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LAÍS LIMA FERREIRA

ASSOCIAÇÃO ENTRE INGESTÃO DIETÉTICA DE ANTIOXIDANTES E DOENÇA CARDIOVASCULAR SUBCLÍNICA EM MULHERES NA PÓS-MENOPAUSA

Porto Alegre, dezembro de 2019.

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Médicas: Endocrinologia da Faculdade de Medicina da Universidade do Rio Grande do Sul como requisito parcial para obtenção do título de Mestra em Endocrinologia

Área de concentração: Endocrinologia, Metabolismo e Nutrição

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RESUMO

Introdução: A transição menopáusica tem sido associada ao aumento do risco de doença cardiovascular (DCV), sendo essa a principal causa de morte nessa população. Evidências sugerem que a produção elevada de espécies reativas de oxigênio (ROS) está envolvida no risco de doenças crônicas. Antioxidantes dietéticos têm sido associados à proteção das células do endotélio vascular contra os danos causados pelas ROS. **Objetivo:** Avaliar a associação entre a presença de DCV subclínica e a ingestão dietética de micronutrientes e polifenóis com ação antioxidante em uma amostra de mulheres pós-menopáusicas sem doença clínica evidente. **Métodos:** Foram arroladas 105 mulheres na pós-menopausa para análise da espessura da camada íntima-média da carótida (C-IMT) em três segmentos e da presença de placas ateromatosas, através de ultrassonografia. A presença de DCV subclínica foi definida como presença de placa e/ou IMT>0,9 mm. A ingestão de micronutrientes e polifenóis foram calculadas através da aplicação de um questionário de frequência alimentar validado. A atividade física habitual foi avaliada com um pedômetro digital por 6 dias. Além disso, também foram avaliados o perfil hormonal e metabólico. **Resultados:** Foram inclusas 96 mulheres, a prevalência de DCV subclínica foi de 35%. A mediana do tempo de menopausa foi de 5,8 (3 – 10) anos, a média de idade de 55,5 ± 5,0 anos e IMC 27,2 ± 5 kg/m^2. Quando as participantes foram estratificadas entre presença ou ausência de DCV subclínica, não houve diferenças na idade, tempo de menopausa, IMC, nível de atividade física, perfil lipídico, glicose e variáveis hormonais. No entanto, o grupo sem DCV subclínica apresentou um maior consumo de micronutrientes com ação antioxidante, como selênio [92,2 (78,8 – 124,8) *vs*. 72,5 (57,3 – 97,6) mcg, p=0,025], magnésio [269,6 (206,5 – 343,5) *vs*. 219,9 (162,1 – 284,2) mg, p=0,047], folato [505,6 (396,1 – 710,1) *vs*. 418,0 (320,6 – 574,7) mcg, p=0,024], vitamina E [4,3 (3,0 – 5.4) *vs.* 3,1 (2,0 – 4,2) mg, p=0,105], polifenóis (3817,7 ± 1914,6 *vs*. 2973,5 ± 1151,5, p=0,881) e isoflavonas [4,9 (2,1 – 9,8) *vs.* 3,5 (2.1 – 4,9) mg, p=0,049] quando comparado ao grupo de mulheres com DCV subclínica, mesmo após ajuste para ingestão calórica. No modelo de regressão multivariado o IMC ≥27,00 kg/m² foi associado a um risco 90% maior de presença de placa e/ou IMT>0,9 mm (*P*=0,017), enquanto maiores níveis de estradiol (*P* =0,004) e ingestão de isoflavonas (*P*=0,021) foram independentemente associados ao menor risco de DCV subclínica. **Conclusão:** Em nossa amostra de mulheres pós-

menopáusicas, uma maior ingestão dietética de isoflavonas foi associada à menor risco de DCV subclínica, independente do IMC e níveis de estradiol.

PALAVRAS-CHAVE: Antioxidantes. Doenças Cardiovasculares. Dieta. Isoflavonas. Polifenóis. Pós-Menopausa.

ABSTRACT

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 aim of our study was to investigate the association between the dietary intake of isoflavones and the presence of subclinical cardiovascular disease (CVD) in postmenopausal women. Ninety-six postmenopausal women (age 55.2±4.9 years, BMI 27.2±4.6 kg/m^2) completed the study protocol. Habitual physical activity was assessed by a digital pedometer, resting metabolic rate was measured by indirect calorimetry and dietary intake was assessed by a validated food frequency questionnaire. Subclinical CVD was defined as carotid artery intima-media thickness (C-IMT) >0.9mm and/or the presence of one or more atherosclerotic plaques in any of the studied segments. Mean 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 was associated with 90% higher risk of having ≥1 plaque and/or C-IMT > 0.9 mm (P=0.017). Higher estradiol levels (P=0.004) and isoflavone intake (P=0.021) were independently associated with lower risk of having subclinical CVD. In the present study, we observed that higher isoflavone dietary intake was associated with lower risk of subclinical CVD in postmenopausal women, independent of BMI, and endogenous estradiol levels.

KEYWORDS: Antioxidants. Cardiovascular Diseases. Diet. Isoflavones. Polyphenols. Postmenopause.

