Relationship between olive oil consumption and ankle-brachial pressure index in a population at high cardiovascular risk

Cristina Sánchez-Quesada [1,2,3,*], Estefanía Toledo[4,5,*], Guadalupe González-Mata [6], Maria Isabel Ramos-Ballesta [6], José Ignacio Peis[7], Miguel Ángel Martínez-González[4,5,8], Jordi Salas-Salvadó[4,10,11,12], Dolores Corella[4,13], Montserrat Fitó[4,14], Dora Romaguera[4,15], Jesús Vioque[9,16] , Ángel M. Alonso-Gómez[4,17], Julia Wärnberg[4,18], J. Alfredo Martínez[4,19,20], Luís Serra-Majem[4,21], Ramon Estruch[4,22], Francisco J Tinahones[4,23], José Lapetra[4,24], Xavier Pintó[4,25], Josep A. Tur[4,15,26], Antonio Garcia-Rios[4,27], Naomi Cano-Ibáñez[9,28], Pilar Matía-Martín[29], Lidia Daimiel[20], Rubén Sánchez-Rodríguez[30], Josep Vidal[31,32], Clotilde Vázquez[4,33], Emilio Ros[4,34], Pablo Hernández-Alonso[4,10,12,23], Rocío Barragan[13], Julia Muñoz-Martínez[14], Meritxell López[15], Sandra González-Palacios[9,16], Jessica Vaquero-Luna[17], Edelys Crespo-Oliva[18], M. Angeles Zulet[4,19], Vanessa Díaz-González[21], Rosa Casas[4,22], José Carlos Fernandez-Garcia[23], José Manuel Santos-Lozano[4,24], Ana Galera[4,25], Tomás Ripoll-Vera[4,15,26,35], Pilar Buil-Cosiales[4,5,36], Silvia Canudas[4,10,12], Raul Martinez-Lacruz[13], Karla-Alejandra Pérez-Vega[14], Ángel Rios[15], Rosario Lloret-Macián[37], Anai Moreno-Rodriguez[17], Miguel Ruiz-Canela[4,5], Nancy Babio[4,10,12], Maria Dolores Zomeño Fajardo[14,38], José J. Gaforio [1,2,3,9].

[1]Center for Advanced Studies in Olive Grove and Olive Oils, University of Jaen, Campus las Lagunillas s/n, 23071, Jaén, Spain.
[2]Immunology Division, Department of Health Sciences, Faculty of Experimental Sciences, University of Jaén, Jaén, Spain;
[3]Agri-food Campus of International Excellence (ceiA3), Córdoba, Spain.
[4]Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Institute of Health Carlos III, Madrid, Spain
[5]University of Navarra, Department of Preventive Medicine and Public Health, IdiSNA, Pamplona, Spain
[6] Preventive Medicine and Public Health Division, Department of Health Sciences, Faculty of Experimental Sciences, University of Jaén, Jaén, Spain;
[7]Centro de Salud Bulevar (Servicio Andaluz de Salud, SAS). Jaén, Spain;
[8]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA
[9]CIBER Epidemiología y Salud Pública (CIBER-ESP), Instituto de Salud Carlos III, Madrid, Spain.
[10]Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Unitat de Nutrició, Reus, Spain
[11]University Hospital of Sant Joan de Reus, Nutrition Unit, Reus, Spain
[12]Institut d'Investigació Sanitària Pere Virgili (IISPV), Reus, Spain
[13]Department of Preventive Medicine, University of Valencia, Valencia, Spain
[14]Unit of Cardiovascular Risk and Nutrition, Institut Hospital del Mar de Investigaciones Médicas Municipal d`Investigació Médica (IMIM), Barcelona, Spain.
[15]Health Research Institute of the Balearic Islands (IdISBa), Palma de Mallorca, Spain.
[16]Miguel Hernandez University, ISABIAL-UMH, Alicante, Spain
[17]Bioaraba Health Research Institute; Osakidetza Basque Health Service, Araba University Hospital; University of the Basque Country UPV/EHU; Vitoria-Gasteiz, Spain.
[18]Department of Nursing, School of Health Sciences, Instituto de Investigación Biomédica de Málaga (IBIMA), Málaga, Spain
[19]Department of Nutrition, Food Sciences, and Physiology, Center for Nutrition Research, University of Navarra, Pamplona, Spain.
[20]Precision Nutrition and Cardiometabolic Health Program. Precision Nutrition and Obesity Program, IMDEA Food, CEI UAM + CSIC, Madrid, Spain
[21]Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria & Centro Hospitalario Universitario Insular Materno Infantil (CHUIMI), Canarian Health Service, Las Palmas de Gran Canaria, Spain.
[22]Department of Internal Medicine, Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clinic, University of Barcelona, Barcelona, Spain
[23]Virgen de la Victoria Hospital, Department of Endocrinology, Instituto de Investigación Biomédica de Málaga (IBIMA). University of Málaga, Málaga, Spain.
[24]Department of Family Medicine, Research Unit, Distrito Sanitario Atención Primaria Sevilla, Sevilla, Spain
[25]Lipids and Vascular Risk Unit, Internal Medicine, Hospital Universitario de Bellvitge, Hospitalet de Llobregat, Barcelona Spain
[26]Research Group on Community Nutrition & Oxidative Stress, University of Balearic Islands, 07122 Palma de Mallorca, Spain
[27]Department of Internal Medicine, Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Reina Sofia University Hospital, University of Cordoba, Cordoba, Spain
[28]Department of Preventive Medicine and Public Health, University of Granada, Granada, Spain
[29]Department of Endocrinology and Nutrition, Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC), Madrid, Spain
[30]Centro de Salud Siero-Sariego, Asturias.
[31]CIBER Diabetes y Enfermedades Metabólicas (CIBERDEM), Instituto de Salud Carlos III (ISCIII), Madrid, Spain

[32]Department of Endocrinology, Hospital Clinic, IDIBAPS, University of Barcelona, Barcelona, Spain.
[33]Department of Endocrinology and Nutrition, Hospital Fundación Jimenez Díaz. Instituto de Investigaciones Biomédicas IISFJD. University Autónoma, Madrid, Spain
[34]Lipid Clinic, Department of Endocrinology and Nutrition, Hospital Clínic, IDIBAPS, Barcelona, Spain
[35]Department of Cardiology, Hospital Son Llàtzer, 07198 Palma de Mallorca, Spain
[36]Servicio Navarro de Salud, Pamplona, Spain.
[37]Centro Salud Cabo Huertas, Alicante.
[38]Blanquerna School of Health Sciences, Universitat Ramon Llull, Barcelona, Spain.

* Both authors equally contributed

Corresponding author:
José J. Gaforio
Health Sciences Department, Immunology Division
Faculty of Experimental Sciences. Campus Las Lagunillas s/n. CP 23071. Jaen (SPAIN)
Email: jgaforio@ujaen.es. Phone: +34 953212002

Abstract

Background and aims: The aim of this study was to ascertain the association between the consumption of different categories of edible olive oils (virgin olive oils and olive oil) and olive pomace oils and ankle-brachial pressure index (ABI) in participants of the PREDIMED-Plus study, a trial of lifestyle modification for weight and cardiovascular event reduction in individuals with overweight/obesity harboring the metabolic syndrome. *Methods*: Cross-sectional analyses of the PREDIMED-Plus trial. Consumption of any category of olive oil ~~total, virgin, olive oil~~ and olive pomace oils were assessed through a validated food-frequency questionnaire. Multivariable linear regression models were fitted to assess associations between olive oil consumption and ABI. Additionally, an ABI ≤1 was considered as the outcome in logistic models with different categories of olive oil and olive pomace oil as exposure. *Results*: Among 4,330 participants, the highest quintile of total olive oil consumption (sum of all categories of olive oil and olive pomace oil) was associated with higher mean values of ABI (beta coefficient: 0.014, 95% confidence interval [CI]: 0.002, 0.027) (*p for trend*=0.010). Logistic models comparing the consumption of different categories of olive oils, olive pomace oil and ABI ≤1 values revealed an inverse association between virgin olive oils consumption and the likelihood of a low ABI (odds ratio [OR] 0.73, 95% CI [0.56, 0.97]), while consumption of olive pomace oil was positively associated with a low ABI (OR 1.22 95% CI [1.00, 1.48]). *Conclusions*: In a Mediterranean population at high cardiovascular risk, total olive oil consumption was associated with a higher mean ABI. These results suggest that olive oil consumption may be beneficial for peripheral artery disease prevention, but longitudinal studies are needed.

Keywords: Olive oil (OO); virgin olive oils (VOO); ankle-brachial pressure index (ABI); peripheral artery disease (PAD); PREDIMED-Plus trial; olive pomace oil.
Abbreviations:
ABI: ankle-brachial pressure index; PAD: peripheral artery disease; CV: cardiovascular; BMI: body mass index; MetS: metabolic syndrome; OR: odds ratio; CI: confidence interval.

1. Introduction

The American College of Cardiology/American Heart Association defines peripheral atherosclerotic vascular diseases as a group of clinical disorders that includes abdominal aortic aneurysm, renal and mesenteric artery disease, extracranial carotid artery disease, and disease of the aortoiliac, femoropopliteal, and infrapopliteal arterial segments. It does not address nonatherosclerotic causes of lower extremity arterial disease, such as vasculitis, fibromuscular dysplasia, physiological entrapment syndromes, cystic adventitial disease and other entities [1].

