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ORIGINAL PAPER

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EQUOL-PRODUCING ABILITY OF POLISH POSTMENOPAUSAL WOMEN AND THE DIETARY DETERMINANTS OF S-(-) EQUOL FORMATION

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ABSTRACT

Background. Equol (Eq) is the most potent soy isoflavone metabolite but individual people differ in their ability to produce it. It has been highlighted that some dietary factors may play a role in Eq formation. This study examines the hypothesis that there are specific dietary factors that determine the ability to produce Eq in Polish postmenopausal women. Moreover, Eq-producing phenotypes were also identified among. Thirty-three healthy, non-smoking Polish postmenopausal women aged 48–68 years were recruited. The women were asked to take soy isoflavone (100 mg/day) for three consecutive days. Mean daily intakes of energy and nutrients were estimated from 3-day food records. After dietary intervention, 24-hour urine samples were collected. Urinary isoflavones and their metabolites were measured using liquid chromatography with the tandem mass spectrometry method. Eq producers were defined using the cut-off of log10-transformed urinary Eq: daidzein (De) ratios > −1.75. 43.75% of participants excreted Eq.

Results. The mean urine Eq excretion ±standard error, corrected for urinary creatinine (Cr), was 41.1 ±9.6 μmol/g-Cr in the Eq producers and 0.2 ±0.07 μmol/g-Cr in the nonproducers. Multivariable logistic regression analysis showed that participants who reported higher pulses intake were more likely to be Eq producers after adjusting for age, BMI and years after menopause (odds ratio: OR = 1.109, 95% CI: 1.033; 1.190; $p = 0.004$).

Conclusions. In summary, higher pulses intake was associated with the ability to produce Eq among Polish postmenopausal women.

Keywords: equol phenotype, postmenopausal women, dietary factors, pulses

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INTRODUCTION

Soy foods contain bioactive compounds such as isoflavones [genistein (Ge), daidzein (De), and glycitein (Gle)] (Bustamante-Rangel et al., 2018). It is already known that regular intake of soy isoflavones reduces menopause symptoms, improves lipid profile and cognitive function in postmenopausal women (Mayo et al., 2019; Shrode et al., 2022). However, soy isoflavones may benefit some but not all menopausal women. The ability to produce equol (Eq) may be the major determinant of its effectiveness (Daily et al., 2019), although the existing data in this field are ambiguous and potentially associated with study design (Barnard et al., 2023). Therefore, increasing the ability to produce Eq may potentially confer protection against breast cancer, cardiovascular disease, bone disease and menopausal symptoms (Crawford et al., 2013; Magee, 2011). Eq is produced from the daidzein by the gut bacteria of humans and animals (Mayo et al., 2019). Although almost every animal species produces Eq when fed soy foods, human daidzein metabolism differs between individuals, thus contributing to variations in the isoflavone profiles (Hong et al., 2012). De-metabolizing phenotypes are divided into two groups: Eq producers and non-Eq producers. A higher frequency of Eq producers (~50%–55%) was found among Asians who regularly consume soy foods (fermented and unfermented) (Utian et al., 2015). In Western populations, the consumption of isoflavones from traditional soy foods is substantially lower and isoflavones are derived mainly from soy protein and soy flour, which is added to a variety of foods (Tousen et al., 2013). This low intake of soy foods in Western countries may be one of the reasons why the prevalence of Eq producers among Western adults is only 20% to 35% (Ideno et al., 2018). Apart from soy food intake, many other factors, such as genetic background, gut microbial environment, habitual dietary pattern, and age, may determine the Eq-producing phenotype (Yoshikata et al., 2019). In the case of the gut microbial environment, more than ten species of gut microbes have been recognized as being responsible for Eq production. Iino et al. (2019) showed that intestinal bacteria that converts daidzein to Eq were present in both Eq producers and nonproducers, but that the relative abundance and prevalence of *Asaccharobactercelatus*, *Slackiaisoflavoniconvertens*, *Bifidobacterium animalis*, *Bacteroides ovatus*, *Enterococcus faecium*, *Lactobacillus mucosae* are significantly higher in Eq producers than in Eq nonproducers (Iino et al., 2019). This means that all adults possess the specific gut bacteria for Eq formation, but in nonproducers, the activity of bacterial enzymes may not be optimal for effective conversion of daidzein to S-(-) Eq (Setchell et al., 2013). Therefore, it has been suggested that some dietary factors may play a role in Eq formation, even if the appropriate gut bacteria are present (Yoshikata et al., 2019). Setchell et al. discovered in their observational study conducted in the US and Australia that Eq production appeared to be associated with a greater intake of maltose and of vitamins A and E (Setchell et al., 2013). Moreover, the Eq producers consumed more dietary fiber than the nonproducers. Bolca et al. revealed that participants with higher polyunsaturated fatty acids and alcohol intakes were more likely to be strong Eq producers (Bolca et al., 2007). Since it has been indicated that the ability to produce Eq is associated with a reduced risk of breast cancer, cardiovascular disease (CVD), improved bone health, and reduced vasomotor symptoms in postmenopausal women (Mayo et al., 2019), determining the specific dietary factors that may stimulate S-(-) equol production in that population is of the utmost importance. This study examines the hypothesis that specific dietary factors determine the ability to produce Eq in Polish postmenopausal women. Moreover, Eq producer phenotypes among aimed population were also identified. The novelty of the present study is the identification of other dietary factors that may play a role in Eq synthesis in Western postmenopausal women. This knowledge can be used to develop appropriate dietary strategies that maximize endogenous Eq production to obtain full benefit from soy food intake, and in this way potentially reduce the risk of breast cancer, CVD, improve bone health and reduce the incidence of hot flushes in that population.

