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ORIGINAL ARTICLE

Dietary patterns are associated with arterial stiffness and carotid atherosclerosis in postmenopausal women

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Abstract

Purpose The increase in cardiovascular risk after the menopausal transition remains partly explained until today. Further research is needed to identify risk factors potentially modifiable by primary prevention practices. This cross-sectional study, part of a larger prospective project, aims to investigate possible associations between dietary patterns and indices of vascular structure and function among healthy postmenopausal women.

Methods Postmenopausal women ($n = 310$) without clinically overt cardiovascular disease were recruited consecutively from a University Menopause Clinic over three years. Dietary intake was assessed by a validated food frequency questionnaire and the MedDietScore. In addition, we assessed anthropometric/biochemical parameters, including the Triglyceride-glucose index (TyG-Index), body fat distribution [triceps skinfold (TSF), mid-upper arm circumference (MUAC)] and physical activity. The vascular assessment included carotid-femoral pulse wave velocity (PWV), carotid and femoral-artery intima-media thickness (IMT) and atheromatous plaques presence.

Results Consumption of non-refined cereals was associated with carotid-bulb IMT ($R^2 = 5.5\%$ b-coefficient $= -0.142$; $p = 0.011$), adjusting for age, physical activity, lipids, systolic blood pressure, smoking, body mass index, insulin resistance, and daily energy intake. PWV was associated with the intake of total dairy products ($R^2 = 27.3\%$, b-coefficient $= -0.117$; $p = 0.017$). Higher red meat consumption was related to a greater TyG-index (Model 1, $R^2 = 14.3\%$, b-coefficient=0.121; $p = 0.048$), an association mediated by total daily energy intake. Higher consumption of alcohol, as well as the MedDietScore, were inversely associated with TSF measurements, significant after Bonferroni correction.

Conclusion Dietary patterns are associated with metabolic indices and subclinical atherosclerosis in postmenopausal women independently of traditional cardiovascular risk factors, total energy intake or physical activity.

Keywords Dietary patterns · Cardiovascular disease · Postmenopausal women · Subclinical atherosclerosis · Arterial stiffness

Introduction

The rate of cardiovascular events is sharply increasing after the age of 50 years in women, which coincides with the time of the natural menopausal transition [1, 2]. The hormonal milieu related to the cessation of ovarian function is linked with significant metabolic derangement, including abdominal adiposity features, pro-atherogenic dyslipidaemia, insulin resistance and new-onset hypertension [3]. As

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we previously reported, in contrast to relatively low calculated traditional cardiovascular risk factor scores in this population, young postmenopausal women present an unexpectedly high prevalence of subclinical atherosclerosis, which is closely associated with increased risk for future clinically overt cardiovascular disease (CVD) [4].

The possible beneficial effect of dietary manipulation on cardiovascular risk has long been recognised [5–7]. Indeed, dietary habits that are characterised by consumption of low amounts of saturated fat, trans-fatty acids, added sugars and sodium in combination with a high intake of low-fat dairy products, fruits, vegetables and non-refined cereals can be highly protective against CVD occurrence [8, 9]. Mainly, the Mediterranean diet and the DASH diet (Dietary Approach to Stop Hypertension) constitute the principal representatives of dietary patterns which are associated with reduced cardiovascular morbidity and mortality amongst middle-aged adults [9, 10]. The cardioprotective role of the aforementioned dietary patterns is mainly based on the high consistency of food groups rich in bioactive compounds, such as unsaturated fatty acids, polyphenols, fibre, phytosterols, vitamins and minerals, which have antioxidant, anti-inflammatory and antithrombotic impact on vascular structure and function [11–13]. On the other hand, the selection of macronutrient intake during the menopausal transition has been reported to associate with the severity of lipidemia, carotid artery intima-media artery (CIMT). It may modify the extent of postmenopausal atherosclerosis [14–16].

Overall, the available studies on the impact of dietary intake during midlife in terms of subclinical atherosclerosis and CVD risk focused on a single nutrient and/or well-established a-priori dietary patterns such as Mediterranean-style or DASH diet. To shed light on the association of real-life dietary habits and subclinical atherosclerosis in healthy Greek postmenopausal women, we have designed a large prospective study which is currently in process [17]. Herein, we aim to explore the cross-sectional associations between specific food categories and the cardiometabolic profile by focusing on three intermediate markers; (1) indices of vascular function and structure, (2) metabolic syndrome and insulin resistance markers and (3) indices of body fat distribution.