LISTA DE ABREVIATURAS

ApoA apoliproteína-A

ApoB apoliproteína-B

CAC calcificação da artéria coronariana

CBG β-glicosidase citosólica

CC circunferência da cintura

CV cardiovascular

C-IMT espessura das camadas intima-média das carótidas

DCNT doenças crônicas não transmissíveis

DCV doença cardiovascular

DCVSub doença cardiovascular subclínica

DXA densitometria óssea

HDL lipoproteínas de alta densidade

IMC índice de massa corporal

LDL lipoproteínas de baixa densidade

LPH lactase-florizina hidrolase

MDR receptores multidrogas

NO óxido nítrico

PAS pressão arterial sistólica

PAD pressão arterial diastólica

PCR proteína-C reativa

ROS espécies reativas de oxigênio

RR risco relativo

SGLT1 transportador de glicose dependente de sódio

SIM sistema de informação de mortalidade

TRH terapia de reposição hormonal

WHO world health organization

Essa Dissertação de Mestrado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, sendo apresentada em duas partes: Parte I (revisão narrativa) e Parte II (artigo original já publicado):

Parte I: Revisão: Associação entre alterações hormonais, metabólicas, de composição corporal, fatores de estilo vida e risco cardiovascular em mulheres pós-menopáusicas.

Parte II: Artigo Original: *Dietary intake of isoflavones is associated with a lower prevalence of subclinical cardiovascular disease in postmenopausal women: cross-sectional study*. *J Hum Nutr Diet. 2019 Dec;32(6):810-818. DOI: 10.1111/jhn.12683.*

SUMÁRIO

PARTE I

Revisão: Associação entre alterações hormonais, metabólicas, de composição corporal, fatores de estilo vida e risco cardiovascular em mulheres pós-menopáusicas.

No ano de 2016, as doenças crônicas não transmissíveis (DCNT) foram responsáveis por 71% das mortes no mundo, sendo as doenças cardiovasculares (DCV) responsáveis por 41% dos óbitos (WHO, 2018). As causas de morte no Brasil se comportam de formas diferenciadas dependendo do sexo. Conforme os dados do Sistema de Informação de Mortalidade (SIM) de 2017, as doenças do aparelho circulatório lideraram as causas de morte nas mulheres, nas cinco regiões do país, enquanto para os homens as causas de morte variaram entre doenças do aparelho circulatório, causas externas e neoplasias, para todas as idades (BRASIL, 2017).

Nas mulheres brasileiras, os óbitos por DCV aumentam a partir dos 40-49 anos apresentando maiores prevalências acima dos 80 anos (BRASIL, 2017). Esses dados são uma importante questão de saúde de pública, visto que as mulheres representam a maior proporção de idosos (FEITOSA FILHO *et al*., 2019). As DCV são multifatoriais, englobam fatores de risco não modificáveis, como idade, carga genética e sexo, e modificáveis, como tabagismo, alimentação inadequada, consumo excessivo de álcool e sedentarismo (SILVA G. A. *et al*., 2017). Além do risco de DCV imposto pelo próprio processo de envelhecimento, os níveis hormonais parecem apresentar uma importante influência na saúde cardiometabólica das mulheres na transição menopáusica. Sabe-se que o risco de DCV é mais elevado em mulheres na pós-menopausa do que entre as mulheres em idade reprodutiva (SOWERS, LA PIETRA, 1995; IEAMTAIRAT *et al*., 2019). Evidências sugerem que maiores níveis de estradiol estão associados ao menor risco de DCV, conferindo um efeito cardioprotetor (MATURANA *et al.*,

2015; EL KHOUDARY *et al*., 2015; WILDMAN *et al*., 2008).

Em relação aos fatores modificáveis, na meta-análise de Colpani e colaboradores (2018) foi avaliado os fatores de risco de mortalidade por DCV e mortalidade por todas as causas em mulheres de meia idade e idosas, deparando-se com o tabagismo (RR 2,76 [IC 95%: 1,62–4,71]) e a obesidade (RR 2.3 [IC 95%: 1,56–3,40]) associados ao maior

risco de mortalidade por DCV, enquanto a prática da atividade física (RR 0.70 [IC 95%: 0,58–0,84]) e consumo moderado de álcool (RR 0.63 [IC 95%: 0,57–0,71]) foram associados ao menor risco de mortalidade por DCV.

1.1 Alterações hormonais, metabólicas, de composição corporal e o risco cardiovascular na pós-menopausa

A menopausa é definida como a ausência permanente das menstruações em consequência do processo fisiológico natural de diminuição da função folicular ovariana ou remoção cirúrgica dos ovários (SOWERS, LA PIETRA, 1995; FERNANDES *et al*., 2008). A menopausa, quando por processo fisiológico natural, acorre em média aos 46-52 anos de idade (SOWERS, LA PIETRA, 1995; SCHOENAKER *et al*., 2014). Ainda, pode ser classificada em pós-menopausa recente, quando se passam os primeiros 3-4 anos depois do último sangramento menstrual, e pós-menopausa tardia, que se refere aos anos posteriores (HARLOW *et al*., 2012).

O marco do período pós-menopáusico é caracterizado pelas modificações hormonais, principalmente a deficiência de estrogênio (SOWERS, LA PIETRA, 1995; CLARKSON, 2007; COYOY; GUERRA-ARAIZA; CAMACHO-ARROYO, 2016), que causam impacto clínico variável entre as mulheres de diferentes etnias devido à influência de fatores socioculturais e psicológicos (OBERMEYER; REHER; SALIBA, 2007). Menores níveis de estrogênio em mulheres jovens (18–40 anos) já foi relacionado ao maior risco de DCV e menopausa precoce (<40 anos) (MORSELLI *et al*., 2017). Mulheres que realizaram ovariectomia bilateral e não receberam terapia de reposição hormonal (TRH) tiveram um risco aumentado de multimorbidades (ROCCA *et al*, 2016), incluindo DCV (SOHRABJI; OKOREEH; PANTA, 2019).