Study design

Participants aged 48–68 years old were recruited though an advertisement post published from 2021 to 2022. Potential participants were screened by a telephone interview. Recruitment for the study was carried out at the Department of Human Nutrition and Dietetics, Poznań University of Life Sciences. The criteria

for inclusion were the absence of menses of over 12 months prior to the study and using a mixed diet without any restrictions on products of animal origin. The exclusion criteria were the following: unnatural menopause as a consequence of surgery or radiotherapy for cervix cancer, a history of chronic liver disease, thyroid disease, kidney disease, gastrointestinal disease, cancer, or allergy to soy, taking exogenous hormonal drugs, supplements containing phytoestrogens such as red clover and other soy isoflavones, smoking cigarettes, using cholesterol-lowering drugs, or bile acid sequestrants or statins, and antibiotics or prebiotics prescribed in the three months prior to participation. Menopause was clinically defined as the natural absence of menstruation over at least 12 months after the final menstrual period (Harlow et al., 2012).

The study protocol, risks, and benefits were explained to each participant. Written informed consent was obtained from all the study participants. The study protocol was approved by the Poznań University of Medical Sciences Bioethics Committee (approval no. 241/20) in line with the World Medical Association Declaration of Helsinki. The trial registration number was DRKS00031637 (https://www.drks.de/drks_web/). Women were asked to take soy isoflavone (Soya Isoflavones, Natures Aid, UK) for three days as a supplement to their habitual diets. Of the 36 respondents screened, 33 met the participation criteria and agreed to participate. One woman was excluded from the study because it was detected that she did not take soy isoflavone supplements on schedule. The analysis thus included data from 32 participants.

