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Daidzein-metabolizing phenotypes in relation to bone density and body composition among premenopausal women in the United States

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Abstract

Background—Bone density has been suggested as a marker of cumulative hormone exposure. Small studies also suggest that patterns of daidzein metabolism may be related to hormone concentrations. To our knowledge, no studies in premenopausal women have compared bone density by daidzein-metabolizing phenotypes in the absence of a soy intervention.

Objective—To evaluate the relationship between daidzein-metabolizing phenotypes [equol and O-desmethylangolensin (ODMA) production] and bone density and body composition in premenopausal women in the United States.

Materials/Methods—Two hundred and three women attended a clinic visit during which their bone density and body composition was measured by DXA, and 200 (99 %) provided a urine sample following a 3-day soy challenge. Samples were analyzed for isoflavones to determine daidzein-metabolizing phenotypes.

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Author contributions

All authors commented on drafts, and read and approved the final manuscript. In addition, CA, KMN, and JWL were involved in the study design, CA oversaw data collection, LL conducted data analyses, CA, KMN, MY, LL, and JWL interpreted the data, FZS provided serum hormone data, and KCW provided urinary hormone data.

Results—In adjusted analyses, there were no differences in hip, spine, femoral neck, or head bone mineral density (BMD) or body composition between producers and non-producers of either equol or ODMA ($p > 0.05$).

Conclusions—In this population of low-soy consuming premenopausal women, there were no associations between daidzein-metabolizing phenotypes and hip, spine, femoral neck, or head BMD or body composition, suggesting that these phenotypes per se do not influence premenopausal bone density or body composition.

Keywords

equol; isoflavones; O-desmethylangolensin; soy challenge

Introduction

While there are clear links between circulating estrogen concentrations, osteoporosis and fracture risk in postmenopausal women, associations between estrogens and bone density in premenopausal women are less clear [1]. Bone density may be a marker of cumulative estrogen exposure, and studies have reported positive associations between bone density and postmenopausal breast cancer risk [2, 3].

Isoflavones are structurally similar to estrogens. Daidzein, a soy isoflavone, is metabolized by intestinal bacteria to equol and O-desmethylangolensin (ODMA). Approximately 30–50% and 80–90% of individuals produce equol or ODMA, respectively [4]. Equol is more biologically active than daidzein *in vitro* suggesting that producers and non-producers may respond differentially to soy or isoflavone interventions regarding hormonally-mediated factors [4]. Alternatively, because intestinal bacteria metabolize estrogens [5], equol and ODMA production may represent intestinal bacterial profiles associated with hormonally-mediated factors independently of soy exposure.

Few studies have looked at daidzein-metabolizing phenotypes and bone density in the absence of soy/isoflavone interventions. In postmenopausal Western women, ODMA-producers had higher total, leg, and head bone mineral density (BMD) than non-producers, but there were no differences between equol-producers and non-producers [6]. Most studies have investigated biological effects of equol by stratifying results of soy/isoflavone interventions by equol-producer status. One study in premenopausal women found no difference between high and low equol excretors in urinary bone turnover markers [7]. Studies in postmenopausal women have reported mixed findings [8–13]. To our knowledge, no studies have investigated associations between daidzein-metabolizing phenotypes per se and body composition. In regards to biological effects, in postmenopausal women taking an isoflavone supplement for one year, equol-producers (n=15) had lower fat mass accumulation than non-producers (n=10) [9].

We sought to determine relationships between daidzein-metabolizing phenotypes and bone density and body composition in low-soy consuming premenopausal women. We hypothesized that bone density would be lower in equol-producers than non-producers. Exploratory analyses examined associations between ODMA-producer status and bone density and between daidzein-metabolizing phenotypes and body composition.

Methods

Premenopausal women (40–45y, n=203), who were not taking hormones and had not taken antibiotics in the past 3 months, attended a clinic visit. Lumbar spine and hip bone mineral content (BMC) and BMD and body composition were measured using dual energy X-ray

absorptiometry (Hologic Delphi, Hologic Inc. Bedford, MA). Weight, height, and waist and hip circumferences were measured, fasting blood and spot urine samples were obtained, and health, demographics and physical activity (PA) questionnaires were collected [14].

For equol and ODMA-producer phenotyping, women consumed a soy bar (38mg daidzein/bar) or soy nuts (approximately 10mg daidzein/serving) on three consecutive days and collected a first-void urine on day 4. We previously showed that equol excretion was detectable with daidzein intakes of 3mg/day [15]. Urine was analyzed for isoflavonoids by gas chromatography-mass spectrometry. Equol- and ODMA-producers were those with detectable equol (87.5 ng/mL, or 362 nmol/L) and ODMA (87.5 ng/mL, or 339 nmol/L) [14].

Serum was measured for estrone, estrone-sulfate, estradiol, dehydroepiandrosterone, dehydroepiandrosterone-sulfate, androstenedione, testosterone, and sex hormone binding globulin, and spot urines were measured for 2-hydroxyestrone and 16á-hydroxyestrone [16].