Methods

Design and sampling procedure

This cross-sectional analysis is consisted of 330 consecutively recruited healthy postmenopausal women without clinically overt CVD or other CV risk factors like hypertension and dyslipidemia from the Menopause Clinic of Aretaieio Hospital of Athens, between September 2018 and July 2020. This is the baseline analysis of a larger prospective study, as previously described [17]. In brief, all healthy postmenopausal women visiting our clinic for advice on menopause and management of bone disease or cardiometabolic complications were invited to participate in a prospective study, which aims to assess dietary patterns of middle-aged Greek women and their association with cardiometabolic parameters, subclinical atherosclerosis and overall adiposity. All women visiting the Menopause clinic of Aretaieio Hospital were invited to participate in this study at the time of their first appointment in the Clinic. As previously analysed [17], exclusion criteria consisted of: i) peri- or premenopausal status, ii) the presence of previously diagnosed clinically overt CVD, diabetes mellitus (DM) of any type, chronic kidney disease, (Glomerular filtration rate by Cockcroft-Gault Formula, GFR > 60 mL/min/m^2) eating disorders or any mental health disorders and physical inability due to injury or chronic disease, history of hypertension and hyperlipidemia iii) obesity class III and above (body mass index, BMI > 40 kg/m^2) or with excessive fluctuation of body weight (>10%). Participants known to be on treatment with hypolipidaemic, hypoglycemic or antihypertensive agents were not included in the present study to minimise the possible confounding effect on vascular measurements. Following strict application of all exclusion criteria, we excluded 20 women who did not provide answers to the food questionnaire so that the final sample included 310 women as of 31 July 2020 (191 women adherent to the Mediterranean diet and 119 non-adherent).

Protocol study procedures

The protocol of this study has been published already in detail elsewhere [17]. Briefly, data were prospectively obtained from each woman consenting to participate in the study. We evaluated anthropometric parameters, haemodynamic parameters, and biochemical and hormonal parameters. In addition, women received a questionnaire on their dietary habits and underwent vascular assessment. All women gave their written consent. Institutional Review Board approval was obtained by the Ethics Committee of Aretaieio Hospital (approval number B − 22/19-12-2013).

Anthropometric measurements

Body mass index (BMI) (kg/m^2), waist and hip circumference, as well as the waist-to-hip circumference ratio (WHR), the Mid Upper Arm Circumference (MUAC) and triceps skinfold thickness (TSF), were estimated as previously described [17].

Medical history and risk factor assessment

Detailed medical history was recorded for each participant, using questionnaires regarding demographic and lifestyle parameters, and obstetrical and gynaecological history. Blood pressure was estimated as the average of two repeated measurements after resting in the sitting position for 5 minutes. Hypertension was defined as a history of antihypertensive treatment or systolic arterial blood pressure (SBP) ≥ 140 mmHg and/or diastolic arterial blood pressure (DBP) ≥ 90 mmHg measured on at least three different occasions [17]. Hyperlipidemia was defined as a history of hypolipidemic treatment or total blood cholesterol level above 200 mg/dL or LDL-cholesterol levels higher than 160 mg/dL and/or plasma triglycerides higher than 200 mg/dL. DM was defined as fasting serum glucose levels > 126 mg/dL or HbA1c > 6.5% [17]. Metabolic syndrome was defined according to the criteria of the joint definition [18] as presence of any three of the following five risk factors: a) elevated waist circumference equal or higher than 80 cm; b) hyper-triglyceridaemia with levels equal or higher than 150 mg/dL or drug treatment; c) levels of high-density cholesterol (HDL-c) less than 50 mg/dL or relative treatment; d) systolic blood pressure equal or higher than 130 mmHg and/or diastolic blood pressure equal or higher than 85 mmHg or anti-hypertensive treatment; e) hyperglycemia with levels equal or higher than 100 mg/dL or hypoglycemic treatment.

Blood assays

Plasma glucose, total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C) and HDL-c concentrations were measured in the fasting state by using standard analysers, as previously described [17]. Insulin resistance was estimated using the triglyceride–glucose index (TyG-Index), calculated as follows; ln[fasting triglycerides (mg/dL) x fasting glucose (mg/dL)/2]. As previously described, the TyG-Index is significantly associated with carotid atherosclerosis and arterial stiffness in postmenopausal women [19].

Vascular measurements

Intima-media thickness (IMT)

IMT was measured in one paired segment, of both right and left carotid bulb (cb), from a fixed lateral transducer angle using B-mode ultrasound imaging (14.0 MHz multi-frequency linear array probe, Vivid 7 Pro; General Electric Healthcare, Milwaukee, Wisconsin, USA). Three measurements of the maximal IMT in the far wall were averaged, and the average IMT was calculated for each segment of the two carotid arteries. The average value of each right and left carotid IMT segment was defined as cbIMT for the carotid bulb. Atherosclerotic plaque's presence was determined as presence of the well-defined plaque, or any focal thickening greater than 1.5 mm. The cbIMT has been selected as the most eligible part of the carotid artery, indicative for sonographical assessment, as this is the segment where atherosclerosis primarily begins [20] and atherosclerotic plaques start developing [20–22]. All scans were digitally recorded for offline analysis (mean carotid IMT coefficient variation-CV 10.6%), both performed by a single operator blinded to the cardiovascular risk profile of the subjects.