A menopausa está associada à alterações cardiovasculares pré-clínicas, como concentrações aumentadas de lipídeos circulantes (MATTHEWS *et al*., 2009), disfunção endotelial (CABRERA-REGO *et al*., 2017) e modificações na distribuição de tecido adiposo (AMBIKAIRAJAH *et al*., 2019) e muscular (GREENDALE *et al*., 2019), é caracterizada pela diminuição da síntese de óxido nítrico (NO), oxidação local das lipoproteínas circulantes e invasão dessas últimas na parede vascular (DAVIGNON; GANZ, 2004). Dados da coorte americana, Study of Women’s Health Across the Nation

(SWAN), demonstram aumento dos níveis de colesterol total, lipoproteínas de baixa densidade (LDL) e apoliproteína-B (ApoB) um ano da cessação das menstruações. As concentrações de lipoproteínas de alta densidade (HDL) e apoliproteína-A (ApoA) foram alteradas após a menopausa (EL KHOUDARY *et al*., 2019). Na mesma coorte, dados obtidos por absorciometria de raios X de dupla energia (DXA), avaliando 1.246 mulheres de diferentes etnias (brancas, negras, chinesas e japonesas), foi demonstrado um ganho médio de 6% de massa de gordura, representando 1,6 kg, ao longo de 3,5 anos durante a transição menopáusica, enquanto uma redução total na massa muscular de 0,5% (0,2 kg) durante a transição menopáusica foi observada (GREENDALE *et al*., 2019). Em uma recente meta-análise de Ambikairajah e colaboradores (2019) foi encontrado que durante a transição menopáusica há um aumento na quantidade de tecido adiposo corporal de 5,49% na região superior do corpo (IC 95% 3,91 – 7,06). No estudo de coorte de Chen e colaboradores (2019), 2683 mulheres pós-menopáusicas com IMC entre 18,5 e 25 kg/m² e sem DCV no início do estudo foram avaliadas durante 17,9 anos. Os resultados obtidos por DXA demonstraram que a combinação de maiores concentrações de gordura corporal na região superior do corpo e menores concentrações de gordura na região inferior do corpo conferiram um risco de DCV três vezes maior do que naquelas com outros perfis de distribuição de gordura corporal.

Fatores relacionados ao estilo de vida, como dieta e atividade física, podem influenciar a saúde cardiometabólica e composição corporal das mulheres na pós-menopausa. Estudos prévios do nosso grupo de pesquisa demostraram que um maior consumo de proteínas (SILVA T. R. *et al*., 2017), bem como maior adesão à dieta do Mediterrâneo (SILVA T. R., SPRITZER, 2019) foram associados a um melhor perfil cardiometabólico e de composição corporal em mulheres na pós-menopausa.

1.2 Desenvolvimento da aterosclerose e métodos de diagnóstico

O desenvolvimento da aterosclerose se inicia a partir de um processo inflamatório crônico (STEVEN *et al*., 2019) caracterizado pelo acúmulo de lipídeos (ApoB e LDL) no lúmen dos vasos sanguíneos, seguido de uma resposta imunológica a esta lesão (MOSS, RAMJI, 2016). Os monócitos deslocam-se até a íntima da artéria, modificam as moléculas de LDL e, em seguida se diferenciam em macrófagos, tornando-se uma célula espumosa que quando se acumula forma estrias gordurosas. As células espumosas podem sofrer apoptose ou necrose, formando um núcleo necrótico rico em lipídeos que continua a estimular o processo pró-inflamatório. As espécies reativas de oxigênio (ROS) são produzidas, fisiologicamente, nas paredes das artérias com a função de sinalização celular, no entanto, em quantidades exacerbadas as ROS são associadas ao envelhecimento celular (ASSAR; ANGULO; RODRÍGUEZ-MAÑAS, 2013). A soma das ROS de origem mitocondrial e celular contribui significativamente para desregulação da produção de óxido nítrico, logo também da função vascular (STEVEN *et al*., 2019).

A DCV subclínica (DCVSub) corresponde ao desenvolvimento aterosclerótico em que ainda não houve sintomatologia da doença cardiovascular. A DCVSub pode ser estimada pela espessura da camada íntima-média das carótidas (C-IMT), local em que há o desenvolvimento de placas ateroscleróticas (NAMBI *et al*., 2010). Existem diferentes métodos não invasivos de diagnóstico, tais como ultrassom modo B, capaz de acessar a dilatação mediada pelo fluxo da artéria braquial, C-IMT e placas carótidas, onde o escore de calcificação da artéria coronariana (CAC) pode ser acessado por tomografia com feixe de elétrons ou tomografia computadorizada multislice (PETERS *et al*., 2012). A combinação da mensuração da C-IMT com a placa carótida parece melhorar a predição para DCV isquêmica em comparação a estas medidas isoladas (PETERS *et al*., 2012). Em um trabalho prévio do nosso grupo de pesquisa foram utilizadas as medidas da C-IMT e CAC para avaliar a prevalência de DCVSub e sua associação com variáveis clínicas e hormonais em 97 mulheres pós-menopáusicas. A prevalência de DCVSub na amostra foi de 35%, ainda a espessura da C-IMT foi positivamente associada à idade e negativamente associada aos níveis endógenos de estradiol (MATURANA *et al*., 2015).