Peripheral artery disease (PAD) is the term used by The European Society of Cardiology to describe all the arterial diseases other than coronary arteries or the aorta.

While several methods are used in the diagnosis of PAD, ankle-brachial index (ABI) is recommended as a non-invasive tool for screening and diagnosis [2~~–5~~]. ABI is calculated as the ratio of the systolic blood pressure measured at the ankle to that measured at the brachial artery [3 ~~6~~]. Usually, the highest ankle systolic pressure is divided by the highest brachial systolic pressure, resulting in an ABI for each leg [2~~–5~~]. The ABI is >1.0 in healthy individuals, among whom the blood pressure wave is amplified as it travels distally from the heart, resulting in a higher ankle than brachial systolic blood pressure. The majority of studies use an ABI of 0.90 as the threshold to define PAD, with borderline ABI defined as that between 0.91 and 0.99 [3 ~~6~~]. ABI has high specificity but suboptimal sensitivity to detect PAD [4 ~~7~~]. However, an ABI of 1.4 or more has been associated with an increased prevalence of PAD and CV risk, since it can be indicative of frequent arterial medial calcifications [5 ~~8~~].

Olive oil consumption is reported to have several beneficial effects on CV disease [6-11 ~~9–13~~]; however to our knowledge, epidemiological evidences on Mediterranean diet and its implications in PAD risk is limited [12-17] and, there is no evidence on the specific effect of total, virgin, olive oil or olive pomace oils in the prevention of PAD.

The PREDIMED trial -a controlled trial for the primary prevention of cardiovascular disease based on a nutritional intervention- was the first primary prevention trial which addressed the effect of a Mediterranean diet enriched with extra-virgin olive oil on the incidence of a first cardiovascular event [15]. Participants allocated to the Mediterranean diet group enriched with extra-virgin olive oil showed a significantly lower risk of cardiovascular disease than participants allocated to the control group. In addition, in the PREDIMED trial, participants allocated to the Mediterranean diet enriched with extra-virgin olive oil showed a significantly lower risk of PAD [14].

This cross-sectional study analyzed the associations between the consumption of different categories of olive oils (total, virgin and olive oil), olive pomace oils and ABI in the PREDIMED-Plus trial.

2. Patients and Methods

2.1. Design and participants

The present study is a cross-sectional assessment conducted within the frame of the PREDIMED-Plus trial. The design and methods of the PREDIMED-Plus trial have been described in detail elsewhere [18] and the protocol is available at www.predimedplus.com. Briefly, PREDIMED-Plus is an ongoing multicenter, randomized controlled trial conducted in Spain to assess the effect of an intensive weight-loss intervention based on an energy-reduced traditional Mediterranean diet (MedDiet), physical activity promotion, and behavioral support on hard CV events, in comparison to that of a control group receiving usual care intervention only with energy-unrestricted Mediterranean diet recommendations. This study was registered with the International Standard Randomized Controlled Trial (ISRCT; http://www.isrctn.com/ISRCTN89898870) with number 89898870 (registration date: July 24, 2014). Written informed consent was obtained from each patient included in the study, the study protocol conforms to the ethical guidelines of the 2013 Declaration of Helsinki and the study protocol has been priorly approved by the Research Ethics Committees from all recruitment centers [18]. The eligible participants were adults (aged 55–75 years in men; 60–75 years in women) with overweight/obesity (body mass index [BMI] ≥27 and <40 kg/m^2, respectively] and who met at least three criteria for metabolic syndrome (MetS) according to the updated harmonized criteria of the International Diabetes Federation and the American Heart Association and National Heart, Lung, and Blood Institute [19]. A total of 6,874 participants were recruited and randomized from 23 recruitment sites at different universities, hospitals, and research institutes in Spain. The present analysis included only those participants whose total energy intakes were within predefined limits (800–4,000 Kcal/day for men, and 500–3,500

Kcal/day for women [20] (n=241 out of limits), participants from recruitment centers with less than 25% missing data on ABI as a quality control (n=1,913 out of criteria), and participants with an ABI <1.4 (n=390 participants with ABI ≥1.4) (Figure 1).

2.2. Exposure Assessment

The consumption of total olive oil, in which are included the following ~~types~~ categories: virgin olive oils (including extra virgin and virgin olive oil), olive oil (described as refined olive oil and virgin olive oil mixture according to the European Commission Regulation (EC) Nº 1019/2002 [21]) and consumption of olive pomace oil was assessed using a validated 143-item semi-quantitative food-frequency questionnaire [22] administered at baseline. In face-to-face interviews, the participants were asked about their frequencies of consumption of each food item during the past year, as well as the usual serving sizes. Nine possibilities of frequency were offered, ranging from never to >6 times/day. Virgin, olive oil, olive pomace oil and total olive oil (which include both categories of olive oils and olive pomace oil) consumptions were adjusted for total energy intake using the residual method and the participants were then grouped into quintiles according to their total, virgin, or olive oil consumption. Due to the low consumption and low variability in olive pomace oil consumption, the participants were classified into tertiles according to their olive pomace oil consumption. Smoke variable was introduced as positive control to assess well known associations with PAD.

2.3. Outcome Assessment

At baseline, trained staff measured ABI in accordance with the PREDIMED-Plus operation protocol as the lowest value of two different measurements in each leg of the participant. For the present analyses, we averaged the ABI values of both legs. A sphygmomanometer cuff is placed just above the ankle and a Doppler instrument is used to measure the pressure of the posterior and anterior tibial (dorsalis pedis) arteries of each foot. In addition to ABI as a continuous variable, we also used a dichotomous outcome defined as an ABI lower or equal to 1. On the other side of the spectrum, since ABI ≥1.4 can be indicative of frequent arterial medial calcifications [5], we excluded patients with ABI ≥1.4.

2.4. Covariate Assessment

Information on socio-demographic variables (e.g., sex, age, marital status, and educational level) and lifestyle-related characteristics (e.g., smoking status, physical activity, or adherence to the Mediterranean diet) were obtained from the baseline questionnaire. Weight and height were measured in duplicate with calibrated scales and a wall-mounted stadiometer, respectively. BMI was calculated as the weight in kilograms divided by the height in meters squared. Leisure-time physical activity was assessed using the short form of the Minnesota Leisure Time Physical Activity Questionnaire validated in Spain [23, 24]. Leisure-time activities were computed by assigning a metabolic equivalent score to each activity [25], multiplied by the time spent for each activity and summing all activities. Adherence to an energy-restricted Mediterranean diet was evaluated with a 17-item questionnaire [26] which we modified with the exclusion of the extra-virgin olive oil component to avoid collinearity (score range 0-16). Self-reported comorbidities (hypertension and type 2 diabetes) were collected from each participant.

2.5. Statistical Analysis

Baseline variables across quintiles of total olive oil consumption were described as means and standard deviations for continuous traits and as percentages for qualitative traits. We estimated the Pearson's correlation coefficient between different categories of olive oil consumption and between continuous traits considered as potential confounders.
Multivariable linear regression models were fitted to assess the association between energy-adjusted consumption of total, virgin, olive oil (quintiles) and olive pomace oil (tertiles) consumption and ABI index. Beta coefficients and their 95% confidence intervals (CI) were calculated with the lowest quintile as the reference category.

To control for potential confounding factors, the results were adjusted for age (continuous), sex, and recruitment center in multivariable Model 1. Multivariable Model 2 was additionally adjusted for BMI (tertiles) and multivariable Model 3 was additionally adjusted for waist circumference (continuous); self-reported prevalent diabetes (yes/no); self-reported hypertension (yes/no); score appraising adherence to an energy-reduced Mediterranean diet (score 0–17); physical activity (metabolic equivalents [METs]-min/ week) (quintiles); educational level (primary school or less, secondary school, university); use of blood pressure-lowering drugs (yes/no); use of lipid-lowering medication (yes/no); use of diuretics (yes/no); use of insulin or oral antidiabetic agents (yes/no); dietary vitamin D intake (tertile); folic acid intake (continuous); family history of coronary heart disease (CHD) (yes/no); smoking (never, current, former ≥5 years, former <5 years); and total energy intake (continuous).

Tests of linear trend across increasing quintiles of exposures were conducted by assigning the quintile-specific median to each participant and treating the resulting variable as continuous trait.

Finally, to assess the robustness of the multivariable linear regressions, we performed sensitivity analysis for Q5 vs. Q1 of total olive oil consumption with different energy limits (percentiles 1 and 99), including participants with ABI ≥1.4, or participants from all recruitment centers. We also assessed the association after stratifying by sex (men and women), BMI (<30 kg/m^2 or ≥30 kg/m^2), age (≤65 or >65 years), baseline adherence to the Mediterranean diet (≤7 points vs ≥8 points), and baseline diabetes.

Additional logistic regression models were fitted to assess the odds ratios (ORs) (95% CI) for an ABI ≤1, as a dichotomous outcome, according to energy-adjusted consumption of total, virgin, and olive oil (quintiles) and olive pomace oil (tertiles). The ORs and their 95% CIs were calculated considering the lowest quintile as the reference category. A sensitivity analysis with ORs for an ABI ≤0.9 is presented in Table 2 of Supplementary files.

