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Dietary intake of isoflavones is associated with a lower prevalence of subclinical cardiovascular disease in postmenopausal women: cross-sectional study

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Keywords
antioxidants, cardiovascular disease, diet, isoflavones, polyphenols, postmenopause.

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Abstract

Background: Menopause has been associated with an increased risk of cardiovascular disease. It has been shown that isoflavones protect vascular endothelial cells against induced oxidative stress injury. Therefore, the present study aimed to investigate the association between the dietary intake of isoflavones and the presence of subclinical cardiovascular disease (CVD) in postmenopausal women.

Methods: Ninety-six postmenopausal women [mean (SD) age 55.2 (4.9) years, body mass index (BMI) 27.2 (4.6) kg m^{-2}] completed the study protocol. Habitual physical activity was assessed using a digital pedometer, resting metabolic rate was measured by indirect calorimetry and dietary intake was assessed via a validated food frequency questionnaire. Subclinical CVD was defined as carotid artery intima-media thickness (C-IMT) >0.9 mm and/or the presence of one or more atherosclerotic plaques in any of the studied segments.

Results: Mean (SD) C-IMT was 0.74 (0.2) mm, 25% of participants were found to have atherosclerotic plaques and the prevalence of subclinical CVD was 35%. Participants with subclinical CVD were more likely to consume less selenium, magnesium, folate and isoflavones, even after adjusting for total energy intake. A multivariate-adjusted regression model showed that a BMI >27 kg m^{-2} was associated with 90% higher risk of having ≥1 plaque and/or C-IMT >0.9 mm ($P = 0.017$). Higher oestradiol levels ($P = 0.004$) and isoflavone intake ($P = 0.021$) were independently associated with a lower risk of having subclinical CVD.

Conclusions: In the present study, we observed that a higher isoflavone dietary intake was associated with a lower risk of subclinical CVD in postmenopausal women, independent of BMI and endogenous oestradiol levels.

Introduction

Menopause has been associated with increased risk of cardiovascular disease (CVD), possibly as a result of changing hormonal status and ageing [1,2]. Atherosclerosis, which underlies the occurrence of cardiovascular events, develops over decades and has a prolonged asymptomatic phase [3]. Some non-invasive procedures are able to detect and measure different stages of atherosclerosis, such as carotid artery intima-media thickness (C-IMT), which is a non-invasive ultrasound measurement of artery wall thickness [4,5], and have been associated with postmenopausal status [6,7].

Several epidemiological studies have described the potential role of diet in CVD prevention [8–10]. Specifically, experimental, epidemiological and clinical data

suggest that the consumption of antioxidants is associated with a reduced risk of CVD [11–13]. Isoflavones (genistein and daidzein) and lignans are dietary-derived polyphenols and the most common phyto-oestrogens. Although isoflavones can be found in soybeans, black beans and barley [14], lignans are found in legumes, vegetables, fruits, flaxseed and whole grains [15]. Isoflavones are known to possess various biological effects that have been associated with cardiovascular protection as a result of their anti-inflammatory properties [16,17], as well as their impacts on endothelial function [18–20]. Isoflavones have also been reported to exert weak oestrogenic activity by binding to oestrogen receptors [21]. Additionally, isoflavones have been shown to exert antioxidative properties [22,23]. An *in vitro* study showed that genistein and daidzein significantly protect vascular endothelial cells against induced oxidative stress injury [24].

To date, few studies have examined the association between dietary isoflavone intake and subclinical CVD, specifically in postmenopausal women. Therefore, the present study aimed to investigate the association between isoflavone dietary intake, metabolic and hormonal variables and C-IMT status in postmenopausal women, with no clinical evidence of CVD.

Materials and methods

Participants and design

In this cross-sectional study, participants were invited by advertisements in local newspapers and radio stations to come to the Gynecological Endocrinology Unit at Hospital de Clínicas de Porto Alegre, Brazil, from October 2010 to February 2012. The inclusion criteria were: (i) menopause, defined as the last menstrual period at least 1 year before the beginning of the study plus follicle-stimulating hormone (FSH) levels >35 IU L^{-1}; (ii) age between 45 and 65 years; and (iii) no use of hormone therapy in the past 3 months. Individuals with diabetes or a previous diagnosis of heart disease and current smokers were excluded. Ninety-six postmenopausal women fulfilling all the inclusion criteria completed the study protocol. The local Ethics Committee approved the study protocol, and written informed consent was obtained from every participant. Detailed information regarding the participants is provided elsewhere [6].