1.3 Associações entre dieta e risco cardiovascular

Diversos estudos epidemiológicos descrevem o papel da dieta como protetor à DCV nas mulheres (STAMPFERM, 2000; KNOOPS *et al*., 2004; AKESSON *et al*., 2007; WANG *et al*., 2018). Sabe-se que padrões alimentares do tipo ocidental, caracterizados por alto consumo de cereais refinados, gorduras saturadas, sal e baixo consumo de fibras, vitaminas e minerais, estão associados à maior inflamação (KORAKAS *et al*., 2018; CASAS *et al*., 2018; FABIANI; NALDINI; CHIAVARINI, 2019), enquanto o padrão alimentar estilo dieta Mediterrânea, rico em vegetais, frutas, castanhas e azeite de oliva têm demonstrado associação protetora à DCV (KORAKAS *et al*., 2018; CASAS *et al*., 2018, ESTRUCH *et al*., 2018, GALBETE *et al*., 2018). Revisões sistemáticas já demonstraram que há associação positiva entre um maior consumo de frutas e vegetais e menor risco de mortalidade por todas as causas (WANG *et al*., 2014; AUNE *et al*., 2017; BLEKKENHORST *et al*., 2018). Atualmente, estudos sugerem que o consumo de compostos bioativos pode estar associado à redução de risco CV, através de ação antioxidante (LEE *et al*., 2005; ADLERCREUTZ, 2007; KIM, JE, 2017; BLEKKENHORST *et al*., 2018). Entre as vitaminas, minerais e compostos bioativos com ação antioxidante se destacam os polifenóis (TORRES *et al*., 2015; CASAS *et al*., 2018), as vitaminas C e E (LEE *et al*., 2005; CASAS *et al*., 2018), beta-caroteno (TRIBBLE, 1999), licopeno (RISSANEN *et al*., 2000; CASAS *et al*., 2018), zinco, selênio e magnésio (CASAS *et al*., 2018).

No recente estudo da coorte americana SWAN, avaliando fatores de estilo de vida de mulheres durante a meia idade, houve uma associação inversa significativa da C-IMT com o escore estilo de vida saudável (WANG *et al*., 2018). Blekkenhorst e colaboradores (2018) mostraram que um consumo maior do que três porções de vegetais por dia (uma porção equivalente a 75g), em relação aos que consumiram menos que duas porções por dia, apresentaram a espessura média da C-IMT ≈0,036 mm (4,6%) menor.

1.3.1 Compostos polifenólicos: conceito e fontes alimentares

Existem mais de oito mil tipos de compostos polifenólicos identificados até o momento, classificados em diferentes subgrupos. São compostos bioativos naturais (BRAVO, 1998) que podem ser encontrados, principalmente, em fontes vegetais. Dentre suas principais funções para as plantas, os polifenóis conferem fotoproteção, defesa contra microrganismos e insetos, e pigmentação, assim como características organolépticas (BRAVO, 1998).

Os compostos polifenólicos estão presentes em hortaliças, frutas, cereais, chás e café, cacau, vinho, leguminosas (COZZOLINO, 2016; ROTHWELL *et al*., 2013) e tsmbém podem ser encontrados no mel (UNITED STATE OF AMERICA, 2018). O seu teor nos vegetais depende do tipo de cultivo, germinação de sementes, solo, fatores genéticos, meio ambiente, processamento e estocagem (BRAVO, 1998; COSTA, ROSA, 2016). O descascamento e o processo de cocção dos alimentos também podem alterar o teor de polifenóis (COZZOLINO, 2016; RANILLA; GENOVESE; LAJOLO, 2009). Não há valor estabelecido para o consumo diário de polifenóis (INSTITUTE OF MEDICINE, 2000), principalmente pela carência de estudos sobre sua biodisponibilidade, dose-efeito e níveis de toxicidade (WILLIAMSON, HOLST, 2008).

Os polifenóis possuem uma estrutura básica composta por, no mínimo, um anel aromático, podendo conter uma hidroxila, formando um fenol. A vasta quantidade de compostos polifenólicos se resulta da sua diversidade estrutural, visto que pode sofrer ligação de diferentes moléculas, como ácidos carboxílicos, aminas, lipídeos e resíduos de diferentes tipos de sacarídeos, e conter mais de um anel aromático (BRAVO, 1998). As estruturas dos polifenóis podem ser divididas em agliconas e glicosídeos (DEL RIO *et al*., 2013; MARÍN *et al*., 2015; COSTA, ROSA, 2016), as quais são de crucial importância o entendimento a fim de compreender a absorção desses compostos no organismo. A estrutura do tipo aglicona corresponde a um polifenol sem a presença da molécula de carboidrato acoplada a sua estrutura, a qual pode ser diretamente absorvida pelo intestino delgado por transporte passivo ou ainda utilizando transportadores multidrogas (MDR1, MDR2, MDR3); algumas subclasses que apresentam esse tipo de estrutura são flavonóis, flavonas, isoflavonas e antocianinas (DEL RIO *et al*., 2013;

MARÍN *et al*., 2015; COSTA, ROSA, 2016; COZZOLINO, 2016). Já as estruturas do tipo glicosídeo possuem glicídio na sua composição que pode ser submetido à ação de hidrólise por enzimas intestinais lactase-florizina hidrolase (LPH), localizada na borda em escova do enterócito, e pela β-glicosidase citosólica (CBG) localizada no interior da célula enterócita, ou pela enzima bacteriana α-ramnosidase no cólon, dando origem a estrutura aglicona (DEL RIO *et al*., 2013; MARÍN *et al*., 2015; COSTA, ROSA, 2016); o processo de absorção pode ser por difusão facilitada através de transportador de glicose dependente de sódio (SGLT1) ou transporte passivo. Quando absorvidas, essas estruturas sofrem processo de conjugação nos hepatócitos, formando metabólitos ativos (DEL RIO *et al*., 2013; MARÍN *et al*., 2015; COSTA, ROSA, 2016; COZZOLINO, 2016). A microbiota intestinal influencia o processo de absorção dos polifenóis (MARÍN *et al*., 2015) e, da mesma forma, o consumo dos polifenóis parece modular a composição da microbiota, diminuindo a razão *Firmicutes*/*Bacteroidetes* e a quantidade de E*nterobacter*, enquanto pode aumentar *Lactobacillus* e *Bifidobacterium* (SHAOLING *et al*., 2019).