Intervention study

Participants consumed one soy isoflavone supplement twice a day (two tablets from Natures Aid, UK; the label indicated that each tablet contains ~50 mg of isoflavones, including 27.8 mg of Ge, 18.4 mg of De, and 3.43 mg of Gle) for three consecutive days to reach steady state isoflavone concentrations prior to testing their urine for soy isoflavones, and their metabolites. The three-day soya challenge had previously been used in other studies (Atkinson et al., 2004; Setchell and Cole, 2006). Moreover, we chose the 3-day study period to avoid potential alteration in gut environment. The maximum dosage (100 mg of soy isoflavones) was recommended by the producer. Consumption of 100 mg of isoflavones also comes from the European Food Safety Authority (EFSA), who conducted a systematic study of the literature, focusing on the correlation between the intake of soy isoflavones and the induced effects on the breast (mammographic density, proliferative marker Ki67 expression), uterus (endometrial thickness, histopathology changes), and thyroid (the thyroid hormone). The results showed that the intake of 35–150 mg isoflavones/day does not affect these organs in peri- and postmenopausal women (EFSA Journal, 2015). Compliance with the supplementation regimen was assessed by showing the dietitian an empty pill box at the end of the study. Moreover, 24-h urinary excretion of isoflavones *per se* was an indicator of dietary isoflavone intake. Soy isoflavones were well tolerated and no serious adverse events were noted during the study.

Collection of urine samples

Participants were instructed to discard their first-morning void in the toilet the next day after soy isoflavones supplementation finished and then to collect subsequent 24-h urine until the first void the next morning. The time of bladder emptying was used as the start time of the urine collection. Urine was collected in a disposable urine collection box 3 L. Participants were instructed to record the start and end times of urine sampling and the 24-hour urine volume. During their visit, subjects provided the recording sheet as well as the 24-hour urine sample. The urine samples were then placed in a 2 ml Eppendorf and stored in a freezer at –20°C for future analysis.

Dietary assessment

During the dietary intervention, each participant began a 3-day dietary record to report all the foods and beverages consumed over a specific period. Before they participated in the survey, the dietitian provided 20 minutes of instructions to each participant on how to evaluate and record meal and food portions, cooking techniques and recipes as well as the brand name of commercial products. The portion sizes of dishes and food products were estimated based on the "Photo Album of Food Products and Dishes". Written copies of record examples were provided to each subject. Analysis of the energy and nutritional value of the daily food rations was carried out using the Dieta 6.0

software (National Institute of Public Health, National Institute of Hygiene, Warsaw, Poland). We also selected the following 15 food groups: meat, fish, eggs, milk and dairy products, vegetable oils, animal fat spreads, refined grains, whole grains, fruits, vegetables, pulses, processed food, confectionary, sweetened drinks and alcoholic beverages.

Anthropometric measurements

Height was measured to the nearest 0.1 cm using a stadiometer (Rad Wag, Radom, Poland). Body weight was measured to the nearest 0.1 kg, with subjects in bathing suits, after an overnight fast using the calibrated scale included in the Bod Pod apparatus (Cosmed, Rome, Italy). Body mass index (BMI) was calculated as weight (kg)/[height (m)]^2.

Assessment of a series of biomarkers in urine

The level of creatinine (Cr), sodium (Na), potassium (K), and magnesium (Mg) in the urine samples were measured by an external laboratory (SRL Inc, Tokyo, Japan). Creatine concentration was measured using the creatinine-sarcosine-oxidase-POD method. Sodium and potassium concentrations were measured using the electrode method, and molecular ratios of Na/K were calculated, while the xylidyl blue method was used for urine magnesium concentrations.

Assessment of isoflavones and their metabolites in the urine samples

Urinary isoflavones were measured in the 24-h urine samples in the laboratory of the Department of Innovative Food Sciences, School of Food Sciences and Nutrition, Mukogawa Women's University using a simple and rapid LC-MS/MS method described by Saha and Kroon (Saha and Kroon, 2020), with a slight modification. The urine sample (200 µL) was mixed with 200 µL of beta-glucuronidase solution (200 U/mL in 0.1 mol/L acetate buffer (pH 5.0); *Helix pomatia*, Sigma-Aldrich, St. Louis, MO, USA) and 200 µL of sulfatase solution (50 U/mL in 0.1 mol/L acetate buffer (pH 5.0); *Helix pomatia*, Fujifilm Wako Pure Chemical, Osaka, Japan), and incubated for 2 h at 37°C for enzymatic hydrolysis. After incubation, 600 µL of N,N-dimethylformamide and 40 µL of formic acid were added (both from Fujifilm Wako Pure Chemical). The samples were allowed to equilibrate for 10 min and subsequently mixed every 5 min. They were then centrifuged (13000 r.p.m., 4°C, 15 min, MRX-150; Tomy Seiko, Tokyo, Japan). The supernatant was injected into the LC-MS/MS system after filtration (0.45 µm).