Anthropometric measurements, body composition and resting metabolic rate

Body weight, height and waist circumference (WC) were measured in duplicate in the standing position. WC was measured at the midpoint between the lower rib margin and the iliac crest, and body mass index (BMI) was calculated as the most recent measured weight (kg) divided by height (m) squared. Resting metabolic rate (RMR) was obtained by indirect calorimetry (Fitmate, Cosmed, Rome, Italy).

Dietary assessment

Dietary intake in the previous month was assessed with a validated food frequency questionnaire (FFQ) consisting of 121 items [25]. Nutritional composition was calculated using the Brazilian Table of Food Composition [26]. Vitamins A, D and E were assessed using the United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference [27].

Total polyphenol, isoflavone and lignan intake were assessed using the Phenol-Explorer database [28]. Food items in the FFQ containing two or more food components were separated according to their individual ingredients and foods that contained no polyphenols were excluded from the analysis. The average food consumption was calculated (g or mL) according to the standard portion sizes used in the FFQ. The individual polyphenol intake from each food was calculated by multiplying the content of each polyphenol by the daily consumption of each food. Total polyphenol, isoflavone and lignan intake were calculated as the sum of all individual polyphenol intakes from all food sources encountered in accordance with this process.

Physical activity assessment

Assessment of habitual physical activity was performed with a digital pedometer (BP 148; Tech Line, São Paulo, Brazil). The device was individually configured according to weight (kg) and individual step length. The equipment was used for six consecutive days, providing the weekly average number of steps. Participants were encouraged not to change their physical activity habits during the study.

Blood pressure and biochemical and hormone tests

Blood pressure was measured with participants in the seated position, with the feet on the floor and the arm supported at heart level after a 10-min rest. Two measurements were obtained, 10 min apart, using an automatic blood pressure monitor (HEM-742INT; Omron, Rio de Janeiro, Brazil). Hypertension was defined as a systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥80 mmHg [29].

Blood samples were collected after a 12-h fast. All samples were obtained between 08.00 h and 10.00 h. FSH was measured with chemiluminescent immunoassays

(Centaur XP; Roche Diagnostics, Mannheim, Germany) with a sensitivity of 0.3 IU L^{-1}. The intra-assay and interassay coefficients of variation were 2.9% and 2.7%, respectively. Oestradiol was measured by electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany), with an assay sensitivity of 5.0 pg mL^{-1} and intra- and interassay coefficients of variation of 5.7% and 6.4%, respectively. Sex hormone-binding globulin (SHBG) was measured by chemiluminescence enzyme immunoassay (Immulite 2000, Centaur XP; Roche Diagnostics), where SHBG had a sensitivity of 0.02 nmol L^{-1} and intra-assay and interassay CVs of 5.3% and 6.6%, respectively.

Assessment of subclinical cardiovascular disease

Measurement of C-IMT was assessed bilaterally with B-mode ultrasonography (Xsario; Toshiba, Tokyo, Japan) by a single expert sonographer operator using a standardised protocol. A 7.5-MHz, fixed angle, multifrequency linear array probe was used. The right and left carotid arteries were scanned to obtain a total of nine images of the far wall of the common carotid (1 cm proximal to the carotid bulb), the carotid bulb (1 cm proximal to the flow divider) and the proximal internal carotid arteries (1 cm distal from the flow divider). In each segment, three measurements of maximum C-IMT were obtained. Subsequently, the average C-IMT of the three segments was calculated for each of the two carotid arteries [4,6,30]. Subclinical atherosclerosis was defined as C-IMT >0.9 mm and/or the presence of one or more atherosclerotic plaques in any of the studied segments [31]. A plaque was defined by at least two of the following three criteria: C-IMT >1.5 mm; shape abnormalities such as protrusion into the lumen or loss of alignment with adjacent arterial wall boundary; and the presence of brighter echoes than adjacent boundaries [4].