Pulse-wave velocity

Carotid-femoral pulse wave velocity (PWV) currently constitutes the gold standard marker for in-vivo assessment of aortic stiffness and reflects arterial ageing [19–21]. PWV was calculated by measuring pulse transit time, and the distance travelled between 2 recording sites with a validated non-invasive device (Complior, Artech Medical) that allows online pulse wave recording and automatic PWV calculation.The common carotid artery and common femoral artery were selected as the recording sites for the measurement of PWV that equals distance (m) divided by transit time (sec). Details about PWV measurement were described elsewhere [17].

Dietary assessment

Dietary habits were evaluated using a validated semi-quantitative food frequency questionnaire comprising 69 questions regarding the frequency of consumption of all main food groups and beverages usually consumed and seven questions regarding eating behaviours, as described in the main protocol of this study [17, 22]. The questionnaire has been translated and validated into the Greek language [23]. Responses were converted to daily food intakes and then extrapolated into macronutrient intakes. Dietary intake was grouped into 20 food groups featuring the core foods of the Greek diet as well as those that have been related to CVD risk, namely non-refined cereals, refined cereals, potatoes, fruits, non-starchy vegetables, vegetable-based cooked meals, legumes, nuts, full-fat dairy products, low-fat dairy products, total dairy products, eggs, red meat, poultry, fish & seafood, processed meat, alcohol drinks, coffee, tea and sweeteners (honey, jam, sugar). We calculated the total energy intake by summing energy intake from macronutrients.

The Mediterranean Dietary Score (MedDietScore) evaluated adherence to Mediterranean dietary pattern. The MedDietScore is a validated 11-item composite index that evaluates adherence level to the Mediterranean dietary pattern. The range of the MedDietScore is between 0 and 55. Consequently, we considered women with scores in the lowest tertile as having low adherence to the Mediterranean Diet and women with scores in the second or third tertile as having high adherence [17].

Physical activity assessment

Physical activity was evaluated by using the International Physical Activity Questionnaire (IPAQ) index, which has already been validated for the Greek population, as previously described [17]. The IPAQ index classifies the extent of physical activity according to the amount of time spent, into walking vs moderate or high – the intensity of physical activity during the previous seven days. MET (metabolic equivalent of task) -minutes were exported for each physical activity category, leading to the total MET-minutes calculation [17].

Statistical analysis

The normal distribution of continuous variables was graphically inspected with histograms and Q-Q plots. Mean, standard deviation (SD), minimum and maximum values were calculated for normally distributed continuous variables and frequencies (%) for categorical variables. The possible associations between meat consumption as well as alcohol and indices of vascular function were also quantified. Analysis has also taken place in a dichotomous fashion, using tertiles of meat consumption and alcohol after applying analysis of variance (ANOVA) and using Bonferroni correction for multiple comparisons. The correlation between continuous variables with normal distribution was assessed with Pearson's correlation coefficient, whereas the correlation between continuous variables without normal distribution was evaluated with Spearman's correlation coefficient. IMT values were analysed as continuous variables, so not excluding possible outliers, with regards to descriptive analysis and correlations. Multivariate stepwise linear regression analysis was performed to evaluate the individual contribution of food group consumption, as opposed to lack of consumption, on vascular structure and function indices, TyG-index and anthropometric indices. Potential confounders of cardiovascular risk were included in the regression models, like age, physical activity, smoking, SBP, LDL-C, BMI and energy intake. Further adjustment for the TyG-index was performed in the final regression model analysis. Only the indicative IMT values (< 1.5 mm) were included in the regression models to eliminate the possible confounding effect of outliers on the assessed associations. Finally, atheromatous plaques presence was not included in the analysis due to the low frequency of plaques in our sample (<1%). A two-sided p-value equal to or less than 0.05 was considered statistically significant. The Statistical Package for Social Sciences (IBM SPSS) program, version 23.0, was used for statistical analysis.

As per the original hypothesis [17], carotid IMT measures are lower by 1.5% in individuals with highest vs lowest adherence to the Mediterranean diet. Consequently, a total sample of at least 327 women is expected to provide a statistical power of 80% to evidence a difference in measures of carotid IMT of at least 1.5% when comparing adherent and non-adherent participants (alpha error 0.05, two-tailed). As we were not able to compensate for the missing values, the current sample of 310 women (191 adherent and 119 non-adherent to the Mediterranean diet) would be sufficient to provide a statistical power of 80% (effect size 0.3, alpha error of 0.1, two-tailed).

Results

Descriptive characteristics

The baseline characteristics of study participants are summarised in Table 1. The mean age of all participants was 58.7 ± 6.9 years old, and the mean menopause age was 49.2 ± 5 years. Overweight and obese women constituted the majority of the sample (36.9% and 28.6%, respectively). Regarding lifestyle characteristics, the mean MedDietScore of participants was equal to 36.6 ± 5.1 (high compliance, 68.4%; low-moderate compliance, 38.6%), whereas the majority of women were characterised as minimally active (total MET-minutes/week, median value: 420; IQR: 140–900).