1.3.2 Associações entre compostos polifenólicos e risco cardiovascular

Numerosos trabalhos, em sua maioria *in vitro*, evidenciam diferentes efeitos biológicos dos polifenóis, principalmente pelas vias antioxidante e anti-inflamatória (YAHFOUFI, 2018), apresentando uma variedade de mecanismos de ação (OLIVEIRA; TAVARES; DAL BOSCO, 2015; COZZOLINO, 2016; DEL BO, 2019).

Dentre seus efeitos conhecidos na saúde está a ação como um potente inibidor do processo de oxidação de LDL, além de sua ação anti-plaquetária e anti-inflamatória com capacidade de melhorar a função endotelial e aumentar as concentrações circulantes de HDL (HERNÁEZ *et al*., 2014). No estudo de Medina-Remón *et al*. (2017), avaliando 1139 homens e mulheres, foi observado que um maior consumo de polifenóis, por meio da dieta mediterrânea, teve associação aos níveis diminuídos de biomarcadores inflamatórios e uma melhora em alguns fatores de risco cardiovascular, como o colesterol LDL e pressão arterial. George *et al*. (2018), avaliando o efeito de compostos polifenólicos presentes no azeite de oliva extra virgem em fatores de risco CV em sua meta-análise, encontrou que o consumo de azeites com maiores quantidades

de polifenóis (366,0 – 753,0 mg/kg de azeite) foi associado à redução de estresse oxidativo através da alteração das concentrações de malondialdeído (MD: -0,07 µmol/L [IC 95%: -0,12; -0,02 µMOL/l]; I^2: 88%, p=0,004]) e do LDL oxidado (SMD: -0,44 [IC 95%: -0,78; -0,10 µmol/L]; I^2: 41%, p=0,01]) em comparação ao consumo de azeites com menores teores de polifenóis (2,7 – 132,0 mg/kg de azeite).

Alguns trabalhos investigaram a associação de polifenóis com DCVsub em mulheres de meia-idade ou idosas. Os resultados das evidências não são conclusivos, com alguns trabalhos demonstrando uma associação inversa entre o maior consumo dietético de polifenóis e menor espessura da C-IMT (ZHANG *et al*., 2008; KWOK *et al*., 2014; BONDONNO *et al*., 2017; BLEKKENHORST *et al*., 2018) e outros nenhum resultado significativo (LI *et al*., 2013; KIRICHENKO *et al*., 2017).

1.3.3 Isoflavonas

Os flavonoides são a subclasse de polifenóis mais pesquisada e conhecida (BRAVO, 1998; COSTA, ROSA, 2016). Entre os tipos de flavonoides encontramos os isoflavonóides (BRAVO, 1998), compostos que conferem uma estrutura básica de dois anéis benzênicos ligados a uma pirona heterocíclica (YUAN; WANG; LIU, 2007) que, dependendo das ligações que realiza, pode conferir diferentes tipos de isoflavonas, como a daidzeína, genisteína, gliciteína, formononetina e biocanina A (KO, 2014). A quantidade de isoflavonas referidas nos alimentos geralmente é apresentada pela soma dos seus diferentes tipos (KO, 2014). Devido a sua conformação estrutural ser semelhante ao 17-β-estradiol é conhecida como um fitoestrógeno (KO, 2014). As isoflavonas podem ser encontradas em diversos vegetais, como kudzu, tremoceiro, cevada, feijão e soja, em maiores quantidades e, em menores quantidades, no trevo vermelho, alfafa e linhaça (KO, 2014).

Em um estudo recente de Xu e colaboradores (2019), a administração de genisteína foi capaz de inibir a síntese da proteína-C reativa (PCR) e da metaloproteinase-9, estimuladas pela angiotensina II, nas células do músculo liso vascular de ratos sendo tempo e dose dependentes. No estudo de Kim e colegas (2018), a atividade de um metabólito da dadzeína diminuiu a expressão gênica de substâncias pró-inflamatórias, como iNOS, COX-2 e TNF-α, através da regulação da atividade do

fator de transcrição NF-κB. Nachvak e colegas (2019) encontraram em sua meta-análise com 23 estudos prospectivos, que inclui 330.826 participantes, que um maior consumo de isoflavonas através da dieta foi associado ao risco 10% menor de mortalidade por qualquer causa. Sathyapalan *et al*. (2018) investigaram a suplementação de 15 g de proteína soja, contendo 66 mg de isoflavonas (SPI) *versus* 15 g de proteína de soja sem isoflavonas (SP), em 200 mulheres na pós-menopausa recente, durante 6 meses, e os resultados observados foram de redução nos parâmetros de risco cardiometabólicos somente no grupo SPI. Poucos trabalhos averiguaram a associação entre a ingestão dietética de isoflavonas e a presença de DCVsub (CHAN *et al*., 2007; CAI *et al*., 2012). No estudo de Chan e colaboradores (2007) com 126 participantes com média de idade de 66 anos (69% do sexo masculino) e de alto risco de eventos cardiovasculares, foi observada uma ingestão média de isoflavona de 5,5 (2,2–13,3) mg/dia. Uma maior ingestão de isoflavona (> 13,3 mg/d) reduziu em 0,17 mm (14,5%) a espessura da C-IMT máxima média (*P*=0,04). Já no estudo de Cai *et al*. (2012), incluindo 572 adultos saudáveis (40-65 anos), uma maior ingestão de isoflavona (>5,4 mg/dia) teve associação à uma menor C-IMT (16,2 %, *P*= 0·035).