LC-MS/MS analysis was performed using a Nexera X2 LC system (Shimadzu, Kyoto, Japan) connected to a triple quadrupole mass spectrometer (LC-MS 8040; Shimadzu) equipped with an electrospray ionization source. The instrument was operated under positive electrospray ionization and with multiple reaction monitoring modes. Chromatographic separation was performed with a Cadenza CD-C18 column with a length of 150 mm and a 3.0 mm inner diameter and with a 3 µm particle size (Imtakt, Kyoto, Japan). The column temperature was 40°C. Elution was performed at a flow rate of 0.3 mL/min with a linear gradient of 0.1% formic acid and methanol (35%–80%, 0–18 min). The total run time was 30 min per sample.

The MS operating conditions were optimized as follows: interface voltage: 4.5 kV; interface temperature: 350°C; desolvation line temperature: 250°C; heating block temperature: 400°C; drying gas: N_2 at 15 L/min; nebulizing gas: N_2 and 2 L/min; and collision-induced dissociation gas: argon at 230 kPa. The m/z values for each analyte were as follows: De: 254.95 > 199.0, 137.00, and 152.00; Gle: 284.95 > 269.90, 241.90, and 118.00; Ge: 270.95 > 153.00, 215.00, and 243.00; Dihydrodaidzein (DHD): 256.95 > 123.00, 162.95, and 77.00; Eq: 242.95 > 133.00, 123.00, and 107.00; and *O*-Demethylangolensin (*O*-DMA): 258.95 > 149.00, 121.00, and 164.95. All analyses and data processing were performed using Lab Solutions V5.60 software (Shimadzu).

Statistical analysis

All statistical analysis was performed using Statistica (version 13.0; http://statistica.io; Tibco Software), and a p-value < 0.05 was considered statistically significant. Sample size calculations were based on Eq production frequencies tested in a previous study of Western adults (35%) (Ideno et al., 2018). In order to detect a statistically significant difference in the proportion of Eq producers, n = 29 postmenopausal women were needed. The test had 95% power at α = .05 (G*Power 3.1.9.6 Universität Kiel, Kiel, Germany). The final sample size in the study (n = 32) exceeded the calculated sample size (n = 29), which was due to low dropout rates.

The Shapiro–Wilk test was used to verify normality. Continuous data were presented as means with standard errors (SE). Variables were compared using the Mann–Whitney *U*-test based on the log10-transformed urinary Eq:De ratios for the individual participants and taking a threshold value of –1.75. Subjects were separated into groups of Eq producers and nonproducers. To measure the association between Eq-producer status and intake of pulses, the odds ratio, its 95% CI, and a significance level (in both a crude model and a model adjusted for age and BMI, as well as for years after menopause), were calculated in a logistic model.

RESULTS

Thirty-two healthy, nonsmoking, Polish postmenopausal women (all Caucasian) aged 48 to 68 years with an average BMI of 26.7 kg/m^2 participated in this study. The log 10-transformed urinary Eq:De ratios for the individual participants are plotted in rank order of value in Figure 1. Using the threshold value of –1.75 as previously defined (Setchell and Cole, 2006) to differentiate Eq producers from nonproducers, 14 of the 32 Polish postmenopausal women (43.75%) were classified as Eq producers.