The descriptive analysis of vascular function and structure indices is presented in Table 1. Moreover, metabolic syndrome was diagnosed in 31.3% of women (97/310).

The dietary parameters of our sample are presented in Supplemental Table 1. The majority of participants (78.7%) consumed daily coffee; notably, 49% of them consumed two or more servings per day and 29.7% consumed at least 1 cup daily. One serving of sweeteners (honey/jam/sugar), non-starchy vegetables and fruits were consumed daily by the 31.3%, 30.7% and 25% of the study sample, respectively. A large proportion of women (62.9%) never consumed alcoholic drinks.

Associations between dietary patterns and metabolic and anthropometric characteristics

Bivariate analysis results between the included food groups and TyG-index and anthropometric indices are presented in Supplmenetal Table 2. Daily consumption of alcohol drinks was negatively related to TyG-index in our sample ($r = -0.135$, $p = 0.03$). Concerning anthropometric parameters, daily consumption of non-starchy vegetables, nuts and alcoholic drinks ($r = -0.151$, $p = 0.02$; $r = -0.209$, $p < 0.001$; $r = -0.159$, $p = 0.01$; respectively), remained significant after Bonferroni correction, as well as tea ($r = -0.133$, $p = 0.04$) were found to be inversely correlated with MUAC in study participants. Non-refined cereals and alcohol consumption was also inversely related to TSF ($r = -0.152$, p-value=0.018; $r = 0.175$, $p = 0.006$, both remained significant even after correction for Bonferroni). Sweeteners consumption

Table 1 Demographic, anthropometric, lifestyle and clinical characteristics of the study sample

	Mean (±SD) or Median (IQR) and frequency (%)
Demographic characteristics	
Age (years)	58.7 (±6.9)
Menopausal age (years)	9.6 (±6.6)
Anthropometric characteristics	
BMI (kg/m^2)	28.2 (11.4)
Waist circumference (cm)	90.4 (±12.2)
Hip circumference (cm)	106.8 (±10)
MUAC (cm)	31.6 (±4.9)
TSF (mm)	29.8 (±7.9)
Lifestyle characteristics	
MedDietScore (0–55)	36.6 (±5.1)
Physical activity	
#*Vigorous physical activity (MET-m/w)*	0.0 (0.0–0.0)
#*Moderate physical activity (MET-m/w)*	0.0 (0.0–120.0)
#*Walking (MET-m/w)*	120.0 (30.0–210.0)
#*Total physical activity (Total MET-m/w)*	420.0 (140.0–900.0)
Smoking	
No smoker (%)	62.6% ($n = 194/310$)
Smoker (%)	23.5% ($n = 73/310$)
Former smoker (%)	11.9% ($n = 37/310$)
Clinical characteristics	
SBP (mmHg)	117.4 (±22.3)
Total cholesterol (mg/dl)	211.8 (±36.1)
TG (mg/dl)	93.9 (±43.5)
HDL-C (mg/dl)	64 (±14.8)
LDL-C (mg/dl)	132.7 (±32.7)
Glucose (mg/dl)	93.4 (±10.3)
Insulin (μU/ml)	7.9 (±4.5)
TyG-Index	8.3 (±0.5)
Metabolic syndrome (%)	31.3% ($n = 97/310$).
Mean vascular structure and function indices	
cbIMT IMT (mm)	0.95 (±0.21)
Plaque presence cb (%)	0
Indices of vascular function	
PWV (m/s)	8.88 (±1.56)

BMI Body Mass Index, *MUAC* Mid-upper muscle circumference, *TSF* Triceps skin-fold thickness, *SBP* Systolic Blood Pressure, *TG* Triglycerides, *HDL-C* High density lipoprotein cholesterol, *LDL-C* Low density lipoprotein cholesterol, *TyG-Index* Triglyceride and Glucose Index, *IQR* Interquartile Range, *IMT* Intima-media thickness, *cc* Combined common carotid artery, *cb* Combined carotid bifurcation, *ic* Combined internal carotid artery, *comb* Combined carotid, *PWV* Pulse wave velocity

Continuous variables are presented as mean (SD) and nominal as count (absolute percentages);

Values were presented as mean (SD) or frequency (%). #Stands for median (IQR)

was inversely related to TSF ($r = -0.140$, $p = 0.03$). Nut intake was inversely related with waist circumference ($r = -0.227$, $p < 0.001$, significant after Bonferroni correction). The MedDietScore values were negatively associated with MUAC ($r = -0.135$, $p = 0.04$), TSF ($r = -0.178$, $p = 0.006$, significant after Bonferroni correction), and waist circumference ($r = -0.126$, $p = 0.035$).