Apesar de a estrutura das isoflavonas se assemelharem ao estradiol, apresentam uma afinidade 100 vezes mais fraca com os seus receptores (KO, 2014). A conformação natural das isoflavonas é glicosídica, sendo assim é biologicamente inativa e somente irá exercer sua atividade biológica quando transformada em aglicona (DEL RIO *et al*., 2013; MARÍN *et al*., 2015; COSTA, ROSA, 2016). O processo de fermentação da soja pode alterar as concentrações de isoflavonas biologicamente ativas, isto porque a fermentação possui micro-organismos que contêm a enzima β-glicosidase. Assim, os alimentos fermentados são as maiores fontes de agliconas livres, quando comparado a outros alimentos à base de soja, principalmente aqueles ultraprocessados (KO, 2014). O processo digestivo das isoflavonas depende da ação de enzimas glicosidases intestinais que hidrolisam a molécula, resultando em um produto aglicona, como a dadzeína, genisteína, gliciteína (KO, 2014; COSTA, ROSA, 2016; COZZOLINO, 2016), podendo ser então absorvidos ou então metabolizados pela microbiota intestinal levando a produção de equol (SHOR *et al*., 2012). O efeito cardioprotetor relacionado às isoflavonas parece ser em função da capacidade de produção do metabolito com ação estrogênica mais forte, o equol (SETCHELL; BROWN; LYDEKING-OLSEN, 2002; SHOR *et al*., 2012; HAZIM *et al*., 2016; YUAN; WANG; LIU, 2007). A capacidade de

produção de equol varia entre os indivíduos, aqueles com uma produção de acima de 83 nmol/L (20 µg/L) são considerados produtores de equol. Yuan e colaboradores (2007) observaram que um maior consumo de isoflavonas foi associado à uma menor C-IMT nos indivíduos produtores de equol, mas esse efeito não foi encontrado nos não produtores (CAI *et al*., 2012). Uma melhora na rigidez arterial de homens também foi encontrada, mas apenas naqueles produtores de equol (HAZIM *et al*., 2016). Em uma amostra de 58 mulheres pós-menopáusicas, as produtoras de equol apresentaram, de forma significativa, uma microbiota mais diversa (YOSHIKATA *et al*., 2019). É interessante a observação de que as bactérias intestinais que convertem daidzeína em equol podem ser encontradas não somente nos produtores de equol, como ainda nos não produtores (YOSHIKATA *et al*., 2019; IINO *et al*., 2019). A diversidade microbiana talvez seja mais importante para a produção do equol do que as quantidades da enzima conversora, assim como bons hábitos de vida e um consumo diverso de grupos alimentares (YOSHIKATA *et al*., 2019).

Considerando a expectativa de vida das mulheres de em média 80 anos, 40% de suas vidas serão na fase pós-menopáusica (AMBIKAIRAJAH *et al*., 2019). Conforme descrito anteriormente, a menopausa é caracterizada por mudanças hormonais, metabólicas e de composição corporal que favorecem um maior risco de DCV, sendo essa a principal causa de óbitos nessa população. A aterosclerose, que precede os eventos cardiovasculares, acontece de maneira silenciosa ao longo de décadas (NAMBI *et al*., 2010). Portanto, métodos de diagnóstico de alterações subclínicas são importantes para a prevenção adequada dessas doenças. A alimentação possui um papel importante na prevenção das DCV e, dessa forma, avaliar como o padrão de dieta, bem como nutrientes e compostos antioxidantes específicos podem influenciar a presença de DCV subclínica é extremamente relevante nessa população.

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PARTE II

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

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

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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 aim of our study was to investigate the association between the dietary intake of isoflavones and the presence of subclinical cardiovascular disease (CVD) in postmenopausal women.

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

Results: Mean 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 was associated with 90% higher risk of having ≥1 plaque and/or C-IMT > 0.9 mm (P=0.017). Higher estradiol levels (P=0.004) and isoflavone intake (P=0.021) were independently associated with lower risk of having subclinical CVD.

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

INTRODUCTION

Menopause has been associated with increased risk of cardiovascular disease (CVD), possibly due to changing hormonal status and aging [1, 2]. Atherosclerosis, which underlies the occurrence of cardiovascular events, develops over decades and has a prolonged asymptomatic phase [3]. Some noninvasive procedures are able to detect and measure different stages of atherosclerosis, such as carotid artery intima-media thickness (C-IMT), which is a noninvasive ultrasound measurement of the 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 diadzein) and lignans are dietary-derived polyphenols, and the most common phytoestrogens. While 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 due to their anti-inflammatory properties [16, 17], and their impacts on endothelial function [18-20]. Isoflavones have also been reported to exert weak estrogenic activity by binding to estrogen receptors (ER) [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 aim of this study was 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.

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 as follows: 1) 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; 2) age between 45 and 65 years; and 3) no use of hormone therapy in the past 3 months. Individuals with diabetes or 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 in kilograms divided by the height in meters 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 (in g or ml) according to the standard portion sizes used in the FFQ. 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 according to 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-hour fast. All samples were obtained between 8 and 10 AM. FSH was measured with chemiluminescent immunoassays (Centaur XP, Roche Diagnostics, Mannheim, Germany) with a sensitivity of 0.3 IU/L. The intra-assay and interassay coefficients of variation were 2.9% and 2.7%, respectively. Estradiol was measured by electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics, Mannheim, Germany), with an assay sensitivity of 5.0 pg/mL 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, Mannheim, Germany), where SHBG had a sensitivity of 0.02 nmol/L and intra-assay and interassay CVs of 5.3% and 6.6%, respectively.

Assessment of subclinical CVD

Measurement of C-IMT was assessed bilaterally with B-mode ultrasonography (Xsario, Toshiba) by a single expert sonographer operator using a standardized 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 mean ± standard deviation (SD) or median and 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, the 2-tailed Student's *t* test was used. Chi square 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 2-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 the Statistical Package for the Social Sciences 19.0 (SPSS, Chicago, IL, USA). Data were considered to be significant at $p \leq 0.05$.