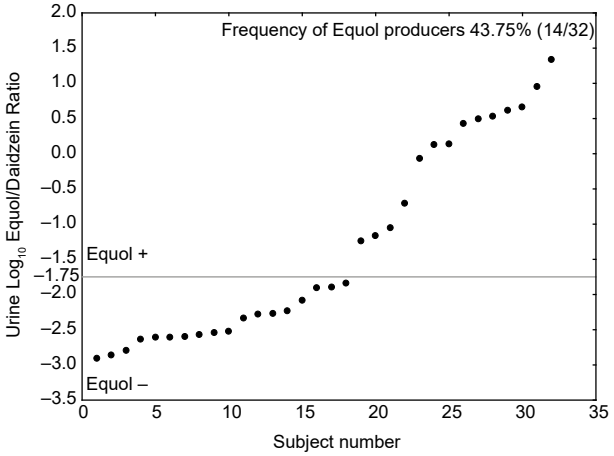

Fig. 1. Log_{10}-transformed urinary S-(-)equol: daidzein ratios in rank order of the individual participants (n = 32). Points below the grey line were classified as equol (Eq) nonproducers and those above the black line were classified as Eq producers. Equol+: equol producer; Equol–: equol nonproducer.

The measured urine concentrations of magnesium (Mg), sodium (Na), and potassium (K), as well as of soy isoflavones and their metabolites, were corrected for urinary creatinine measured on the same urine samples. As expected, some urinary biomarkers reflecting soy isoflavone supplementation showed statistically significant differences between Eq producers and nonproducers, including DHD [Eq (+) 3.9 ±0.7 µmol/g-Cr vs Eq (–) 37.9 ±6.8 µmol/g-Cr, $p < 0.001$], Eq [Eq (+) 41.5 ±9.4 µmol/g-Cr vs Eq (–) 0.2 ±0.07 µmol/g-Cr, p<0.001], *O*-DMA [Eq (+) 5.9 ±3.0 µmol/g-Cr vs Eq (–) 7.8 ±1.3 µmol/g-Cr, $p = 0.012$], Log_{10} Eq/De ratio [Eq(+) 0.07 ±0.22 vs Eq(–) –2.4 ±0.1, $p < 0.001$], and soy isoflavone [Eq(+) 38.7 ±6.8 µmol/g Cr vs. Eq (–) 69.4 ±9.2 µmol/g Cr, $p = 0.026$] (Table 1).

Table 1. Selected anthropometrical and urine biomarkers in a group of Eq producers (Eq+) and Eq nonproducers (Eq–) after three-day soy isoflavone supplementation

Variable	Eq+ (n = 14)	Eq– (n = 18)	*p*-value*
	mean ±SE	mean ±SE	
Age, years	60.5 ±1.1	58.6 ±1.3	0.360
BMI, kg/m^2	28.1 ±1.3	25.6 ±1.2	0.167
Cr, g/L	0.7 ±0.1	0.56 ±0.05	0.231
Na, mEq/g-Cr	141.1 ±14.1	126.2 ±11.5	0.436
K, mEq/g-Cr	65.2 ±8.8	60.9 ±3.9	0.662
Na/K	2.4 ±0.3	2.4 ±0.3	0.582
Mg, mEq/g-Cr	7.3 ±0.6	8.9 ±0.6	0.864
De, µmol/g-Cr	31.2 ±5.5	61.3 ±8.1	0.008
Gle, µmol/g-Cr	4.8 ±0.9	4.7 ±0.8	0.690
Ge, µmol/g-Cr	2.7 ±0.7	3.4 ±0.9	0.531
DHD, µmol/g-Cr	3.9 ±0.7	37.9 ±6.8	<0.001
Eq, µmol/g-Cr	41.5 ±9.4	0.2 ±0.07	<0.001
O-DMA, µmol/g-Cr	5.9 ±3.0	7.8 ±1.3	0.012
Log_{10} Eq/De	0.07 ±0.22	–2.4 ±0.1	<0.001
Soy isoflavones (Σ De, Gle, Ge), µmol/g Cr	38.7 ±6.8	69.4 ±9.2	0.026

Values are presented as means ±standard errors (SEs).
*Mann–Whitney *U*-test.
Abbreviations: BMI – body mass index; UN – urea nitrogen; Cr – creatinine; K – potassium; Mg – magnesium; Na – sodium; DHD – dihydrodaidzein; Eq – eqwol, *O*-DMA – *O*-desmethylangolensin.