The dietary patterns did not differ significantly between women with metabolic syndrome and women without metabolic syndrome. The mean number of servings per fish or seafood intake as well as per red meat intake was marginally different in women with vs without metabolic syndrome (fish or seafood servings: 0.09 ± 0.07, $p = 0.063$; red meat servings: 0.09 ± 0.05, $p = 0.054$). The MedDietScore results or daily energy intake did not differ between women with and without metabolic syndrome. The presence of metabolic syndrome was not significantly associated with any of the nutrients assessed or with the MedDietScore (data not shown)

As presented in Table 2, multivariate stepwise linear regression analysis models showed that alcoholic drink consumption was inversely associated with TSF (b-coefficient = −0.128, $p = 0.047$), after adjustment for age, BMI, physical activity, LDL-C, SBP, smoking, TyG-index and energy consumption. The daily red meat consumption was positively related to TyG-index (Model 1, Model $R^2 = 14.3\%$, b-coefficient = 0.121, p-value = 0.048), but the significance became only borderline after additional adjustment for daily energy intake (Model 2). The MedDietScore was inversely associated with TSF in all regression models (Model 1: $R^2 = 23.4\%$, b-coefficient = −0.172 significant after Bonferroni correction, $p = 0.006$; Model 2: $R^2 = 22.4\%$, b-coefficient = −0.153, $p = 0.017$).

Associations between dietary patterns and vascular indices

Correlation coefficients between the daily consumption of 20 food groups and study vascular indices are presented in Supplementary Table 3. Regarding arterial stiffness, full-fat dairy products ($r = -0.190$, $p = 0.001$), total dairy ($r = -0.136$, p-value = 0.019), tea ($r = -0.149$, $p = 0.010$) and coffee ($r = -0.171$, $p = 0.003$), all significant after Bonferroni correction, as well as processed meat ($r = -0.129$, $p = 0.026$) were found to be inversely associated with PWV in postmenopausal women. Daily coffee consumption was also inversely related to cbIMT ($r = -0.141$, $p = 0.014$, significant after Bonferroni).

Regarding arterial stiffness, daily intake of full-fat dairy (Model 2, $R^2 = 27.7\%$, b-coefficient = −0.133, $p = 0.007$) and total dairy products (Model 2, $R^2 = 27.3\%$, b-coefficient = −0.117, $p = 0.017$) were inversely associated with PWV (Table 3). Multivariate stepwise linear regression analysis was conducted, including each of the assessed indices of vascular function and structure as dependent variables, excluding the outliers in IMT values (IMT > 1.5 mm) (Table 4). Non-refined cereals were also negatively associated with cbIMT (Model 2, b-coefficient = −0.142, $p = 0.011$). Physical activity was not

Table 2 Multivariate stepwise linear regression analysis including each of the assessed vascular indices of metabolic indices as dependent variables after controlling for potential confounders and further adjustment for daily energy intake

	TyG – Index					
	Model 1			Model 2		
	Model R^2	beta	*p*-value	Model R^2	beta	*p*-value
Red meat consumption	14.3%	**0.121**	**0.048**	13%	0.119	0.054
Age (years)		**0.158**	**0.010**[(#)]		**0.136**	**0.028**
Body mass index (kg/m^2)		**0.278**	**<0.001**[(#)]		**0.295**	**<0.001**[(#)]
physical activity *(Total MET-m/w)*		0.045	0.465		0.040	0.514
LDL-C (mg/dL)		**0.153**	**0.012**[(#)]		0.141	**0.022**
SBP (mmHg)		0.052	0.395		0.062	0.316
Smoking		0.044	0.474		0.040	0.525
daily energy intake (kcal)		–	–		−0.031	0.612

	TSF (mm)					
	Model R^2	beta	*p*-value	Model R^2	beta	*p*-value
Alcohol drinks consumption	20.8%	−0.122	0.054	21.7%	−0.**128**	**0.047**
Age (years)		−0.079	0.214		−0.086	0.180
Body mass index (kg/m^2)		**0.416**	**<0.001**		**0.386**	**<0.001**[(#)]
physical activity *(Total MET-m/w)*		−0.073	0.254		−0.075	0.251
LDL-C (mg/dL)		**0.172**	**0.007**		**0.194**	**0.003**[(#)]
SBP (mmHg)		0.044	0.487		0.034	0.595
Smoking		0.020	0.757		0.031	0.633
daily energy intake (kcal)		–	–		−0.027	0.689
TyG-index		–	–		0.073	0.303
Total MedDiet score (0–55)	23.4%	−**0.172**	**0.006**[(#)]	22.4%	−**0.153**	**0.017**
Age (years)		−0.063	0.314		−0.070	0.278
Body mass index (kg/m^2)		**0.393**	**<0.001**[(#)]		**0.388**	**<0.001**[(#)]
physical activity *(Total MET-m/w)*		−0.048	0.455		−0.048	0.461
LDL-C (mg/dL)		**0.189**	**0.003**[(#)]		**0.194**	**0.003**[(#)]
SBP (mmHg)		0.063	0.317		0.061	0.345
Smoking		0.013	0.835		0.016	0.798
daily energy intake (kcal)		–	–		−0.036	0.582
TyG-index		–	–		0.087	0.215