To control for potential confounding factors in logistic regression models, the results were adjusted for the same confounding factors as the multiple linear regression models. We also conducted tests of linear trend across increasing quintiles of exposure. Smoke variable was introduced as positive control to assess a well-known association with PAD.

Finally, we assessed correlations between total, virgin, olive oil and olive pomace oil consumption in the PREDIMED-Plus trial.

3. Results

This study included 4,330 out of 6,874 participants recruited in the PREDIMED-Plus trial, according to previously defined exclusion criteria (Figure 1). The mean age of study subjects was 65 and 47.9% were women. Table 1 shows the baseline characteristics of the participants included according to energy-adjusted quintiles of total olive oil consumption. Participants in the highest category of total olive oil consumption had a higher total energy intake, higher adherence to the Mediterranean diet, higher fat intake (especially monounsaturated fatty acids), lower consumption of meat, fish, and dairy products and a decrease in fruit, vegetables, cereals and legumes, respect to lowest category of total olive oil intake.

The results of correlation analyses between total, virgin, olive oil and olive pomace oil consumption are shown in Table 2. We observed a significant inverse correlation between virgin and olive oils consumption (-0.41). Correlations between all continuous traits considered as potential confounders are shown in Table 1 of Supplementary files.

The associations between energy-adjusted consumption of total, virgin, olive oil and olive pomace oil and ABI are shown in Table 3. The ABI was 0.014 points (95% CI: 0.002, 0.027) higher among participants in the highest category of total olive oil consumption compared to participants in the lowest consumption quintile in the fully-adjusted model (*p* for trend *0.010*). Associations between specific categories of olive oil, olive pomace oil and ABI were not statistically significant.

Table 4 shows the ORs (95% CI) between categories of total, virgin, olive oil and olive pomace oil consumption and a low or borderline ABI (defined as ABI ≤1). Participants in Q5 of virgin olive oils intake showed a significantly lower odds of borderline ABI (0.73, 95% CI 0.56, 0.97;

p for trend *0.031*), whereas participants in Q5 of olive oil (OR 1.43, 95% CI 1.07, 1.90; *p* for trend 0.114) or olive pomace oil (OR 1.22, 95% CI 1.00, 1.48; *p* for trend *0.050*) showed a significantly higher odds of borderline ABI compared to participants in the Q1. When ABI ≤0.9 was analyzed in a logistic model (Table 2-Supplementary data) any category or total olive oil intake were statistically significative.

In order to assess the robustness of our findings, we performed sensitivity analyses of the differences in mean ABI across categories of olive oil and olive pomace oil consumption from all recruitment centers included but removing those with missing values of ABI in the right or the left leg (Table 3-Supplementary files). We also conducted sensitivity analysis for total olive oil consumption (comparing extreme quintiles), after applying different energy limits (percentiles 1 and 99), from all recruitment centers and other analysis including participants with ABI ≥1.4. We also conducted stratified analyses by sex (men and women), BMI (<30 kg/m^2 or ≥30 kg/m^2), age (≤65 or >65 years), baseline adherence to the Mediterranean diet (≤7 points *vs* ≥8 points), and baseline diabetes (Figure 2). These analyses supported the robustness of our main results.

We also analyzed the association between smoking habit and the ABI or an ABI ≤1 as a positive control. When compared to never smokers (n=1873), smokers who quitted >5 years ago (n=1586) showed an ABI difference of -0.008 (-0.017, 0.000), smokers who quitted <5 years ago (n=96) an ABI difference of -0.030 (-0.054, -0.005) and current smokers (n=755) an ABI difference of -0.032 (-0.043, -0.021) in multivariable adjusted models. Compared to never smokers, the OR of an ABI ≤1 was 1.32 (1.08, 1.61) for smokers who quitted >5 years ago, 1.50 (0.88, 2.57) for smokers who quitted <5 years ago and 2.09 (1.66, 2.64) for current smokers in the multivariable adjusted models.

4. Discussion

The results of this cross-sectional analysis of the PREDIMED-Plus trial showed a direct association between total olive oil consumption (including olive pomace oil) and ABI. In addition, virgin olive oils consumption was inversely associated with borderline ABI whereas olive oil and olive pomace oil consumption were directly associated with borderline ABI.

ABI is a non-invasive, simple, and reliable diagnostic method used as a simple method to screen for PAD and to evaluate CV prognosis in the general population [27]. The range of normal ABI values is between 0.90 and 1.4. In fact, an ABI below 0.90 represents an independent marker of CV risk [28]. This threshold of ≤0.90 is based on studies reporting >90% sensitivity and specificity to detect PAD compared to angiography [29, 30]. Otherwise, with an ABI ≥1.0 used as a threshold for detecting PAD, sensitivities as high as 100% have been reported [28, 30]. Thus, ABI should be carefully interpreted according to *a priori* probability of PAD and values between 0.91 and 1.00 should be considered borderline [3]. In this study, our evaluation of the probability of an ABI ≤1 included all participants considered to be borderline. Total and virgin olive oils consumption appeared to be inversely associated with the risk of an ABI ≤1 (OR 0.86 and OR 0.73, respectively). However, olive pomace oil and olive oil consumption appeared to be associated with a higher odd of borderline ABI (OR 1.22, 95% CI 1.00, 1.48 and OR 1.43, 95% CI 1.07, 1.90, respectively).

There is no clear association between the role of nutrition in preventing PAD [31]; although the Mediterranean diet may reduce the risk of PAD [32-34], more trials with an experimental design to confirm this protective effect are required. Regarding the consumption of extra-virgin olive oil –the main fat source of the Mediterranean diet– has been associated with beneficial effects on PAD [6, 34] and ~~the~~ this association may be related to several components of virgin olive oils. Indeed, the InCHIANTI study reported that increasing HDL cholesterol levels could exert some protective effect against PAD [33]. Considering that virgin olive oils increase HDL cholesterol levels in humans, as reported by the VOHF study [8], it could indirectly prevent PAD. Pedret *et al.* recently described the activity of the phenolic fraction of virgin olive oils in the HDL proteome because it may lead to the up-regulation of proteins related to cholesterol homeostasis, protection against oxidation, and blood coagulation [8]. The authors also reported the down-regulation of acute-phase response, lipid transport, and immune response [8]. Some studies have described the effects of virgin olive oils or its components in preventing CV disease [7-10], which could also be related to the potential protective effect of olive oil consumption against PAD.

Overall, our results suggest that a higher total olive oil consumption was associated with better values for the ABI but there was no significant association with borderline ABI used as a dichotomous variable (ABI≤1). When we further addressed if consumption of specific olive oil categories was associated with ABI, we found significant association with virgin olive oils and olive pomace oil. Higher virgin olive oil consumption was significantly associated with a lower odds of borderline ABI, whereas olive oil consumption and olive pomace oil consumption were associated with a higher odds of borderline ABI compared to the lowest consumption category, with a statistically significant finding for olive pomace oil. It could be hypothesized that replacing more processed categories of olive oil and olive pomace oils by virgin olive oils may help improve the ABI. The observed results across the different olive oil categories could be due to minor compounds present in virgin olive oil, which are practically absent in refined olive oil and olive pomace oil [32, 33]. Given that the lipid profile is mainly the same in all categories of olive oils and olive pomace oil, this idea becomes coherent. In fact, minor compounds of virgin olive oils have been described to be responsible for different beneficial properties against several diseases, including CV disease prevention [7, 9, 34-36]. Contrarily, olive pomace oil loses the majority of minor compounds and some chemical contaminants could be present in it as a result of manufacturing processes [37]. Some of these contaminants have been described as harmful compounds [38]. Nevertheless, we have to notice that differences between categories of olive oils and olive pomace oils may be due not only to their composition, but also to their differential culinary use. It is known that the fatty acid profile of olive pomace oil is similar to the fatty acid profile of virgin olive oils, but it is generally used for frying foods, including ultraprocessed foods, which have been reported to increase CV risk [39]. These hypotheses could explain the differences observed between extreme quintiles and tertiles of consumption in virgin olive oils as compared to olive pomace oil or refined olive oil in their association with the ABI index; however, prospective studies are needed to corroborate these associations.

This study has several strengths, including the large sample size, inclusion of both men and women in the trial, adjustment for a wide array of potential confounders, and the use of validated tools to assess information. However, we acknowledge some limitations such as its cross-sectional design, which does not allow the establishment of any causal association between olive oil consumption and ABI. In addition, the age of the participants was limited to 55–75 years and all of our participants had metabolic syndrome; therefore, generalization of the results to other age groups may be limited. Furthermore, as this is not a prospective study, results should be treated carefully and potential causal inferences need to be confirmed by future prospective studies. Also, olive oil consumption was addressed only once. The food-frequency questionnaire covered one year of exposure over the previous year. The implicit assumption is that past year consumption tends to be correlated with lifetime consumption. However, we admit that appraising a longer exposure to olive oil may be important to fully disentangle the association between olive oil consumption and ABI or PAD. Finally, we were not able to distinguish between the minor components of the different categories of olive oils and olive pomace oils. Concentration of minor components does not only depend on olive oil category but also on other characteristics such as its origin, olive variety or ripeness of the olives from which olive oil was extracted [40].