No differences in terms of the intake of the selected dietary constituents were observed between equol producers and nonproducers. Eq producers declared a 7.6-fold greater ($p < 0.001$) intake of pulses (34.4 ±6.3 g/d) than Eq nonproducers (4.5 ±2.0 g/d) (Table 2).

Table 2. Comparison of the dietary and selected food groups intakes of postmenopausal women classified as Eq producers (Eq+) and Eq nonproducers (Eq–)

Variables	Eq+ (n = 14)	Eq– (n = 18)	*p-value
	mean ±SE	mean ±SE	
1	2	3	4
Dietary intake			
Energy, kcal/d	1740.8 ±117.2	1683.9 ±130.1	0.761
Total fat, g/d	53.8 ±5.2	57.9 ±4.9	0.595
Calories from fat, %	28.1 ± 2.05	30.3 ±1.4	0.362
Total SFA, g/d	22.0 ±2.2	23.1 ±2.7	0.879
Total MUFA, g/d	19.7 ±2.1	20.5 ±1.9	0.704
Total PUFAs, g/d	7.4 ±0.9	8.4 ±0.8	0.196
PUFA: SFA ratio	0.4 ±0.05	0.4 ±0.04	0.470
Omega 3, g/d	1.5 ±0.2	2.0 ±0.34	0.382
Omega 6, g/d	5.9 ±0.7	6.4 ±0.6	0.470
Total carbohydrate, g/d	243.0 ±22.9	221.7 ±21.6	0.160
Calories from carbohydrates, %	49.9 ±2.73	47.7 ±1.4	0.254
Digestible carbohydrates, g/d	222.1 ±21.57	202.1 ±20.4	0.184
Dietary fiber, g/d	22.1 ±1.6	18.7 ±1.7	0.119
Total fat: dietary fiber ratio	2.9 ±0.4	3.2 ±0.3	0.518
Lactose, g/d	7.9 ±1.1	6.5 ±1.3	0.224
Starch, g/d	115.7 ±14.3	107.0 ±11.3	0.287
Total protein, g/d	73.4 ±5.3	74.5 ±5.2	0.970
Calories from protein, %	17.4 ±1.21	18.4 ±1.0	0.459
Vitamin A, IU/d	995.9 ±119.83	1434.2 ±257.9	0.595
β-Carotene, mg/d	4111.2 ±661.5	4239.9 ±971.5	0.470
Vitamin B_1, mg/d	1.1 ±0.06	1.0 ±0.08	0.271
Vitamin B_6, mg/d	1.8 ±0.1	1.7 ±0.12	0.569
Vitamin C, mg/d	134.9 ±23.2	118.5 ±19.7	0.342
Vitamin E, IU/d	7.7 ±0.8	7.6 ±0.6	0.820
Magnesium, mg/d	322.7 ±19.2	316.9 ±20.7	0.704
Calcium, mg/d	711.8 ±63.9	748.9 ±62.3	0.621
Iron, mg/d	11.5 ±0.6	12.8 ±1.5	0.820
Sodium, mg/d	2902.3 ±156.3	2988.3 ±240.5	1.000
Potassium mg/d	3767.2 ±258.4	3279.1 ±263.1	0.111
Dietary isoflavones, mg/d	0.84 ±0.25	0.65 ±0.08	0.635
Intake of selected food groups			
Meat, g/d	43.8 ±11.8	30.3 ±7.9	0.301
Fish, g/d	34.8 ±11.5	36.1 ±11.1	0.955
Eggs, g/d	14.6 ±4.8	21.4 ±4.9	0.344
Milk and dairy products, g/d	503.2 ±95.0	714.3 ±99.0	0.145
Refined grains, g/d	79.0 ±14.8	76.0 ±15.8	0.694
Whole grains, g/d	89.1 ±12.9	62.4 ±11.8	0.251
Fruits, g/d	210.4 ±35.1	265.3 ±48.0	0.398
Vegetables, g/d	297.0 ±38.8	259.9 ±32.9	0.319
Pulses, g/d	34.4 ±6.3	4.4 ±2.1	<0.001
Vegetable oils g/d	5.5 ±3.2	5.9 ±1.7	0.913
Animal fat spreads, g/d	9.5 ±2.0	5.1 ±1.7	0.084
Processed food, g/d	98.7 ±20.0	126.7 ±24.1	0.442
Confectionary, g/d	63.0 ±14.4	76.4 ±18.1	0.866
Sweetened drinks, ml/d	14.6 ±12.0	25.0 ±15.9	0.694
Alcoholic beverages, ml/d	53.4 ±27.9	41.1 ±20.3	0.896