Food groups' consumption is presented as servings per day

In Model 1 regression analysis, covariates included: age, physical activity, LDL-C, SBP, smoking, BMI

In Model 2 regression analysis, covariates included: age, physical activity, LDL-C, SBP, smoking, BMI and TyG-index, daily energy intake

TyG-Index Triglyceride and Glucose Index, *MUAC* Mid-Upper Arm Circumference, *TSF* Triceps Skin Fold, *LDL-C* Low-density lipoprotein, *SBP* Systolic Blood Pressure, *SFA* Saturated Fatty Acids, *MUFA* Monounsaturated Fatty Acids, *EI* Energy Intake

Bold indicates statistical significance which was defined as *p*-values < 0.05

[(#)]Remained significant after Bonferroni correction

related statistically significant to any of the vascular indices among correlations in all regression models.

Discussion

In this cross-sectional study, we report that in postmenopausal women, a population in need of more drastic primary prevention measures for CVD, the consumption of specific food groups is associated with vascular indices and metabolic and anthropometric parameters. The daily consumption of nuts, tea, dairy products and non-refined cereals was associated with better outcomes in vascular arterial function and structure after controlling for potential confounders. It should be considered that the postmenopausal population assessed in this study showed only minimal

Table 3 Multivariate stepwise linear regression analysis including PWV as dependent variable after controlling for potential confounders and further adjustment for daily energy intake

	PWV (m/s)					
	Model 1			Model 2		
	Model R^2	beta	*p*-value	Model R^2	beta	*p*-value
Total dairy consumption	26.3%	**−0.136**	**0.016**	27.3%	**−0.117**	**0.017**
Age (years)		**0.322**	**<0.001**$^{(\#)}$		**0.365**	**<0.001**$^{(\#)}$
Body mass index (kg/m^2)		**0.121**	**0.03**		0.095	0.069
physical activity *(Total MET-m/w)*		0.014	0.805		−0.013	0.798
LDL-C (mg/dL)		−0.011	0.840		−0.019	0.700
SBP (mmHg)		**0.171**	**0.003**$^{(\#)}$		**0.158**	**0.001**$^{(\#)}$
Smoking		**−0.196**	**0.001**$^{(\#)}$		**−0.182**	**<0.001**$^{(\#)}$
daily energy intake (kcal)		–	–		−0.011	0.828
TyG-index		–	–		**0.151**	**0.002**$^{(\#)}$
Full-fat dairy consumption	27.1%	**−0.164**	**0.004**$^{(\#)}$	27.7%	**−0.133**	**0.007**
Age (years)		**0.307**	**<0.001**$^{(\#)}$		**0.350**	**<0.001**$^{(\#)}$
Body mass index (kg/m^2)		**0.116**	**0.045**		0.090	0.085
physical activity *(Total MET-m/w)*		−0.004	0.940		−0.023	0.632
LDL-C (mg/dL)		−0.021	0.709		−0.023	0.651
SBP (mmHg)		**0.176**	**0.002**$^{(\#)}$		**0.157**	**0.002**$^{(\#)}$
Smoking		**−0.195**	**0.001**$^{(\#)}$		**−0.184**	**<0.001**$^{(\#)}$
daily energy intake (kcal)		–	–		−0.007	0.886
TyG-index		–	–		**0.153**	**0.002**$^{(\#)}$

Food groups' consumption is presented as servings per day

In Model 1 regression analysis covariates included: age, physical activity, LDL-C, SBP, smoking, BMI

In Model 2 regression analysis covariates included: age, physical activity, LDL-C, SBP, smoking, BMI and TyG-index, daily energy intake

PWV Pulse wave velocity, *TyG-Index* Triglyceride and Glucose Index, *LDL-C* Low-density lipoprotein, *SBP* Systolic Blood Pressure, *SFA* Saturated Fatty Acids, *MUFA* Monounsaturated Fatty Acids, *EI* Energy Intake

Bold indicates statistical significance which was defined as *p*-values < 0.05

$^{(\#)}$Remained significant after Bonferroni correction

evidence of atherosclerotic plaque presence. Notably, a diet including higher amounts of nuts and non-refined cereals was associated with a lower extent of subclinical atherosclerosis in the carotid arteries. In contrast, a diet rich in tea and dairy products was related similarly to lower arterial stiffness. This is the first study investigating the association of real-life dietary patterns with vascular indices during menopause. Postmenopausal women are a population with a high residual risk for CVD, and proper identification of modifiable risk factors such as dietary habits could be of clinical value for the optimal prevention of CVD. Regarding metabolic parameters, daily red meat consumption seems to be closely related to insulin resistance, as indicated by the association with TyG-index. Anthropometric indices during menopause may be positively affected by the frequent consumption of tea, nuts, non-refined cereals and alcohol. In contrast, red meat consumption seems to have the opposite effect. The observed associations occurred independently of traditional risk factors for CVD, physical activity, total energy intake and TyG-index.