Statistical analysis

The sample size was estimated based on a previous study [32], considering a power of 80% and alpha of 5%. Seventy-six postmenopausal women were required to detect a difference of 0.19 mm in C-IMT between higher and lower isoflavone dietary intake.

Results are presented as the mean (SD) or median (interquartile range), depending on the Gaussian or non-Gaussian distribution of variables (Shapiro–Wilk test). Non-Gaussian variables were log-transformed for statistical analysis and reported as back-transformed into their original units. To compare the differences between participants with no plaque and C-IMT ≤0.9 mm to those with subclinical CVD, a two-tailed Student's *t*-test was used. Chi-squared was calculated for comparisons of dichotomous variables. Prevalence ratios (PR) were determined for subclinical CVD according to demographic, lifestyle and dietary factors that were associated with C-IMT >0.9 mm and/or the presence of atherosclerotic plaque in a two-tailed Student's *t*-test. A multivariate-adjusted Poisson regression model with robust estimates was used to assess the association between demographic, lifestyle and dietary factors and subclinical CVD. All analyses were performed using SPSS, version 19.0 (IBM Corp., Armonk, NY, USA). $P \leq 0.05$ was considered statistically significant.

Results

Of one hundred and nineteen postmenopausal women enrolled in the present study, 96 participants fulfilling all the inclusion criteria completed the study protocol. Eighteen candidates were excluded (five with diabetes, one with hyperthyroidism, two with untreated hypothyroidism, two with breast cancer, one who was premenopausal and seven current smokers). An additional five participants dropped out because they were unable to commit to the study (no time for blood collection, dual X-ray absorptiometry and indirect calorimetry). The mean (SD) carotid C-IMT of 0.74 (0.2) mm and the overall prevalence of subclinical CVD was 35% of the sample population; of these, 25% of patients were found to have one or more atherosclerotic plaques. The characteristics of the postmenopausal women according to carotid intima-media thickness status are provided in Table 1. The groups were similar regarding age, time subsequent to menopause, years of schooling, BMI and the prevalence of overweight/obesity, waist circumference, physical activity, and RMR. There were no differences between groups regarding traditional CVD risk factors, such as glucose, lipids (data not shown) and previous smoking behaviour. However, the frequency of hypertension was higher in women with subclinical CVD (56% *vs* 32%, $P = 0.018$). Oestradiol and the proportion of previous users of hormone therapy were also similar in the two groups.

Participants presenting at least one plaque in the carotid and/or C-IMT >0.9 mm were more likely to consume less calories ($P = 0.027$), total protein and plant-based protein, although, after total kcal intake adjustment, this difference did not remain significant (Table 2). Furthermore, women with subclinical CVD consumed less selenium ($P = 0.025$), magnesium ($P = 0.047$), folate ($P = 0.024$) and isoflavones ($P = 0.049$), even after adjustment for energy intake.

The main factors related to the presence of subclinical CVD are shown in Table 3. BMI ≥27 kg m^{-2} (defined as the median of participants in the sample) and

Table 1 Characteristics of postmenopausal women according to carotid intima-media thickness status