Values are means ±standard errors (SEs).
*Mann–Whitney *U*-test.

Table 3. Intake of pulses in relation to the equol producer phenotype

Independent variable	Crude model			Model 1*			Model 2**		
	OR	95% CI	p-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Pulses intake (g/d)	1.113	1.037; 1.193	0.003	1.105	1.031; 1.184	0.005	1.109	1.033; 1.190	0.004

Values are odds ratio (OR) and 95% confidence interval (CI).
*Model 1 adjusted for age and BMI value.
**Model 2 adjusted for age and BMI value and years after menopause.

Multivariable logistic regression analysis showed that postmenopausal women who stated that they consumed pulses were more likely to be Eq producers in both the crude model ($p = 0.006$) and after adjusting for age and BMI ($p = 0.010$) than postmenopausal women who stated that they did not consume pulses. Multivariable logistic regression analysis showed that postmenopausal women who reported a higher pulses intake were more likely to be Eq producers in both the crude model [odds ratio (OR) = 1.113, 95% CI: 1.037; 1.193; $p = 0.003$)] and after adjusting for age and BMI (OR = 1.105, 95% CI: 1.031; 1.184; $p = 0.005$) and additionally years after menopause (OR = 1.109, 95% CI: 1.033; 1.190; $p = 0.004$ (Table 3).

DISCUSSION

The dietary factors that trigger the ability to harbor Eq-producing bacteria are not well known (Lampe, 2009), although such associations between Eq-producer status and some nutrients have been observed. Our study shows that Polish postmenopausal women who reported a higher pulses intake were more likely to be Eq producers, even after adjusting for age, BMI and years after menopause. Eq producers reported a 7.6-times greater intake of pulses than their non-Eq-producing counterparts. Nutrition dietary guidelines in Poland recommend replacing animal sources of protein with pulses, due to health and environmental benefits (Jarosz et al., 2020). Recently, the Dietary Guidelines for Americans for 2020–2025 recommended 1.5 cup (~360 g) equivalents of beans, peas, and lentils per week for individuals following a 2000-calorie healthy U.S.-style dietary pattern or a 2000-calorie healthy Mediterranean-style dietary pattern (Mitchell et al., 2022). In our study, the mean daily intake of pulses among the Eq producers was 34.4 ±6.3 g; extrapolating this data to a week provides ~241 g of pulses. The pulses that were commonly consumed in our study included beans, peas, lentils, and chickpeas. In general, legumes, including pulses, are promoted in healthy dietary patterns as nutrient-dense foods (Didinger and Thompson, 2022). Pulses have the potential to modulate the composition and function of the gut microbiota, regulate appetite, and attenuate inflammation (John et al., 2023). The ability of pulses to modulate the gut microbiota is caused by its their high content of non-digestible carbohydrates (NDCs) and polyphenols. (John et al., 2023). In a study by Yoshikata et al. (2019), Eq producers showed significantly higher gut microbiota diversity compared with Eq nonproducers. An association between alpha diversity and microbial beta glucosidase activity was also observed. This enzyme is responsible for the polyphenol metabolism and is also involved in the fermentation of soluble fibers and indigestible carbohydrates to produce short-chain fatty acids (SCFAs) as an important energy sources for optimal functioning of intestinal cells and gut microbiota in the host. Thus, SCFAs can affect the gut environment to enhance isoflavone metabolism and consequently Eq production (Yoshikata et al., 2019). However, we did not find any studies that test the relationship between pulses intake, gut microbial populations and the ability to produce Eq. As was mentioned earlier, there are other dietary factors that can be associated with the ability to produce Eq. It was also suggested that Eq concentrations in low-soy-consuming populations may reflect Eq intake from mammalian milk sources and not the endogenous production of Eq from the microbial metabolism of daidzein (Frankenfeld, 2011). Indeed, milk Eq, which originates from cattle feed, may contribute to the human Eq supply (Mustonen et al., 2009). However, our study does not