Based on our findings, higher amounts of nuts, non-refined cereals, tea and dairy products seem to be linked with less subclinical atherosclerosis and lower arterial stiffness, independently of the presence of traditional risk factors for CVD and total daily energy intake in postmenopausal women. The association between dietary patterns and indices of subclinical atherosclerosis has not received significant attention yet. Observational data in general [23] and postmenopausal subgroups [24] indicate a link between frequent tea consumption and lower prevalence of CVD events [23, 25], possibly related to the number of catechins [26]. Further supporting the adverse association between tea consumption and arterial stiffness, as observed in this sample of postmenopausal women, our results also point out that higher tea consumption was also related to better body weight status in terms of lower MUAC measurements. Cross-sectional evidence on green tea consumption has been associated with lower body fat mass and better cardio-ankle vascular index [26]. Moreover, the anti-inflammatory and anti-oxidative properties of tea,

Table 4 Multivariate stepwise linear regression analysis including markers of carotid atherosclerosis as dependent variable after controlling for potential confounders and further adjustment for daily energy intake

	Combined carotid bifurcation IMT (mm)					
	Model 1			Model 2		
	Model R^2	Beta	*p*-value	Model R^2	beta	*p*-value
Non-refined cereals consumption	5.0%	**−0.139**	**0.028**	5.5%	**−0.142**	**0.011**
Age (years)		0.035	0.583		**0.139**	**0.013**
Body mass index (kg/m^2)		0.013	0.838		0.042	0.454
physical activity *(Total MET-m/w)*		−0.040	0.524		−0.023	0.673
LDL-C (mg/dL)		−0.102	0.104		−0.077	0.167
SBP (mmHg)		0.005	0.934		**0.162**	**0.004**$^{(\#)}$
Smoking		**0.206**	**0.001**$^{(\#)}$		0.017	0.766
daily energy intake (kcal)		–	–		−0.056	0.338
TyG – index		–	–		−0.011	0.842

Food groups' consumption is presented as servings per day

In Model 1 regression analysis covariates included: age, physical activity, LDL-C, SBP, smoking, BMI

In Model 2 regression analysis covariates included: age, physical activity, LDL-C, SBP, smoking, BMI and TyG-index, daily energy intake

All the included IMT values in the regression models were < 1.5 mm

IMT Intima-media thickness, *TyG-Index* Triglyceride and Glucose Index, *LDL-C* Low-density lipoprotein, *SBP* Systolic Blood Pressure, *SFA* Saturated Fatty Acids, *MUFA* Monounsaturated Fatty Acids, *EI* Energy Intake

Bold indicates statistical significance which was defined as *p*-values < 0.05

$^{(\#)}$Remained significant after Bonferroni correction

nuts and non-refined dietary fibres may regulate the chronic systemic low-grade inflammation related to metabolic complications established in postmenopausal women [27–30]. In our study, body fat distribution parameters, namely TSF and MUAC, were inversely associated with daily consumption of tea, nuts and non-refined cereals. The positive effect of the aforementioned dietary parameters on achieving better body fat consistency and regulation of metabolic mechanisms during menopause may link to the observed protective role on vascular health.

The observed associations between food groups and vascular or anthropometric indices were largely unrelated to insulin resistance. Adjustment for TyG-index in all regression models did not substantially change the above associations except for the food group of non-refined cereals. The results are not surprising, taking into account that the amount of carbohydrate intake and the combination between increased daily energy intake and decreased physical activity in our study population may influence the development of insulin resistance [31]. The preference for non-refined cereals in the case of our participants was related to lower TSF measurements, even after further adjustment for TyG-index in the existing regression model analysis. Available studies suggest that dietary patterns, including nuts and non-refined cereals, are related to a lower risk for generalised and abdominal obesity [8, 27]. The low glycemic index and the fibre content of non-refined cereals and nuts have been associated with improved insulin resistance. They might indirectly mediate the accumulation of visceral fat [27, 28]. Thus, fibre content and low glycemic index may be the principal nutritional components of non-refined cereals that contribute to their vascular protective role. However, the excessive daily carbohydrate intake may reduce the positive vascular impact of non-refined cereals due to the possible detrimental effect of insulin resistance mechanism patterns during menopause.

On the other hand, the association between consumption of dairy products and CVD events seems to vary according to the fat content and the fermentation status of the products consumed [27, 28]. Interestingly, a recent randomised clinical trial could not demonstrate an adverse add-hoc effect of dairy consumption on arterial stiffness of middle-aged hypertensive adults [29]. In pathophysiological terms, polar milk lipids decrease fasting and postprandial plasma cholesterol concentrations through reduced intestinal cholesterol absorption [30]. In our sample, daily consumption of dairy and, primarily, full-fat dairy products were related to better arterial stiffness even after further adjustment for TyG-index in the existing regression model analysis.