In conclusion, the main findings of the current study suggest a direct association between total olive oil consumption and a better ABI. Moreover, consumption of virgin olive oils could be useful to promote a better ABI instead other olive oils categories or olive pomace oils. Nevertheless, longitudinal studies are needed to confirm these results and to definitively establish the role of total olive oil consumption, different categories of olive oil and olive pomace oils consumption in the development of PAD.

5. Conflicts of Interest

Jordi Salas-Salvadó reports serving on the board of the International Nut and Dried Fruit Council, the Danone International Institute, and the Eroski Foundation and receiving grant support from these entities through his institution. He also reports serving on the Executive Committee of the Instituto Danone Spain. He has also received the olive oil and nuts used in PREDIMED and PREDIMED-plus studies from the Patrimonio Comunal Olivarero, Spain; La Morella Nuts, Spain; and Borges S.A., Spain. He reports receiving consulting fees or travel expenses from Danone; the California Walnut Commission, the Eroski Foundation, the Instituto Danone - Spain, Nuts for Life, the Australian Nut Industry Council, Nestlé, Abbot Laboratories, and Font Vella Lanjarón.

Dr Estruch reported receiving grants from Instituto de Salud Carlos III and olive oil for the trial from Fundacion Patrimonio Comunal Olivarero during the conduct of the study and personal fees from Brewers of Europe, Fundación Cerveza y Salud, Interprofesional del Aceite de Oliva, Instituto Cervantes, Instituto Cervantes, Pernaud Richar, Fundación Dieta Mediterránea, Wine and Culinary International Forum; nonfinancial support from Sociedad Española de Nutrición and Fundación Bosch y Gimpera; and grants from Uriach Laboratories outside the submitted work.

6. Financial support

The PREDIMED-Plus trial was supported by the official funding agency for biomedical research of the Spanish government, ISCIII, through the Fondo de Investigación para la Salud (FIS), which is co-funded by the European Regional Development Fund (five coordinated FIS projects led by J.S.-S. and J.Vid., including the following projects: PI13/00673, PI13/00492, PI13/00272, PI13/01123, PI13/00462, PI13/00233, PI13/02184, PI13/00728, PI13/01090, PI13/01056, PI14/01722, PI14/00636, PI14/00618, PI14/00696, PI14/01206, PI14/01919, PI14/00853, PI14/01374, PI14/00972, PI14/00728, PI14/01471, PI16/00473, PI16/00662, PI16/01873, PI16/01094, PI16/00501, PI16/00533, PI16/00381, PI16/00366, PI16/01522, PI16/01120, PI17/00764, PI17/01183, PI17/00855, PI17/01347, PI17/00525, PI17/01827, PI17/00532, PI17/00215, PI17/01441, PI17/00508, PI17/01732, PI17/00926, PI19/00957, PI19/00386, PI19/00309, PI19/01032, PI19/00576, PI19/00017, PI19/01226, PI19/00781, PI19/01560, and PI19/01332), the Especial Action Project entitled: Implementación y evaluación de una intervención intensiva sobre la actividad física Cohorte PREDIMED-Plus grant to J.S.-S., the European Research Council (Advanced Research Grant 2013–2018, 340918) to M.Á.M.-G., the Recercaixa Grant to J.S.-S. (2013ACUP00194), a grant from the Generalitat Valenciana (APOSTD/2019/136 to RB), grants from the Consejería de Salud de la Junta de Andalucía (PI0458/2013, PS0358/2016, and PI0137/2018), a grant from the Generalitat Valenciana (PROMETEO/2017/017), a SEMERGEN Grant, EU-COST Action CA16112, a grant of support to research groups no. 35/2011 from the Balearic Islands Government, Grants from Balearic Islands Health Research Institute (IDISBA), funds from the European Regional Development Fund (CIBEROBN CB06/03 and CB12/03) and from the European Commission (EAT2BENICE_H2020_SFS2016). PH-A is supported by a postdoctoral fellowship (Juan de la Cierva-Formación, FJCI-2017-32205). GG-M is supported by a grant of Diputación de Jaén. The funding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of the data; in the writing of the manuscript, and in the decision to publish the results.

7. Authors contributions

CS-Q and ET conducted the statistical analyses and drafted the article. ET, JJG and MAM-G made substantial contributions to the conception and design of the work. All authors contributed substantially in the acquisition of data or analysis and interpretation of data. All authors revised the article critically for important intellectual content. All authors approved the final version to be published.

8. Acknowledgments

The authors especially thank the PREDIMED-Plus participants for their enthusiastic collaboration, the PREDIMED-Plus personnel for outstanding support, and the personnel of all associated primary care centres for their exceptional effort. CIBEROBN, CIBERESP and CIBERDEM are initiatives of Instituto de Salud Carlos III, Spain.

9. References

1. Gerhard-Herman, MD.; Gornik, H.L.; Barrett, C.; Barshes, N.R.; Corriere, M.A.; Drachman, DE.; Fleisher, L.A.; Fowkes, FGR.; Hamburg, N.M. Kinlay, S.; et al. 2016 AHA/ACC Guideline on the Management of Patients With Lower Extremity Peripheral Artery Disease: Executive Summary. Circulation, 2017, 135, e-686-e725.

2. European Stroke Organisation, Tendera, M.; Aboyans, V.; Bartelink, ML.; Baumgartner, I.; Clement, D. ESC Guidelines on the diagnosis and treatment of peripheral artery diseases: Document covering atherosclerotic disease of extracranial carotid and vertebral, mesenteric, renal, upper and lower extremity arteries: The Task Force on the Diagnosis and Treatment of Peripheral Artery Diseases of the European Society of Cardiology (ESC). Eur Heart J 2011, 32, 2851-2906.

3. Aboyans, V.; Criqui, M.H.; Abraham, P.; Allison, M.A.; Creager, MA.; Diehm, C. Measurement and interpretation of the ankle-brachial index: A scientific statement from the American Heart Association. Circulation, 2012, 126, 2890 -2909.

4. Dachun, X.; Jue, L.; Liling, Z.; Yawei, X.; Dayi, H.; Pagoto, S.L. Sensitivity and specificity of the ankle-brachial index to diagnose peripheral artery disease: A structured review. Vasc Med 2010, 15, 361-369.

5. Potier, L.; Abi Khalil, C.; Mohammedi, K.; Roussel, R. Use and utility of ankle brachial index in patients with diabetes. Eur J Vasc Endovasc Surg. 2011, 41(1), 110-116.

6. Ramirez-Tortosa, M.C.; Urbano, G.; López-Jurado, M.; Nestares, T.; Gomez, M.C.; Mir, A.; Ros, E.; Mataix, J.; Gil, A. Extra-virgin olive oil increases the resistance of LDL to oxidation more than refined olive oil in free-living men with peripheral vascular disease. J Nutr. 1999, 129(12), 2177-2183.

7. Covas, M.; Nyyssönen, K.; Poulsen, HE.; Kaikkonen, J.; Zunft, H-JF.; Kiesewetter, H. The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. Ann Intern Med. 2006, 145, 333-341.

8. Pedret, A.; Catalán, U.; Fernández-Castillejo, S.; Farràs, M.; Valls, RM.; Rubió, L.; Canela, N.; Aragonés, G.; Romeu, M.; Castañer, O.; de la Torre, R.; Covas, M.; Fitó, M.; Motilva, M.J; Solà, R. Impact of Virgin Olive Oil and Phenol-Enriched Virgin Olive Oils on the HDL Proteome in Hypercholesterolemic Subjects: A Double Blind, Randomized, Controlled, Cross-Over Clinical Trial (VOHF Study). PLoS One. 2015, 10(6), e0129160.

9. Widmer, R.J.; Freund, M.A.; Flammer, A.J.; Sexton, J.; Lennon, R.; Romani, A.; Mulinacci, N.; Vinceri, F.F.; Lerman, L.O.; Lerman, A. Beneficial effects of polyphenol-rich Olive Oil in patients with early atherosclerosis. Eur J Nutr. 2013, 52(3): 1223-1231.

10. Gaforio JJ, Visioli F, Alarcón-de-la-Lastra C, Castañer O, Delgado-Rodríguez M, Fitó M, Hernández AF, Huertas JR, Martínez-González MA, Menendez JA, Osada J, Papadaki A, Parrón T, Pereira JE, Rosillo MA, Sánchez-Quesada C, Schwingshackl L, Toledo E, Tsatsakis AM. Virgin Olive Oil and Health: Summary of the III International Conference on Virgin Olive Oil and Health Consensus Report, JAEN (Spain) 2018. Nutrients. 2019, 11(9), E2039.

11. Guasch-Ferré, M.; Liu, G.; Li, Y.; Sampson, L.; Manson, JE.; Salas-Salvadó, J.; Martínez-González, MA.; Stampfer, MJ.; Willett, WC.; Sun, Q.; Hu, FB. Olive Oil Consumption and Cardiovascular Risk in U.S. Adults. J Am Coll Cardiol, 2020, 75(15), 1729-1739.

12. Ruiz-Canela, M.; Martínez-González, M.A. Lifestyle and Dietary Risk Factors for Peripheral Artery Disease. Circulation Journal, 2014, 78, 553-559.

13. Antonelli-Incalzi, R.; Pedone, C.; McDermott, M.M.; Bandinelli, S.; Miniati, B.; Molino Lova, R.; Lauretani, F.; Ferruccie, L. Association between nutrient intake and peripheral artery disease: Results from the InCHIANTI study. Atherosclerosis. 2006, 186(1), 200-206.