show any relation between the ability to produce Eq and milk or dairy products intake.

We identified the frequency of Eq producers among the Polish postmenopausal women as 43.75%, which is consistently higher than reported for Western populations generally (20% to 35%), and more similar to that observed in Asian countries (50% to 55%), where soy foods are commonly consumed (Hong et al., 2012). It should be noted that all the enrolled postmenopausal women were omnivores who did not habitually consume soy foods. To define the term "Eq producer phenotype", we used the method described by Setchell and Cole (2006), employing their formula and cut-off threshold value of ≥ -1.75. However, this approach to defining the Eq phenotype is independent of isoflavone intake and the analytical technique used. Bolca et al. (2007) observed that phenotyping of postmenopausal women (all Caucasian) based on the daidzein metabolism by fecal cultures resulted in 39% Eq producers, as expected from the literature. On the other hand, using the formula described by Setchell and Cole (2006), 61% of the subjects would be defined as Eq nonproducers. According to the latter authors, the discrepancy was mainly due to the contribution of DHD and *O*-DMA. However, Franke et al. found that Setchell and Cole's cut-off was useful, even if overnight urine samples were used instead of 24-h urine samples (Franke et al., 2012).

In our study, we did not characterize the gut microbiota of Eq producers and Eq nonproducers, which can be consider as limitation of the study. Additionally, our composition analysis of a random sample of soy supplements used in this study showed some discrepancy between their isoflavone content and the claims from the label. On the other hand, our analysis showed that the participants took enough daidzein to demonstrate whether they were producers of Eq or not. Moreover, our analysis confirmed that the soy supplements did not contain Eq.

However, this study has a number of strengths. Even though the sample size of our study may seem relatively small, we were able to indicate Eq producers and nonproducers. Since we used specific inclusion and exclusion criteria, our participants were not on antibiotic treatment and did not have any intestinal disease, which are factors that can affect gut diversity and subsequently the production of metabolites, which may skew the classification of the different phenotypes.

This data may be used to develop appropriate dietary strategies that maximize endogenous Eq production in Western postmenopausal women to obtain full benefit from soy intake, and in this way, potentially reduce the risk of breast cancer, CVD, improve bone health and reduce the incidence of hot flushes in that population. Moreover, future studies are needed to determine the effects of dietary pulses on gut microbial populations and their ability to Eq producing.

CONCLUSION

This study expands our knowledge by identifying the dietary factors that may determine the ability to produce Eq in Polish postmenopausal women. In this matter, postmenopausal women who reported higher dietary pulses intake were more likely to be Eq producers, even after adjusting for age, BMI and years after menopause. Due to the important role of the gut microbiome in equol production, further investigations are required to test the changes in gut microbiota activity of Polish postmenopausal women during the soy isoflavones challenge, especially when the consumption of pulses is present.

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AUTHOR CONTRIBUTIONS

Conceptualization: JB, YY, MM, TT, and SM; Methodology: YY, MM, TT, NM, JB; Data curation: JB, AS-R, KŁ, AB-D, JN; analysis and interpretation: YY, MM, TT, NM, JB; Writing – Original draft preparation: JB; Writing – Review & Editing: YY, MM, TT, and SM.

All authors agreed the final version of the manuscript and the content has not been published anywhere.

DATA AVAILABILITY

Datasets from the current study are available from the corresponding author upon request.

DECLARATIONS

Ethical Approval
Not applicable.

Competing Interests
The authors declare that they have no conflicts of interest.

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