaga (IBIMA), 29071, Málaga, Spain.

[17]University of Navarra, Department of Nutrition, Food Science and Physiology, IDISNA, Pamplona, Spain.

[18]Precision Nutrition Program, IMDEA Food, CEI UAM + CSIC, Madrid, Spain.

[19]University of Las Palmas de Gran Canaria, Research Institute of Biomedical and Health Sciences (IUIBS), Preventive Medicine Service, Centro Hospitalario Universitario Insular Materno Infantil (CHUIMI), Canarian Health Service, Las Palmas, Spain.

[20]Department of Internal Medicine, IDIBAPS, Hospital Clinic, University of Barcelona, Barcelona, Spain.

[21]Servicio de Medicina Interna , Hospital Regional Universitario de Malaga, Instituto de Investigación Biomédica de Málaga (IBIMA), Universidad de Málaga (UMA), Málaga, Spain.

[22]Department of Family Medicine, Research Unit, Distrito Sanitario Atención Primaria Sevilla, Sevilla, Spain.

[23]Lipids and Vascular Risk Unit, Internal Medicine, Hospital Universitario de Bellvitge, Hospitalet de Llobregat, Barcelona Spain.

[24]Research Group on Community Nutrition & Oxidative Stress, University of Balearic Islands, Palma de Mallorca, Spain.

[25]Department of Internal Medicine, Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Reina Sofia University Hospital, University of Cordoba, Cordoba, Spain.

[26]Department of Preventive Medicine, University of Granada, Granada, Spain.

[27]Division of Preventive Medicine, Faculty of Medicine, University of Jaén, Jaén, Spain.

[28]Department of Endocrinology and Nutrition, Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC), Madrid, Spain.

[29]Nutritional Genomics and Epigenomics Group, IMDEA Food, CEI UAM+CSIC, Madrid, Spain.

[30]Institute of Biomedicine (IBIOMED), University of León, León, Spain.

[31]CIBER Diabetes y Enfermedades Metabólicas (CIBERDEM), Instituto de Salud Carlos III (ISCIII), Madrid, Spain.

[32]Departament of Endocrinology, IDIBAPS, Hospital Clínic, University of Barcelona, Barcelona, Spain.

[33]Department of Endocrinology, Fundación Jiménez-Díaz, Madrid, Spain.

[34]Lipid Clinic, Department of Endocrinology and Nutrition, Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clínic, Barcelona, Spain.

[35]Research support Unit Tarragona-Reus. Institut Universitari d´investigació en l´atenció Primària Jordi Gol, (IDIAP Jordi Gol), Institut Catalá de la Salut; Tarragona, Spain.

[36]Universitat Rovira i Virgili. Departament de Medicina i Cirugia; Reus, Spain.

[37]Unidad de Gestión Clínica Arroyo de la Miel. Distrito de Atención Primaria Costa del Sol. Servicio Andaluz de Salud. Benalmádena, Málaga.

[38]Centro de Salud Santa Pola, Alicante, Spain.

[39]Unidad de Gestión Clínica de Endocrinología y Nutrición, Instituto de Investigación Biomédica de Málaga (IBIMA), Hospital Universitario de Málaga (Virgen de la Victoria), Universidad de Málaga, Málaga, Spain.

[40]Atención Primaria. Servicio Navarro de Salud, Navarra, Spain.

*Corresponding author: Universitat Rovira i Virgili. Facultat de Medicina i Ciències de la Salut. Departament de bioquímica i biotecnología. Unitat de Nutrició Humana, C/ Sant Llorenç 21, 43 201 Reus (Spain). *Email address*: jordi.salas@urv.cat

**Corresponding author: Universitat Rovira i Virgili. Facultat de Medicina i Ciències de la Salut. Departament de bioquímica i biotecnología. Unitat de Nutrició Humana, C/ Sant LLorencç 21, 43 201, Reus (Spain). *Email address*: nancy.babio@urv.cat

¥Leyre López González and Dr. Nerea Becerra Tomás contributed equally to this work.

Abstract

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 was 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 (-0.07kg (-0.13,-0.02)) and waist circumference (WC) (-0.08cm (-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 (-0.11kg (-0.15,-0.07)) and WC (-0.10cm (-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 (-0.40kg (-0.58,-0.23)) and WC (-0.50cm (-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.

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 grams 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 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 behavioural 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 centres from the National Health System, like universities, research institutes hospitals and primary health care centres (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 analysed 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 refers 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 means±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 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 centre, family members and patients 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 100g/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 centre (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 **Figure 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 Figure 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 Figure 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 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 Figure 3**), fruit variety and quantity (**Supplementary Figure**

4), and vegetable variety and quantity (**Supplementary Figure 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 explained 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 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). 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.

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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.

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Ethical 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).

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.

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Table and Figure legends

Table 1. Baseline characteristics of the study population.

Table 2. Cardiometabolic changes over one 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.

Figure 1. Beta-coefficients and 95% CI for cardiometabolic changes by one-year changes in categories of fruit and vegetable quantity and variety.