RESULTS

Of one hundred and nineteen postmenopausal women enrolled in the study, ninety-six participants fulfilling all the inclusion criteria completed the study protocol. Eighteen candidates were excluded (5 with diabetes, 1 with hyperthyroidism, 2 with untreated hypothyroidism, 2 with breast cancer, 1 who was premenopausal, and 7 current smokers). An additional five participants dropped out because they were unable to commit to the study (no time for blood collection, DXA and indirect calorimetry). The mean 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. Characteristics of the postmenopausal women according to carotid intima-media thickness status are shown in table 1. The groups were similar regarding age, time since 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 behavior. However, the frequency of hypertension was higher in women with subclinical CVD (56% *vs* 32%, *P*=0.018). Estradiol 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 hypertension were related to the presence of subclinical CVD (PR: 1.83; 95% 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 fiber, ≥21g, according Dietary Reference Intakes (DRIs) [33], (PR: 0.58; 95% CI: 0.34–0.97), selenium, ≥55µg, according DRIs, (PR: 0.54; 95% CI: 0.30–0.98) and isoflavones, ≥4.9mg, 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 estradiol 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, estradiol (per 1 pg/ml increase), and higher isoflavone intake were independently associated with 3% (*P*=0.004), and 50% (*P*=0.021) lower risk of having subclinical CVD, respectively.

DISCUSSION

In the present study, we observed that higher isoflavone dietary intake and endogenous estradiol levels were independently associated with 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, 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 was observed, and a higher isoflavone intake predicted an absolute 0.17 mm decrease in mean maximum C-IMT [32]. In the other study of 572 healthy adults

(aged 40–65 years), higher intake of isoflavone (>5.4 mg/day) was associated with lower C-IMT [34]. However, the isoflavone intake in occidental countries seems to be much lower than that in comparison with Asian countries, with most people eating on average less than 1 mg/d compared to 20-50 mg/d or higher, respectively [35]. In the present study, the median isoflavone intake was 4.9 (2.1-5.1) mg/day, and less than this amount was consumed by women with subclinical CVD. Additionally, while in most studies the consumption of soy products was the main source of isoflavones, in our 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].

During the menopausal transition, changes in endogenous estradiol levels may impact the vulnerability of the vessels in the postmenopausal period. In the present study, although endogenous estradiol 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 estradiol levels was associated with 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 estradiol was associated with an increase in C-IMT, independent of blood pressure, BMI, lipids and other covariates [38]. Increased C-IMT prevalence has also been associated with postmenopausal status [7,39]. However, menopause, aging, and an increase in CVD risk occur somewhat synchronously, and cross-sectional studies are unable to elucidate which factor exerts the main role on C-IMT changes.

Two clinical trials evaluated isoflavone supplementation, in women who were <5 years since menopause [40], or more than 5 years since 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 estrogenic activity through binding to ER and were postulated to improve endothelial function by acting on nitric oxide release by endothelial cells[42][43]. In fact, a meta-analysis of randomized 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 are not confined, to estrogen modulation only, but also by anti-inflammatory and antioxidant properties. A recent

study suggests that the anti-inflammatory properties of the isoflavone genistein plays a beneficial role in the cardiovascular system through the inhibition of angiotensin II-stimulated C-reactive protein and matrix metalloproteinase-9 expressions in vascular smooth muscle cells [16]. Besides that, isoflavones are polyphenols derived from the diet, and recently interest in food phenolics has greatly increased due to their antioxidant capacity (free radical scavenging and 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 recognized to exert a protective effect against atherogenesis and CVD [45]. Similarly, a systematic review showed that high intakes and/or circulatory levels of magnesium, and 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 $\geq$ 0.9 mm. Isoflavones seem to be more effective than antioxidant vitamins and minerals in the scavenging of reactive oxygen species and lipid peroxidation [22, 23]. Taken together, data from the literature and our present results 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 estrogenic activity.

Besides, some studies have pointed out that positive cardiovascular effects of isoflavones may be a function of the ability to produce equol, an active metabolite, produced by gut microbiota [47] [48] [49], and presenting affinity for estrogen receptors, antiandrogenic properties, and good antioxidant activity [50]. Indeed, equol-producing ability varies greatly among individuals and higher dietary isoflavone consumption was associated with lower IMT in equol producers, but not in equol non-producers, in a study with 572 healthy Chinese adults [34]. Interestingly, recent reports suggest that the equol production status might be associated with dietary isoflavones intake and healthy diet pattern, by increasing gut microbiome diversity [51] [52]. While our participants with higher isoflavone dietary intake could present a better gut microbiome profile, the equol

producing ability could not be assessed in this study due to 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 age, 63.1±7.7 years old) living in the Mediterranean region, 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 and colleagues (2018)[(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, defined according to 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 show the relationship between isoflavone intake and subclinical CVD. Limitations are the cross-sectional design, which precludes 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 suggest the potential contribution of improved psychological health to better population-level cardiovascular health[(57)]. Another limitation was the impossibility of measuring urine equol, since 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 this study, a robust validated FFQ was administered by trained nutritionists who interviewed each participant for approximately 50 minutes.

In conclusion, our results showed that higher isoflavone dietary intake was associated with lower risk of subclinical CVD in postmenopausal women, independent of BMI and endogenous estradiol 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 Statement.

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.