Variables	Subclinical cardiovascular disease		
	No plaque and C-IMT ≤0.9 mm	≥1 plaque and/or C-IMT >0.9 mm	*P*
n	62	34	
Age (years)	54.7 (4.7)	56.5 (5.2)	0.095
Years of schooling†	8.5 (5.0–12.7)	7.5 (4.7–11)	0.147
White, *n* (%)‡	53 (85)	30 (88)	0.706
Time subsequent to menopause (years)†	5.5 (3–10)	7.5 (3–10.5)	0.259
Waist circumference (cm)	86.4 (13.2)	87.2 (9.8)	0.754
BMI (kg m^{-2})	27.2 (5.1)	27.3 (4.2)	0.904
Obesity, *n* (%)‡	10 (16)	10 (29)	0.125
Overweight and obesity, *n* (%)‡	39 (63)	23 (68)	0.642
Mean (SD) steps/day	6111.0 (3007.9)	5471.1 (2840.7)	0.312
RMR (kJ day^{-1}) [kcal day^{-1}]	5264.7 (920.9) [1258.3 (220.1)]	5286.5 (688.7) [1263.5 (164.6)]	0.904
Hypertension, *n* (%)‡	19 (31)	19 (56)	0.018
Previous smoking behaviour, *n* (%)‡	20 (32.2)	15 (44.1)	0.248
Hormonal variables:			
Oestradiol (pg mL^{-1})†	21.1 (10.8–30.3)	19.2 (12.5–23.8)	0.252
FSH (IU L^{-1})	80.5 (29.2)	86.8 (28.8)	0.310
SHBG (nmol $L^{-1)}$†	48.2 (34.0–61.7)	43.1 (36.5–58.8)	0.610
Previous hormone therapy, *n* (%)‡	18 (29)	13 (38)	0.371

BMI, body mass index; C-IMT, carotid intima-media thickness; DBP, diastolic blood pressure; FSH, follicle-stimulating hormone; HDL, high-density protein; LDL, low-density protein; RMR, resting metabolic rate; SBP, systolic blood pressure; SHBG, sex hormone-binding globulin.
Data are the mean (SD) or median (IQR).Student's *t* test.
†Variables analysed after log transformation.
‡Chi-squared test.

hypertension were related to the presence of subclinical CVD [PR = 1.83; 95% confidence interval (CI) = 1.06–3.19 and PR = 1.93; 95% CI = 1.13–3.32, respectively]. Assessment of the dietary factors showed that the presence of one or more plaques and C-IMT ≥0.9 mm was less frequent in women consuming higher levels of fibre, ≥21 g, according to dietary reference intakes [33], (PR = 0.58; 95% CI: 0.34–0.97), selenium, ≥55 μg, according to dietary reference intakes, (PR = 0.54; 95% CI = 0.30–0.98) and isoflavones, ≥4.9 mg, defined as the median of participants in the sample (PR = 0.52; 95% CI = 0.30–0.92).

The multivariate-adjusted Poisson regression model with robust estimate is shown in Table 4. BMI, endogenous oestradiol levels and isoflavone intake were independently related to the presence of subclinical CVD. Higher BMI was independently associated with 90% ($P = 0.017$) higher risk of having ≥1 plaque and/or C-IMT >0.9 mm. On the other hand, oestradiol (per 1 pg mL^{-1} increase), and higher isoflavone intake were independently associated with a 3% ($P = 0.004$) and 50% ($P = 0.021$) lower risk of having subclinical CVD, respectively.

Discussion

In the present study, we observed that a higher isoflavone dietary intake and endogenous oestradiol levels were independently associated with a lower risk of subclinical CVD in postmenopausal women and BMI was independently related to higher risk of subclinical CVD. To date, only two cross-sectional studies, both from Chinese population, have examined the association between dietary isoflavone intake and C-IMT status [32,34]. In the first study, comprising 126 participants aged 66.5 (11.1) years old (69% male) at high risk of cardiovascular events, a median isoflavone intake of 5.5 (2.2–13.3) mg day^{-1} was observed, and a higher isoflavone intake predicted an absolute 0.17 mm decrease in mean maximum C-IMT [32]. In the other study comprising 572 healthy adults (aged 40–65 years), a higher intake of isoflavone (>5.4 mg day^{-1}) was associated with a lower C-IMT [34]. However, the isoflavone intake in occidental countries appears to be much lower compared to that in Asian countries, with most people eating on average less than 1 mg day^{-1} compared to 20–50 mg day^{-1} or higher, respectively [35]. In the present study, the median (interquartile range) isoflavone intake was 4.9 (2.1–5.1) mg day^{-1} and less than this amount was consumed by women with subclinical CVD. Additionally, although, in most studies, the consumption of soy products was the main source of isoflavones, in the present study, the major source of isoflavones was beans. Interestingly, the most frequently consumed beans in Brazil is the black type, which contains the highest concentrations of isoflavonoids and daidzein [36,37].