14. Ruiz-Canela, M.; Estruch, R.; Corella, D.; Salas-Salvadó,J.; Martínez-González, MA. Association of Mediterranean Diet With Peripheral Artery Disease: The PREDIMED Randomized Trial. JAMA, 2014, 311, 4, 415-416.

15. Estruch R.; Ros E.; Salas-Salvadó J.; Covas MI.; Corella D.; Arós F.; Gómez-Gracia E.; Ruiz-Gutiérrez V.; Fiol M.; Lapetra J.; e*t al*. Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts. N Engl J Med. 2018, 378, 25, e34.

16. Guasch-Ferré M.; Hu FB.; Martínez-González MA.; Fitó M.; Bulló M.; Estruch R.; Ros E.; Corella D.; Recondo J.; Gómez-Gracia E.; *et al.* Olive oil intake and risk of cardiovascular disease and mortality in the PREDIMED Study. BMC Med. 2014,12:78.

17. Ruiz-Canela M. and Martínez-González M.A. Olive oil in the primary prevention of cardiovascular disease. Maturitas. 2011, 68, 3, 245-250.

18. Martínez-González, MA.; Buil-Cosiales, P.; Corella, D.; Bulló, M.; Fitó, M.; Vioque, J.; Romaguera, D.; Martínez JA.; Wärnberg, J.; López-Miranda, J. *et al*. Cohort Profile: Design and methods of the PREDIMED-Plus randomized trial. Int J Epidemiol. 2018, doi: 10.1093/ije/dyy225.

19. Alberti, K.G.; Eckel, R.H.; Grundy, S.M.; Zimmet, P.Z.; Cleeman, J.I.; Donato, K.A.; Fruchart, J.C.; James, W.P.; Loria, C.M.; Smith, S.C., Jr. 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, 1640-1645.

20. Willett, W. Nutritional Epidemiology. 3rd ed.: Oxford University Press; 2012.

21. Commission Implementing Regulation (EU) Nº 29/2012 of 13 January 2012 on marketing standards for olive oil. https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX:32012R0029. Accesed on February 5th, 2020.

22. Fernández-Ballart, J.D.; Piñol, J.L.; Zazpe, I.; Corella, D.; Carrasco, P.; Toledo, E.; Perez-Bauer, M.; Martínez-González, M.A.; Salas-Salvadó, J.; Martí-Moreno, J.M. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br. J. Nutr. 2010, 103, 1808-1816.

23. Elosua, R.; Garcia, M.; Aguilar, A.; Molina, L.; Covas, M.I.; Marrugat, J. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish Women. Investigators of the MARATHOM Group. Med. Sci. Sports Exerc. 2000, 32, 1431-1437.

24. Elosua, R.; Marrugat, J.; Molina, L.; Pons, S.; Pujol, E. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish men. The MARATHOM Investigators. Am. J. Epidemiol. 1994, 139, 1197-1209.

25. Ainsworth, B.E.; Haskell, W.L.; Herrmann, S.D.; Meckes, N.; Bassett, D.R.; Tudor-Locke, C.; Greer, J.L.; Vezina, J.; Whitt-Glover, M.C.; Leon, A.S. 2011 compendium of physical activities: A second update of codes and MET values. Med. Sci. Sports Exerc. 2011, 43, 1575-1581.

26. Galilea-Zabalza, I.; Buil-Cosiales, P.; Salas-Salvadó, J.; Toledo, E.; Ortega-Azorín, C.; Díez-Espino, J.; Vázquez-Ruiz, Z.; Zomeño, MD.; Vioque,J.; Martínez, JA.; *et al.* Mediterranean diet and quality of life: Baseline cross-sectional analysis of the PREDIMED-PLUS trial. Plos One. 2018, 13, 6, e0198974.

27. Rac-Albu, M.; Iliuta, L.; Guberna, S.M.; Sinescu, C.; The Role of Ankle-Brachial Index for Predicting Peripheral Arterial Disease. MAEDICA 2014, 9(3), 295-302.

28. Carter, S.A. Indirect systolic pressures and pulse waves in arterial occlusive diseases of the lower extremities. Circulation. 1968, 37, 624-637.

29. Yao, S.T.; Hobbs, J.T.; Irvine, W.T. Ankle systolic pressure measurements in arterial disease affecting the lower extremities. Br J Surg. 1969, 56, 676-679.

30. Baxter, GM.; Polak, J.F. Lower limb colour flow imaging: a comparison with ankle: brachial measurements and angiography. Clin Radiol. 1993, 47, 91-95.

31. Delaney CL.; Smale, MK.; Miller, MD. Nutritional Considerations for Peripheral Arterial Disease: A Narrative Review. Nutrients. 2019, 11(6):1219.

32. Cert,A.; Moreda,W.; Perez-Camino,M.C. Chromatographic analysis of minor constituents in vegetable oils. J.Chromatogr.A, 2000, 881, 1-2, 131-148.

33. Ambrosewicz-Walacik, M.; Tańska, M.; Rotkiewicz, D. Comparison of the quality of two classes of olive oil: Extra virgin and refined oil. Polish Journal of Natural Science. 2012, 27(2), 229-241.

34. Lou-Bonafonte, J.M.; Arnal, C.; Navarro, M.A.; Osada, J. Efficacy of Bioactive Compounds from Extra Virgin Olive Oil to Modulate Atherosclerosis Development. Mol. Nutr. Food Res. 2012, 56, 1043-1057.

35. Storniolo, C.E.; Rosello-Catafau, J.; Pinto, X.; Mitjavila, M.T.; Moreno, J.J. Polyphenol Fraction of Extra Virgin Olive Oil Protects Against Endothelial Dysfunction Induced by High Glucose and Free Fatty Acids through Modulation of Nitric Oxide and Endothelin-1. Redox Biol. 2014, 2, 971-977.

36. Bullon, P.; Quiles, J.L.; Morillo, J.M.; Rubini, C.; Goteri, G.; Granados-Principal, S.; Battino, M.; Ramirez-Tortosa, M. Gingival Vascular Damage in Atherosclerotic Rabbits: Hydroxytyrosol and Squalene Benefits. Food Chem. Toxicol. 2009, 47, 2327-2331.

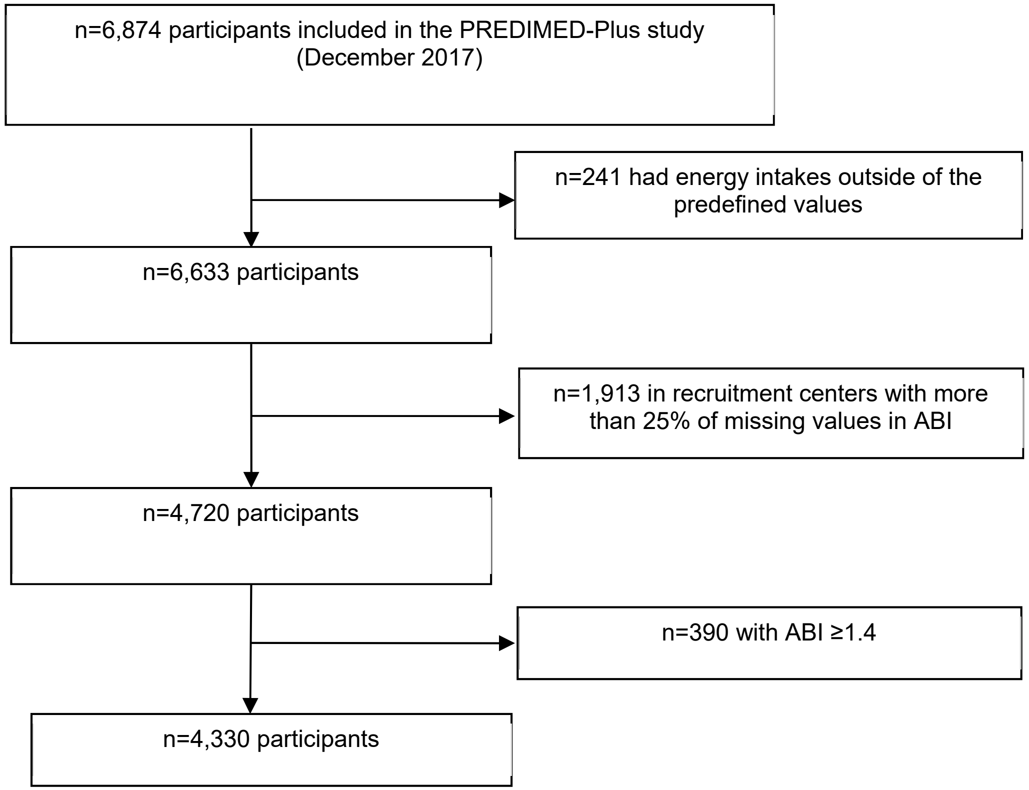
37. Guillén MD.; Sopelana P.; Palencia G. Polyciclic Aromatic Hydrocarbons and Olive Pomace Oil. J Agric Food Chem, 2004, 52, 2123-2132.