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

Variables	Subclinical cardiovascular disease		*P*
	No plaque and C-IMT ≤ 0.9 mm	**≥1 plaque and/or C-IMT > 0.9 mm**	
n	62	34	
Age, years	54.7 ± 4.7	56.5 ± 5.2	0.095
Years of schooling[a]	8.5 (5.0 – 12.7)	7.5 (4.7 – 11)	0.147
White, n (%)[b]	53 (85)	30 (88)	0.706
Time since menopause, years[a]	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/m²	27.2 ± 5.1	27.3 ± 4.2	0.904
Obesity, n (%)[b]	10 (16)	10 (29)	0.125
Overweight and obesity, n (%)[b]	39 (63)	23 (68)	0.642
Mean steps/day	6111.0 ± 3007.9	5471.1 ± 2840.7	0.312
RMR, kJ/day (kcal/day)	5264.7 ± 920.9 (1258.3 ± 220.1)	5286.5 ± 688.7 (1263.5 ± 164.6)	0.904
Hypertension, n (%)[b]	19 (31)	19 (56)	**0.018**
Previous smoking behavior, n (%)[b]	20 (32.2)	15 (44.1)	0.248
Hormonal variables:			
Estradiol, pg/mL[a]	21.1 (10.8 – 30.3)	19.2 (12.5 – 23.8)	0.252
FSH, IU/L	80.5 ± 29.2	86.8 ± 28.8	0.310
SHBG, nmol/L[a]	48.2 (34.0 – 61.7)	43.1 (36.5 – 58.8)	0.610
Previous hormone therapy, n (%)[b]	18 (29)	13 (38)	0.371

CIMT, carotid intima-media thickness; BMI, body mass index; RMR, resting metabolic rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low density protein; HDL, high density protein; FSH, follicle stimulating hormone; SHBG, sex hormone-binding globulin.
Student's *t* test
[a] Variables analyzed after log transformation
[b] χ^2 test.

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*** [b]
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	
Glycemic 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[a]	227.3 (161.3 – 306.0)	150.1 (124.7 – 230.5)	0.177	
Fiber, g[a]	27.5 (20.9 – 38.8)	24.7 (17.1 – 35.3)	0.070	
Vitamin B12, µg[a]	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[a]	**92.2 (78.8 – 124.8)**	**72.5 (57.3 – 97.6)**	**0.008**	**0.025**
Magnesium, mg[a]	**269.6 (206.5 – 343.5)**	**219.9 (162.1 – 284.2)**	**0.013**	**0.047**
Zinc, mg[a]	8.7 ± 3.4	7.3 ± 2.4	0.033	0.872
Folate, µg[a]	**505.6 (396.1 – 710.1)**	**418.0 (320.6 – 574.7)**	**0.031**	**0.024**
Vitamin D, mg[a]	4.6 (2.6 – 10.6)	4.0 (1.9 – 10.5)	0.705	
Vitamin E, mg[a]	4.3 (3.0 – 5.4)	3.1 (2.0 – 4.2)	**0.047**	0.105
Vitamin C, mg[a]	209.6 (122.5 – 373.2)	133.1 (106.8 – 253.8)	0.194	
Vitamin A, µg[a]	962.5 (556.3 – 1330.9)	690.6 (326.7 – 1392.7)	0.499	
Alcohol, g[a]	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[a]	**4.9 (2.1 – 9.8)**	**3.5 (2.1 – 4.9)**	**0.019**	**0.049**
Lignans, mg[a]	29.9 (12.1 – 54.4)	20.4 (6.4 – 39.7)	0.831	

CIMT, carotid intima-media thickness.
[a] Variables analyzed after log transformation, Student's *t* test.
[b] Adjusted for energy intake

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.)	
	≥ 55	1.37 (0.77 – 2.43)	0.287
Time since menopause, years	< 5	(ref.)	
	≥ 5	1.21 (0.70 – 2.10)	0.485
Previous hormone therapy	No	(ref.)	
	Yes	1.30 (0.75 – 2.23)	0.347
BMI, kg/m² [b]	< 27	(ref.)	
	≥ 27	1.83 (1.06 – 3.19)	**0.031**
Estradiol, pg/ml		0.98 (0.95 – 1.00)	0.067
Hypertension	No	(ref.)	
	Yes	1.93 (1.13 – 3.32)	**0.017**
Fiber, g[a]	< 21.0	(ref.)	
	≥ 21.0	0.58 (0.34 – 0.97)	**0.038**
Selenium, µg[a]	< 55.0	(ref.)	
	≥ 55.0	0.54 (0.30 – 0.98)	**0.042**
Magnesium, mg[a]	< 320.0	(ref.)	
	≥ 320.0	0.46 (0.20 – 1.07)	0.072
Folate, µg[a]	< 400.0	(ref.)	
	≥ 400.0	0.65 (0.38 – 1.10)	0.110
Polyphenol intake, mg[b]	< 3073.6	(ref.)	
	≥ 3073.6	0.57 (0.32 – 1.01)	0.056
Isoflavones intake, mg[b]	< 4.9	(ref.)	
	≥ 4.9	0.52 (0.30 – 0.92)	**0.024**

PR, prevalence ratio; BMI, body mass index.

Each model (each block) was evaluated independently. Unadjusted models were used. Determinants were identified on the basis of a backward

prevalence ratio model. Estradiol was considered a continuous variable. The other variables are categorical.

[a] Dietary Reference Intakes (DRIs)[33].

[b] 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 >0.9mm

Factors	PR (95% CI)	*P* [a]
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
Estradiol, pg/ml	0.97 (0.95 – 0.99)	**0.004**
Fiber, g[a]	0.78 (0.40 – 1.50)	0.778
Selenium, ≥ 55.0 µg[a]	0.76 (0.37 – 1.55)	0.450
Isoflavones, ≥ 4.9 mg[b]	0.50 (0.27 – 0.90)	**0.021**

PR, prevalence ratio; CI, confidence interval.
[a] Multivariate adjusted Poisson regression model with robust estimate.
Age and estradiol were considered continuous variables. The others variables are categorical.
[a] Dietary Reference Intakes (DRIs)[33].
[b] Defined as the median of participants in this sample