Table 2 Dietary intake of postmenopausal women according to carotid intima-media thickness status

Variables	Subclinical cardiovascular disease			
	No plaque and C-IMT ≤0.9 mm	≥1 plaque and/or C-IMT >0.9 mm	*P*	*P*‡
Total energy intake, kJ (kcal)	8186.4 (2788.6) [1956.6 (666.5)]	6947.1 (2158.1) [1660.4 (515.8)]	0.027	
Protein (%)	17.1 (3.0)	16.3 (3.1)	0.223	
Total protein (g)	83.1 29.0)	66.7 21.5)	0.005	0.894
Plant-based protein (g)	19.6 (9.4)	15.5 (8.0)	0.040	0.346
Carbohydrate (%)	58.4 (7.0)	58.0 (8.0)	0.762	
Glycaemic index (%)	55.1 (4.4)	56.5 (5.0)	0.157	
Lipids (%)	23.6 (5.2)	24.8 (5.8)	0.313	
Saturated fatty acids (%)	6.8 (2.0)	6.8 (2.2)	0.963	
Monounsaturated fatty acids (%)	7.0 (2.4)	7.5 (3.7)	0.400	
Polyunsaturated fatty acids (%)	3.1 (0.9)	3.4 (1.0)	0.218	
Cholesterol (mg)†	227.3 (161.3–306.0)	150.1 (124.7–230.5)	0.177	
Fibre (g)†	27.5 (20.9–38.8)	24.7 (17.1–35.3)	0.070	
Vitamin B_{12} (μg)†	4.8 (3.4–6.5)	3.3 (2.2–4.6)	0.132	
Calcium (mg)	9.1 (0.4)	9.0 (0.3)	0.306	
Selenium (μg)†	92.2 (78.8–124.8)	72.5 (57.3–97.6)	0.008	0.025
Magnesium (mg)†	269.6 (206.5–343.5)	219.9 (162.1–284.2)	0.013	0.047
Zinc (mg)†	8.7 (3.4)	7.3 (2.4)	0.033	0.872
Folate (μg)†	505.6 (396.1–710.1)	418.0 (320.6–574.7)	0.031	0.024
Vitamin D (mg)†	4.6 (2.6–10.6)	4.0 (1.9–10.5)	0.705	
Vitamin E (mg)†	4.3 (3.0–5.4)	3.1 (2.0–4.2)	0.047	0.105
Vitamin C (mg)†	209.6 (122.5–373.2)	133.1 (106.8–253.8)	0.194	
Vitamin A (μg)†	962.5 (556.3–1330.9)	690.6 (326.7–1392.7)	0.499	
Alcohol (g)†	5.0 (0.0–28.0)	0 (0.0–17.5)	0.610	
Polyphenol (mg)	3817.7 (1914.6)	2973.5 (1151.5)	0.008	0.881
Isoflavones (mg)†	4.9 (2.1–9.8)	3.5 (2.1–4.9)	0.019	0.049
Lignans (mg)†	29.9 (12.1–54.4)	20.4 (6.4–39.7)	0.831	

C-IMT, carotid intima-media thickness. Data are the mean (SD) or median (IQR).
†Variables analysed after log transformation, Student's *t* test.
‡Adjusted for energy intake.

During the menopausal transition, changes in endogenous oestradiol levels may impact the vulnerability of the vessels in the postmenopausal period. In the present study, although endogenous oestradiol levels did not differ between the participants with no plaque and C-IMT ≤0.9 mm and those with subclinical CVD, each 1 pg mL^{-} increase in oestradiol levels was associated with a 3% lower risk of subclinical CVD, independent of age, BMI and blood pressure. Similar to these results, the Study of Women's Health Across the Nation (SWAN) also found that lower oestradiol was associated with an increase in C-IMT, independent of blood pressure, BMI, lipids and other covariates [38]. An increased C-IMT prevalence has also been associated with postmenopausal status [7,39]. However, menopause, ageing, and an increase in CVD risk occur somewhat synchronously, and cross-sectional studies are unable to clarify which factor exerts the main role on C-IMT changes.