According to our findings, adherence to the Mediterranean diet seems to impact body fat distribution rather than the development of subclinical atherosclerosis. Most postmenopausal women in this study were characterised by high adherence to Mediterranean-style dietary patterns. Further adjustment for insulin resistance resulted in a loss of significant results (after Bonferroni correction), implying possible mediation. According to our knowledge, the association between the MedDietScore and the development or progression of subclinical vascular function and structure in postmenopausal women has not been explored yet. Our results show a negative association between MedDietScore and indices of fat distribution, namely MUAC and TSF, but no association with indices of vascular structure and function, indicating an indirect effect of the diet to fat accumulation rather than direct interaction with the vasculature. As described in the latest guidelines of the European Menopause and Andropause Society, adherence to the Mediterranean diet can offer cardiometabolic protection in terms of blood pressure regulation, better lipidemic profile and severity of vasomotor symptoms management [31].

Given that the Mediterranean-style dietary pattern is characterised by moderate alcohol consumption, there is a justifiable background to our results about the impact of alcohol drinks on body fat distribution. Specifically, average daily alcohol intake was associated with lower TSF and MUAC measurements in our participants. No such associations were detected with vascular indices. Existing data on postmenopausal women suggest that moderate alcohol consumption may have a beneficial role in inflammation and hemostasis, possibly by inducing an increase in plasminogen activator inhibitor 1 [32]. Moreover, fish consumption was not related to any of the endpoints assessed in this study. A fish-based diet was effective against increased arterial stiffness in a mixed-gender sample, as assessed by PWV [33]. High fish intake (≥ 3 times vs 1–1.9 times per week) was associated with a higher risk of larger atherosclerotic plaque area and a greater risk of novel plaque formation (OR 1.32, 95% CI: 1.01–1.73) in mixed-gender participants without atherosclerosis at baseline [34]. In a cross-sectional study of 1270 healthy males, high fish intake was associated with lower non-HDL-c levels.[35]. Of note, women in our study consumed relatively low portions of fish, estimated at 0.1 serving per day, which can likely explain the poor correlates with indices of vascular disease.

The limitations of this study need to be acknowledged. The sample size of this study was relatively small. The cross-sectional design of our study does not allow for cause-effect relationships. Moreover, participating women were recruited from a single-centre and might therefore be more health-aware than the general population. Consequently, more than half of our study sample showed high adherence to the Mediterranean diet. The prevalence of atheromatous plaques was very low to be incorporated in statistical analysis, while we did not assess for coronary artery calcium, which represents stronger predictors of cardiovascular risk [36]. Furthermore, since we used a food frequency questionnaire to imprint dietary habits, the information provided might be affected by recall bias. Moreover, as with all food frequency questionnaires, the questionnaire has a specific food list, thus not allowing the exploration of other than the specified food items. Finally, our data collection was completed in July 2020. The Sars-CoV-2 pandemic has affected the follow-up of our women. Lockdown and restriction in the number of participants examined per day have delayed the completion of the follow-up visits. However, most of the participants were recruited before the lockdown restrictions were enforced, while only 10% of ladies were recruited after the outbreak (March 2020–July 2020). This study also has certain strengths. We assessed a homogenous population since a sample consisted only of apparently healthy postmenopausal women without clinically overt CVD or traditional cardiovascular risk factors, including hypertension, hyperlipidemia, diabetes mellitus, chronic kidney disease and obesity or concomitant medications. Moreover, all vascular studies were conducted by the same experienced operator with high rates of reproducibility. Overall, these characteristics of our studied population and design provide consistency in several major confounders allowing relatively low requirements in terms of sample size, as also indicated by our a priori sample size calculations.

In summary, our findings indicate that the consumption frequency of nuts, non-refined cereals, tea and dairy products may have a protective impact on the extent of subclinical atherosclerosis and arterial stiffness in postmenopausal women. The beneficial role of specific food groups on vascular health during menopause is amplified by the absence of atherosclerotic plaques in our study population. In addition, food choices and the adherence level to Mediterranean Diet, as estimated by MedDietScore, were associated with body fat accumulation. Further longitudinal analysis of our data will confirm the validity of these initial observations and shed light on the association between dietary patterns and subclinical CVD.

Author contributions All authors contributed to the study's conception and design. Material preparation and data collection was performed by IK, DDP, AA, GM, LA, and TP. Data analysis was performed by EA, DD, IK, DB, and KP. The first draft of the manuscript was written by IK, DD, MY, and EA and all authors commented on previous versions of the manuscript. KS and IL supervised the data collection, study investigations, manuscript preparation, and statistical analysis of the results. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare no competing interests.

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