38. Kim KH.; Jahan SA.; Kabir E.; Brown RJ. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ Int. 2013, 60, 71-80.

39. Srour B.; Fezeu LK.; Kesse-Guyot E.; Allès B.; Méjean C.; Andrianasolo RM.; Chazelas E.; Deschasaux M.; Hercberg S.; Galan P.; *et al.* Ultra-processed food intake and risk of cardiovascular disease: prospective cohort study (NutriNet-Santé). BMJ. 2019, 365, l1451.

40. Allouche,Y.; Jimenez,A.; Uceda,M.; Aguilera,M.P.; Gaforio,J.J.; Beltran,G. Triterpenic content and chemometric analysis of virgin olive oils from forty olive cultivars. J.Agric.Food Chem., 2009, 57, 9, 3604-3610.

Figure 1. Flow chart of participants recruited in the PREDIMED-Plus project.



ABI = ankle-brachial pressure index. Total energy intakes were within predefined limits (800–4,000 Kcal/day for men, and 500–3,500 Kcal/day for women).

Table 1. Baseline characteristics of 4,330 participants in the PREDIMED-Plus study according to quintiles of total olive oil consumption.

	Quintiles of total olive oil consumption				
	Q1	Q2	Q3	Q4	Q5
N	866	866	866	866	866
Median total olive oil consumption (g/day)[a]	30.9 (6.2)	41.4 (3.4)	56.1 (2.7)	62.2 (1.5)	72.0 (8.0)
Age (years)	65 (5)	66 (5)	65 (5)	65 (5)	65 (5)
Women (%)	50.5	53.8	50.0	39.7	45.6
Waist circumference (cm)	109 (10)	108 (9)	107 (9)	107 (9)	108 (10)
Height (cm)	163.2 (9.2)	161.9 (9.4)	162.8 (9.3)	164.1 (9.2)	162.8 (9.3)
BMI (kg/m^2)	32.8 (3.5)	32.7 (3.4)	32.4 (3.4)	32.1 (3.3)	32.8 (3.5)
Systolic blood pressure (mm Hg)	136.3 (16.3)	137.2 (16.9)	139.1 (15.1)	139.8 (15.5)	139.7 (16.5)
Diastolic blood pressure (mm Hg)	81.9 (9.3)	81.8 (9.9)	83.0 (8.8)	82.6 (8.7)	82.1 (9.3)
Leisure-time physical activity (METs-min/week)	2451 (2345)	2628 (2369)	2536 (2404)	2695 (2258)	2605 (2458)
Smoking (%)					
Former smoker	39.4	40.3	43.0	47.0	46.3
Current smoker	12.6	12.4	12.9	14.1	13.5
Educational level					
Primary or less	53.9	51.8	49.3	44.8	46.4
Secondary	26.2	28.1	27.6	30.5	27.7
University	18.8	18.9	22.3	24.1	24.9
Family history of CHD[b] (%)	43.0	42.6	42.1	38.7	43.2
Self-reported diabetes (%)	25.9	26.0	21.8	22.5	27.3
Self-reported hypertension (%)	93.3	92.8	93.4	92.1	92.3
Self-reported medication use					
Diuretics (%)	0.4	0.5	0.4	0.2	0.4
Insulin and other glucose-lowering agents (%)	45.4	46.5	34.2	37.5	47.3

Lipid lowering medication (%)	52.2	52.4	48.7	47.1	50.5
Blood pressure-lowering drugs (%)	79.0	78.9	77.8	75.8	78.1
Adherence to energy-reduced Mediterranean diet (0-16 score)	7.5 (2.5)	7.9 (2.6)	7.8 (2.6)	7.9 (2.6)	8.2 (2.6)
Total energy intake (kcal/day)	2489 (579)	2007 (509)	2775 (463)	2275 (237)	2316 (602)
Macronutrient intake					
Carbohydrate intake (% energy)	45.1 (6.7)	42.5 (6.4)	40.7 (5.5)	37.9 (5.3)	35.2 (5.5)
Protein intake (% energy)	17.1 (2.9)	17.8 (3.0)	15.9 (2.4)	16.2 (2.4)	15.5 (2.4)
Fat intake (% energy)	34.9 (5.9)	36.9 (5.2)	40.0 (4.7)	42.4 (4.7)	46.2 (5)
Monounsaturated fatty acids (% energy)	16.3 (3.7)	18.5 (3.0)	20.9 (2.8)	23.1 (3.0)	26.0 (3.3)
Polyunsaturated fatty acids (% energy)	6.4 (2.2)	6.0 (1.9)	6.3 (1.7)	6.5 (1.6)	6.8 (1.5)
Saturated fatty acids (% energy)	9.8 (2.4)	9.6 (2.1)	10.0 (1.8)	10.0 (1.8)	10.3 (1.8)
Alcohol intake (g/day)	11.1 (15.6)	8.4 (12.8)	14.1 (17.4)	11.5 (14.0)	10.8 (14.6)
Fruit consumption (g/day)	394 (225)	333 (204)	392 (207)	344 (174)	341 (187)
Vegetable consumption (g/day)	340 (156)	316 (136)	335 (137)	303 (115)	305 (130)
Cereal consumption (g/day)	162 (82)	129 (70)	185 (86)	142 (63)	128 (67)
Legume consumption (g/day)	23.8 (13.1)	19.8 (10.7)	21.7 (11.9)	18.8 (8.3)	19.6 (10.9)
Meat product consumption (g/day)	149 (66)	132 (55)	165 (60)	144 (48)	135 (53)
Fish product consumption (g/day)	100.1 (52.8)	88.8 (45.2)	108.5 (45.7)	98.9 (43.0)	98.1 (45.3)
Dairy product consumption (g/day)	405 (220)	328 (202)	380 (217)	303 (169)	293 (181)
Micronutrient intake					
Vitamin D intake (mg/day)	6.3 (3.4)	5.4 (3.3)	6.7 (3.5)	6.0 (3.3)	6.1 (3.4)
Folic acid intake (mg/day)	384 (113)	327 (98)	382 (95)	328 (75)	320 (99)

Values are expressed as mean (SD), unless otherwise stated
METs: metabolic equivalents
CHD: coronary heart disease
[a] Energy-adjusted consumption
[b] Information from parents and siblings

Table 2. Correlations between virgin, olive oil and olive pomace oil consumption in the PREDIMED-Plus trial. (*) $p<0.05$.

	Virgin olive oil consumption	**Olive oil consumption**	**Olive pomace oil consumption**
Virgin olive oil consumption		-0.41*	-0.03
Olive oil consumption			-0.14*

Table 3. Differences in ankle-brachial indexes across categories of energy-adjusted total, virgin, olive oil, olive pomace oil consumption in the PREDIMED-Plus trial. n=4,330

Quintiles of total olive oil consumption	***Q1***	***Q2***	***Q3***	***Q4***	***Q5***	***p* for trend**
Median total olive oil consumption (g/day)	31	41	56	62	72	
N	866	866	866	866	866	
Multivariable Model 1	0 (ref)	0.002 (-0.009, 0.013)	0.010 (-0.002, 0.022)	0.010 (-0.002, 0.022)	0.014 (0.001, 0.026)	*0.011*
Multivariable Model 2	0 (ref)	0.002 (-0.010, 0.013)	0.010 (-0.002, 0.022)	0.010 (-0.002, 0.022)	0.014 (0.001, 0.026)	*0.011*
Multivariable Model 3	0 (ref)	0.002 (-0.010, 0.014)	0.010 (-0.002, 0.022)	0.010 (-0.003, 0.022)	0.014 (0.002, 0.027)	*0.010*
Quintiles of virgin olive oil consumption	***Q1***	***Q2***	***Q3***	***Q4***	***Q5***	
Median virgin olive oil consumption (g/day)	15	35	44	61	68	
N	866	866	866	866	866	
Multivariable Model 1	0 (ref)	-0.003 (-0.014, 0.008)	0.001 (-0.011, 0.013)	0.003 (-0.010, 0.015)	0.010 (-0.003, 0.022)	0.114
Multivariable Model 2	0 (ref)	-0.003 (-0.014, 0.008)	0.001 (-0.011, 0.013)	0.003 (-0.009, 0.015)	0.010 (-0.003, 0.022)	0.110
Multivariable Model 3	0 (ref)	-0.002 (-0.014, 0.009)	0.002 (-0.010, 0.014)	0.002 (-0.010, 0.014)	0.010 (-0.002, 0.022)	0.127
Quintiles of olive oil consumption	***Q1***	***Q2***	***Q3***	***Q4***	***Q5***	
Median olive oil consumption (g/day)	3	4	4	5	30	
N	866	866	866	866	866	
Multivariable Model 1	0 (ref)	0.002 (-0.009, 0.013)	-0.004 (-0.015, 0.007)	-0.004 (-0.015, 0.008)	0.0003 (-0.011, 0.012)	0.743
Multivariable Model 2	0 (ref)	0.002 (-0.009, 0.013)	-0.004 (-0.015, 0.007)	-0.004 (-0.015, 0.008)	0.0003 (-0.011, 0.012)	0.743
Multivariable Model 3	0 (ref)	-0.002 (-0.015, 0.010)	-0.010 (-0.025, 0.005)	-0.011 (-0.029, 0.006)	-0.003 (-0.017, 0.010)	0.698
Tertiles of olive pomace oil consumption	***T1***	***T2***	***T3***			
Median olive pomace oil consumption (g/day)	0.003	0.02	0.03			
N	1,444	1,443	1,443			
Multivariable Model 1	0 (ref)	-0.003 (-0.012, 0.007)	0.0003 (-0.008, 0.009)			0.960
Multivariable Model 2	0 (ref)	-0.003 (-0.012, 0.007)	0.0003 (-0.008, 0.009)			0.959
Multivariable Model 3	0 (ref)	-0.003 (-0.012, 0.007)	0.0002 (-0.009, 0.009)			0.980

Results from multivariable lineal regression models. Q, quintile; T, tertile.
Multivariable Model 1: adjusted for age (continuous), recruitment center, and sex.
Multivariable Model 2: Model 1 additionally adjusted for BMI (tertiles).
Multivariable Model 3: Model 2 additionally adjusted for waist circumference (continuous); self-reported prevalent diabetes (yes/no); self-reported hypertension (yes/no); adherence to a Mediterranean diet (score 0–16); physical activity (metabolic equivalents-min/ week) (quintiles); educational level (primary school, secondary school, university); blood pressure-lowering drug use (yes/no); lipid-lowering medication use

(yes/no); diuretics use (yes/no); insulin and oral antidiabetic agents use (yes/no); vitamin D intake (tertile); folic acid intake (continuous); family history of coronary heart disease (yes/no); smoking (never, current, former >5 years, former <5 years); and total energy intake (continuous).