Two clinical trials evaluated isoflavone supplementation, in women who were <5 years subsequent to menopause [40], or more than 5 years subsequent to menopause [41], and found isoflavones were associated with lower C-IMT progression compared to the placebo. In addition, *in vitro* studies have shown isoflavones exert a weak oestrogenic activity through binding to oestrogen receptors and were postulated to improve endothelial function by acting on nitric oxide release by endothelial cells[42,43]. A meta-analysis of randomised clinical trials suggests that exposure to soy isoflavones can modestly but significantly improve endothelial function as measured by flow-mediated vasodilation [19].

In turn, the physiological effects of isoflavones not only influence oestrogen modulation, but also anti-inflammatory and antioxidant properties. A recent study suggests that the anti-inflammatory properties of the isoflavone genistein play a beneficial role in the cardiovascular system via the inhibition of angiotensin II-stimulated C-reactive protein and matrix metalloproteinase-9 expressions in vascular smooth muscle cells [16]. Furthermore, isoflavones are polyphenols derived from the diet and, recently, interest in food phenolics has greatly increased as a result of their antioxidant capacity (free radical scavenging and

Table 3 Demographic, lifestyle and dietary factors related to the presence of ≥1 plaque and/or C-IMT >0.9 mm

Factors	Categories	PR (95% CI)	*P*
Age (years)	<55	(ref.)	0.287
	≥55	1.37 (0.77–2.43)	
Time subsequent to menopause (years)	<5	(ref.)	0.485
	≥5	1.21 (0.70–2.10)	
Previous hormone therapy	No	(ref.)	0.347
	Yes	1.30 (0.75–2.23)	
BMI (kg m^{-2})‡	<27	(ref.)	0.031
	≥27	1.83 (1.06–3.19)	
Oestradiol (pg mL^{-1})		0.98 (0.95–1.00)	0.067
Hypertension	No	(ref.)	0.017
	Yes	1.93 (1.13–3.32)	
Fibre (g)†	<21.0	(ref.)	0.038
	≥21.0	0.58 (0.34–0.97)	
Selenium (μg)†	<55.0	(ref.)	0.042
	≥55.0	0.54 (0.30–0.98)	
Magnesium (mg)†	<320.0	(ref.)	0.072
	≥320.0	0.46 (0.20–1.07)	
Folate (μg)†	<400.0	(ref.)	0.110
	≥400.0	0.65 (0.38—1.10)	
Polyphenol intake (mg)‡	<3073.6	(ref.)	0.056
	≥3073.6	0.57 (0.32–1.01)	
Isoflavones intake (mg)‡	<4.9	(ref.)	0.024
	≥4.9	0.52 (0.30–0.92)	

BMI, body mass index; CI, confidence interval; C-IMT, carotid intima-media thickness; PR, prevalence ratio.
Each model (each block) was evaluated independently. Unadjusted models were used. Determinants were identified on the basis of a backward prevalence ratio model. Oestradiol was considered a continuous variable. The other variables are categorical.
†Dietary reference intakes [33].
‡Defined as the median of participants in this sample.

Table 4 Adjusted of prevalence ratios for the presence of ≥1 plaque and/or C-IMT >0.9 mm

Factors	PR (95% CI)	*P*†
Age (years)	1.03 (0.98–1.08)	0.271
BMI: ≥27 kg m^{-2}	1.90 (1.12–3.22)	0.017
Hypertension	1.44 (0.80–2.58)	0.222
Oestradiol (pg mL^{-1})	0.97 (0.95–0.99)	0.004
Fibre (g)‡	0.78 (0.40–1.50)	0.778
Selenium: ≥55.0 μg‡	0.76 (0.37–1.55)	0.450
Isoflavones: ≥4.9 mg§	0.50 (0.27—0.90)	0.021

BMI, body mass index; CI, confidence interval; PR, prevalence ratio.
Age and oestradiol were considered continuous variables. The others variables are categorical.
†Multivariate-adjusted Poisson regression model with robust estimate.
‡Dietary reference intakes [33].
§Defined as the median of participants in this sample.