Table 4. Odds ratios (95% CI) for ankle-brachial index (ABI) ≤1 across categories of energy-adjusted total, virgin, olive oil, olive pomace oil consumption in the PREDIMED-Plus trial.

	Odds Ratio (95% CI)					
Quintiles of total olive oil consumption	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	***p* for trend**
Range of total olive oil consumption (g/day)	(< 36.63)	(36.63, 49.32)	(49.32, 59.53)	(59.53, 64.93)	(64.93, 119.89)	
N	866	866	866	866	866	
Multivariable Model 1	1 (ref)	1.04 (0.82, 1.33)	0.97 (0.75, 1.26)	1.00 (0.77, 1.29)	0.86 (0.66, 1.12)	0.258
Multivariable Model 2	1 (ref)	1.04 (0.82, 1.33)	0.98 (0.76, 1.26)	1.00 (0.77, 1.30)	0.86 (0.66, 1.12)	0.253
Multivariable Model 3	1 (ref)	1.04 (0.81, 1.34)	0.99 (0.76, 1.29)	1.01 (0.77, 1.32)	0.86 (0.65, 1.13)	0.278
Quintiles of virgin olive oil consumption	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
Range of virgin olive oil consumption (g/day)	(< 25.91)	(25.91, 38.68)	(38.68, 57.68)	(57.68, 63.49)	(63.49, 92.62)	
N	866	866	866	866	866	
Multivariable Model 1	1 (ref)	0.93 (0.74, 1.18)	0.95 (0.74, 1.21)	0.83 (0.64, 1.08)	0.74 (0.56, 0.96)	*0.022*
Multivariable Model 2	1 (ref)	0.94 (0.74, 1.19)	0.96 (0.75, 1.22)	0.83 (0.64, 1.08)	0.74 (0.56, 0.96)	*0.022*
Multivariable Model 3	1 (ref)	0.92, (0.72, 1.17)	0.94 (0.73, 1.20)	0.84 (0.65, 1.10)	0.73 (0.56, 0.97)	*0.031*
Quintiles of olive oil consumption	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
Range of olive oil consumption (g/day)	(< 3.15)	(3.15, 3.90)	(3.90, 4.63)	(4.63, 15.52)	(15.52, 74.66)	
N	866	866	866	866	866	
Multivariable Model 1	1 (ref)	0.98 (0.76, 1.26)	1.21 (0.95, 1.56)	1.16 (0.90, 1.50)	1.28 (0.99, 1.65)	0.084
Multivariable Model 2	1 (ref)	0.98 (0.76, 1.26)	1.21 (0.95, 1.56)	1.15 (0.89, 1.49)	1.27 (0.99, 1.64)	0.090
Multivariable Model 3	1 (ref)	1.10 (0.83, 1.46)	1.46 (1.06, 2.03)	1.45 (1.00, 2.10)	1.43 (1.07, 1.90)	0.114
Tertiles of olive pomace oil consumption	**T1**	**T2**	**T3**			
Range of olive pomace oil consumption (g/day)	(< 0.01)	(0.01, 0.02)	(0.02, 49.99)			
N	1,444	1,443	1,443			
Multivariable Model 1	1 (ref)	1.18 (0.96, 1.46)	1.22 (1.00, 1.47)			*0.045*
Multivariable Model 2	1 (ref)	1.18 (0.96, 1.45)	1.21 (1.00, 1.47)			*0.047*
Multivariable Model 3	1 (ref)	1.18 (0.96, 1.46)	1.22 (1.00, 1.48)			*0.050*

Results from logistic models. Q, quintile; T, tertile.

Logistic Model 1: adjusted for age (continuous), recruitment center, and sex.

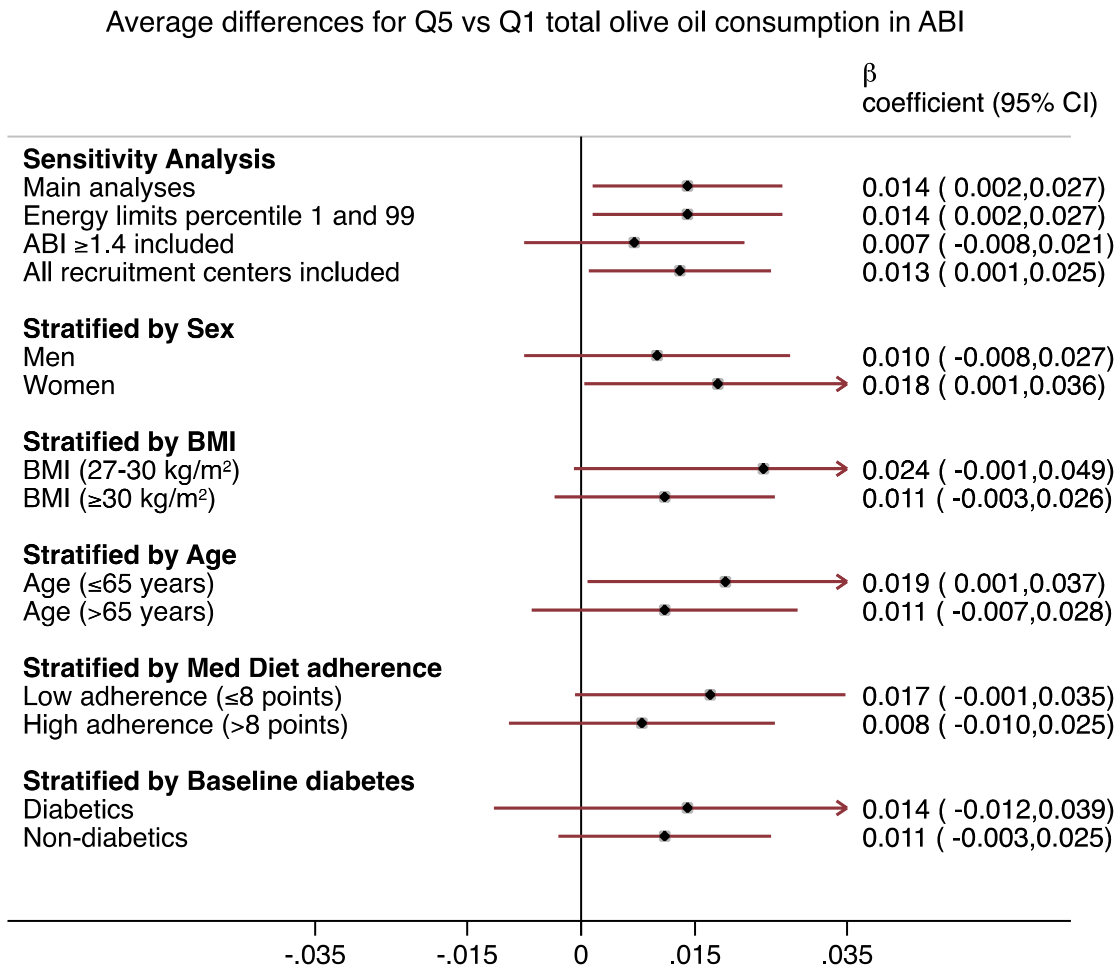
Logistic Model 2: Model 1 additionally adjusted for body mass index (tertiles).

Logistic Model 3: Model 2 additionally adjusted for waist circumference (continuous); self-reported prevalent diabetes (yes/no); self-reported hypertension (yes/no); adherence to a Mediterranean diet (score 0–16); physical activity (metabolic equivalents-min/ week) (quintiles);

educational level (primary school, secondary school, university); blood pressure-lowering drug use (yes/no); lipid-lowering medication use (yes/no); diuretics use (yes/no); insulin and oral antidiabetic agents use (yes/no); vitamin D intake (tertile); folic acid intake (continuous); family history of coronary heart disease (yes/no); smoking (never, current, former >5 years, former <5 years); and total energy intake (continuous).

[a] Model 3 adjusted without smoking variable.

Figure 2. Sensitivity analyses. Average differences for Q5 vs. Q1 total olive oil consumption in ABI.



ABI, ankle-brachial index; BMI, body mass index; Med Diet, Mediterranean diet; Q, quintile.

Supplementary files.

Supplementary Table 1. Correlations between cofounding variables of analyses in the PREDIMED-Plus trial. (*) *p*<0.05.

	Age	**Body-Mass index**	**Waist circumference**	**Adherence to a Meddiet**	**Physical activity**	**Vitamin D intake**	**Folic acid intake**
Body- Mass index	-0.02						
Waist circumference	-0.10*	0.62*					
Adherence to a Meddiet	0.12*	-0.06*	-0.14*				
Physical activity	0.04*	-0.15*	-0.10*	0.14*			
Vitamin D intake	0.01	-0.03	-0.01	0.22*	0.11*		
Folic acid intake	0.09*	-0.01	-0.05*	0.36*	0.14*	0.21*	
Total energy intake	-0.12*	-0.02	0.13*	-0.12*	0.05*	-0.00	-0.04*

Meddiet: Mediterranean diet.

Supplementary Table 2. Sensitivity analysis. Odds ratios (95% CI) for ankle-brachial index (ABI) ≤0.9 across categories of energy-adjusted total, virgin, olive oil and olive pomace oil consumption in the PREDIMED-Plus trial.

	Odds Ratio (95% CI)					
Quintiles of total olive oil consumption	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	***p* for trend**
Range of total olive oil consumption (g/day)	(< 36.63)	(36.63, 49.32)	(49.32, 59.53)	(59.53, 64.93)	(64.93, 119.89)	
Multivariable Model 1	1 (ref)	1.52 (0.92, 2.51)	1.52 (0.90, 2.54)	1.02 (0.59, 1.77)	1.22 (0.72, 2.09)	0.979
Multivariable Model 2	1 (ref)	1.52 (0.92, 2.51)	1.53 (0.91, 2.56)	1.03 (0.59, 1.79)	1.21 (0.70, 2.06)	0.954
Multivariable Model 3	1 (ref)	1.45 (0.85, 2.46)	1.52 (0.89, 2.58)	1.02 (0.58, 1.79)	1.16 (0.67, 2.02)	0.940
Quintiles of virgin olive oil consumption	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
Range of virgin olive oil consumption (g/day)	(< 25.91)	(25.91, 38.68)	(38.68, 57.68)	(57.68, 63.49)	(63.49, 92.62)	
Multivariable Model 1	1 (ref)	0.95 (0.60, 1.50)	0.96 (0.60, 1.52)	0.84 (0.51, 1.38)	0.69 (0.41, 1.15)	0.172
Multivariable Model 2	1 (ref)	0.95 (0.60, 1.51)	0.98 (0.62, 1.56)	0.85 (0.52, 1.40)	0.68 (0.41, 1.14)	0.169
Multivariable Model 3	1 (ref)	0.96 (0.60, 1.54)	0.96 (0.60, 1.54)	0.91 (0.55, 1.51)	0.69 (0.41, 1.18)	0.244
Quintiles of olive oil consumption	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
Range of olive oil consumption (g/day)	(< 3.15)	(3.15, 3.90)	(3.90, 4.63)	(4.63, 15.52)	(15.52, 74.66)	
Multivariable Model 1	1 (ref)	0.71 (0.42, 1.19)	1.04 (0.65, 1.67)	0.86 (0.51, 1.43)	1.16 (0.72, 1.85)	0.168
Multivariable Model 2	1 (ref)	0.70 (0.42, 1.18)	1.04 (0.65, 1.67)	0.84 (0.50, 1.40)	1.13 (0.71, 1.81)	0.187
Multivariable Model 3	1 (ref)	0.68 (0.39, 1.21)	1.00 (0.54, 1.86)	0.81 (0.39, 1.70)	1.08 (0.62, 1.86)	0.265
Tertiles of olive pomace oil consumption	**T1**	**T2**	**T3**			
Range of olive pomace oil consumption (g/day)	(< 0.01)	(0.01, 0.02)	(0.02, 49.99)			
Multivariable Model 1	1 (ref)	0.90 (0.60, 1.34)	1.11 (0.77, 1.59)			0.590
Multivariable Model 2	1 (ref)	0.89 (0.59, 1.33)	1.10 (0.76, 1.57)			0.622
Multivariable Model 3	1 (ref)	0.85 (0.56, 1.29)	1.07 (0.74, 1.55)			0.723

Results from logistic models. Q, quintile; T, tertile.

Logistic Model 1: adjusted for age (continuous), recruitment center, and sex.

Logistic Model 2: Model 1 additionally adjusted for body mass index (tertiles).

Logistic Model 3: Model 2 additionally adjusted for waist circumference (continuous); self-reported prevalent diabetes (yes/no); self-reported hypertension (yes/no); adherence to a Mediterranean diet (score 0–16); physical activity (metabolic equivalents-min/ week) (quintiles); educational level (primary school, secondary school, university); blood pressure-lowering drug use (yes/no); lipid-lowering medication use (yes/no); diuretics use (yes/no); insulin and oral antidiabetic agents use (yes/no); vitamin D intake (tertile); folic acid intake (continuous); family history of coronary heart disease (yes/no); smoking (never, current, former >5 years, former <5 years); and total energy intake (continuous).

Supplementary Table 3. Differences in ankle-brachial index (ABI) across categories of energy-adjusted total, virgin, olive oil and olive pomace oil consumption in the PREDIMED-Plus trial. (n=4729, with all recruitment centers included but without missing ABI values in the right or left leg).

Quintiles of total olive oil consumption	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	***p* for trend**
Median total olive oil consumption (g/day)	30	40	54	61	69	
Multivariable Model 1	0 (ref)	-2.84 e-06 (-0.011, 0.011)	0.006 (-0.005, 0.018)	0.009 (-0.003, 0.020)	0.012 (0.00006, 0.024)	*0.018*
Multivariable Model 2	0 (ref)	0.00004 (-0.011, 0.011)	0.006 (-0.005, 0.018)	0.009 (-0.003, 0.020)	0.012 (0.00002, 0.024)	*0.019*
Multivariable Model 3	0 (ref)	0.0005 (-0.011, 0.012)	0.007 (-0.005, 0.018)	0.009 (-0.003, 0.021)	0.013 (0.001, 0.025)	*0.011*
Quintiles of virgin olive oil consumption	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
Median virgin olive oil consumption (g/day)	14	33	40	60	66	
Multivariable Model 1	0 (ref)	-0.002 (-0.013, 0.009)	0.0003 (-0.011, 0.012)	0.002 (-0.010, 0.013)	0.009 (-0.003, 0.021)	0.168
Multivariable Model 2	0 (ref)	-0.002 (-0.013, 0.009)	0.0003 (-0.011, 0.012)	0.002 (-0.010, 0.013)	0.009 (-0.003, 0.021)	0.166
Multivariable Model 3	0 (ref)	-0.002 (-0.013, 0.009)	0.001 (-0.010, 0.013)	0.0009 (-0.011, 0.013)	0.010 (-0.002, 0.022)	0.155
Quintiles of olive oil consumption	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
Median olive oil consumption (g/day)	2	4	5	7	30	
Multivariable Model 1	0 (ref)	0.003 (-0.008, 0.014)	-0.003 (-0.014, 0.008)	-0.003 (-0.014, 0.009)	0.002 (-0.011, 0.011)	0.870
Multivariable Model 2	0 (ref)	0.003 (-0.008, 0.013)	-0.003 (-0.014, 0.008)	-0.003 (-0.014, 0.008)	0.0002 (-0.011, 0.012)	0.859
Multivariable Model 3	0 (ref)	-0.0002 (-0.012, 0.012)	-0.006 (-0.020, 0.008)	-0.007 (-0.023, 0.009)	-0.002 (-0.014, 0.011)	0.759
Tertiles of olive pomace oil consumption	**T1**	**T2**	**T3**			
Median olive pomace oil consumption (g/day)	0.01	0.02	0.02			
Multivariable Model 1	0 (ref)	0.002 (-0.007, 0.011)	0.002 (-0.006, 0.011)			0.587
Multivariable Model 2	0 (ref)	0.002 (-0.007, 0.011)	0.002 (-0.006, 0.011)			0.591
Multivariable Model 3	0 (ref)	0.002 (-0.007, 0.011)	0.002 (-0.006, 0.011)			0.584

Results from multivariable lineal regression models. Q, quintile; T, tertile.
Multivariable Model 1: adjusted for age (continuous), recruitment center and sex.
Multivariable Model 2: Model 1 additionally adjusted for body mass index (tertiles).
Multivariable Model 3: Model 2 additionally adjusted for waist circumference (continuous); self-reported prevalent diabetes (yes/no); self-reported hypertension (yes/no); adherence to a Mediterranean diet (score 0–16); physical activity (metabolic equivalents-min/ week) (quintiles); educational level (primary school, secondary school, university studies); blood pressure-lowering drugs use (yes/no); lipid-lowering medication use (yes/no); diuretics use (yes/no); insulin and oral antidiabetic agents use (yes/no); vitamin D intake (tertile); folic acid intake (continuous); family history of coronary heart disease (yes/no); smoking (never, current, former >5 years, former <5 years); and total energy intake (continuous).