metal chelating activities) [14]. The generation of reactive oxygen species (ROS) in endothelial cells causes the rapid degradation of nitric oxide, and thus the quenching of ROS should, in theory, improve endothelial function. In a study with 1683 postmenopausal women, an inverse association was observed between total polyphenol intake and lower prevalence of CVD [44]. Other dietary antioxidants have been recognised to exert a protective effect against atherogenesis and CVD [45]. Similarly, a systematic review showed that high intakes and/or circulatory levels of magnesium, as well as the vitamin B group, may be associated with lower C-IMT or reduced progression of C-IMT [46]. In the present study, women with subclinical CVD had lower dietary intake of magnesium, selenium and folate, although only lower isoflavone intake was independently associated with the presence of plaque and C-IMT ≥0.9 mm. Isoflavones appear to be more effective than antioxidant vitamins and minerals in the scavenging of reactive oxygen species and lipid peroxidation [22,23]. Taken together, the data from the literature and our the results obtained in the present study suggest that the association found between higher isoflavones intake and lower risk of subclinical CVD in postmenopausal women, might be related to its antioxidant effect and putative weak oestrogenic activity.

Some studies have indicated that the positive cardiovascular effects of isoflavones may be a function of the ability to produce equol, an active metabolite, produced by gut microbiota [47–49], as well as presenting affinity for oestrogen receptors, anti-androgenic properties and good antioxidant activity [50]. Indeed, equol-producing ability varies greatly among individuals and higher dietary isoflavone consumption was associated with a lower IMT in equol producers but not in equol non-producers in a study involving 572 healthy Chinese adults [34]. Interestingly, recent reports suggest that the equol production status might be associated with intake of dietary isoflavones and a healthy diet pattern by increasing gut microbiome diversity [51,52]. Although our participants with higher isoflavone dietary intake could present a better gut microbiome profile, the equol-producing ability could not be assessed in the present study because of the lack of specific biological (urine) samples.

Our results show that BMI ≥27 kg m^{-2} was associated with 90% higher risk of having ≥1 plaque and/or C-IMT >0.9 mm. In a previous study with 390 postmenopausal women [mean (SD) age, 63.1 (7.7) years old] living in the Mediterranean region, a high BMI was associated with increased common carotid C-IMT and lumen diameters, and this association persisted after adjustment for the presence of metabolic syndrome [53]. Recently, Hruskova *et al.* [54] demonstrated that, in younger individuals (n = 102, aged 25–64 years old, with no current or past

CVD history), BMI and blood pressure were significantly and positively associated with C-IMT. In line with these results, in our sample of recent postmenopausal women, hypertension, as defined in accordance with new guidelines [29] was also associated with the presence of subclinical CVD.

The strength of the present study is the sampling of postmenopausal women with no clinical disease, who were mostly non-obese, allowing us to demonstrate the relationship between isoflavone intake and subclinical CVD. Study limitations are the cross-sectional design, which precludes making conclusions regarding the direction of cause and effect. Also, we did not assess psychological factors, such as anxiety or depression and their potential impact on the study findings [55,56]. Indeed, emerging evidence suggests the potential contribution of improved psychological health to better population-level cardiovascular health [57]. Another limitation was the impossibility of measuring urine equol because it could modify the association of isoflavone consumption and subclinical CVD. An additional limitation was the use of a semiquantitative FFQ to assess nutrition intake. However, FFQs are still widely used as the primary dietary assessment in research studies [58] and, in the present study, a robust validated FFQ was administered by trained nutritionists who interviewed each participant for approximately 50 min.

In conclusion, the results obtained in the present study show that a higher isoflavone dietary intake was associated with lower risk of subclinical CVD in postmenopausal women, independent of BMI and endogenous oestradiol levels. Considering that IMT status has been proposed as an independent predictor of future cardiovascular events, a higher intake of isoflavones combined with other healthy lifestyle habits in postmenopausal women may have a beneficial effect on the primary prevention of CVD.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.

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All authors were involved in the conception and design of the study and contributed to data collection and analysis. LLF, TRS and PMS drafted the article. All the authors read and approved the final manuscript submitted for publication.

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