Using a ratio of 1:2 equol-producers to non-producers and a standard deviation of 0.06 g/cm^3 for spine BMD [17], we estimated that a sample of 200 women would give >99% power to detect a 0.1 g/cm^3 difference between producers and non-producers, which was considered clinically meaningful in terms of breast cancer risk [18]. Study procedures were approved by Institutional Review Boards of the Fred Hutchinson Cancer Research Center and Group Health, and participants provided written informed consent.

Data analysis

Differences between producers and non-producers of equol and ODMA were assessed using t-tests, chi square and Fishers exact tests. T-tests and multiple regression assessed relationships between equol and ODMA production and bone density and body composition. Adjustment variables were those significantly associated with bone density or body composition and equol or ODMA-producer status in univariate analyses. Adjusted analyses were conducted with and without adjustment for hormones. Data were analyzed using SAS (version 9.1; SAS Institute, Cary, NC).

Results

Two hundred women (99%) provided a soy challenge urine sample. Data on amount of soy bar or soy nuts (n=5 participants) consumed were available for 190, 190 and 186 women, respectively. Of these, 186 (98%), 184 (97%), and 181 (97%) consumed ≥ half the assigned portion on days 1–3, respectively. Fifty-five (27.5%) and 182 (91%) women were equol- and ODMA-producers, respectively.

Equol-producers were more likely to be Hispanic or Latino, and were better educated than non-producers. ODMA-producers were taller and less likely to be Asian than non-producers (Table 1). In unadjusted analyses, ODMA-producers had higher whole body total lean mass than non-producers [46.12kg (95% CI 45.24, 47.00)] vs. 42.73kg (95% CI 39.31, 46.16), respectively, *p=0.03*]; this attenuated in adjusted analyses. In all other analyses there were no differences between producers and non-producers of ODMA or equol in bone density or body composition (Table 2). Additional adjustment for hormones or physical activity during age 22–33y (which was associated with equol-production and spine BMD in univariate analyses), did not alter these findings (data not shown).

Discussion

We found no associations between daidzein-metabolizing phenotypes and hip, spine, femoral neck, or head BMD. In unadjusted analyses ODMA-producers had higher whole body total lean mass than non-producers but this attenuated in adjusted analyses and was based on small numbers of ODMA non-producers.

Associations between equol-producer status and education and ethnicity, and between ODMA-producer status and race and height have been discussed elsewhere [14]. Briefly, the nature of the association between equol production and education is unclear, but could represent an unmeasured exposure. Geographic differences in bacterial populations exist [19, 20], and intestinal microbiota may influence adult height (e.g., via competition for nutrients or effects on hormone concentrations involved in the onset of puberty) [21], suggesting potential explanations for these associations.

Our study was designed to examine effects of daidzein-metabolizing phenotypes per se rather than biological effects of isoflavones. A soy intervention in premenopausal women reported lower estrogen and androgen concentrations in equol-producers than non-producers regardless of soy/isoflavone dose [22], and we hypothesized we would see lower bone density in equol-producers than non-producers. The lack of an association suggests that ability to produce equol or ODMA per se has no effect on BMD in premenopausal women. In a study of postmenopausal women, ODMA-producers had higher total, leg, and head BMD than non-producers, but there were no differences between equol-producers and non-producers [6]. Although not directly comparable with our study (due to being interventions and studying postmenopausal women), some [8, 9, 13], although not all [10–12], studies suggest that soy/isoflavones in addition to being an equol-producer may be protective. Potential reasons for conflicting findings across studies include small sample sizes, differences in ethnicity, intervention type and dose, study length, and methods used to define equol- and ODMA-producers [23].

We found no association between daidzein-metabolizing phenotypes per se and premenopausal body composition. Although again not directly comparable, a study in postmenopausal women showed that equol-producers taking an isoflavone supplement for one year had lower accumulation of fat mass than non-producers [9].

There are some strengths of our study. Conducting it in a low-soy consuming population and using a soy challenge to determine phenotypes allowed us to examine effects of daidzein-metabolizing phenotypes per se (rather than biological effects of isoflavones). Women were well characterized, blood and urine collection was timed to menstrual cycle phase, and we explored many potential covariates. To our knowledge, this is the largest study to date to assess bone density and body composition in relation to daidzein-metabolizing phenotypes in premenopausal women.

Our study has some weaknesses. It was a cross-sectional study and causal inferences cannot be made. Findings regarding differences between ODMA-producers and non-producers should be interpreted cautiously due to small numbers of non-producers (n=18) compared to producers (n=182). Most women were Caucasian, well-educated, and recruited according to BIRADS score (see [14]), and our findings may be generalizable only to similar populations. We recruited women who reported regular menstrual periods, but some may have been in the menopause transition. Finally, post-hoc power calculations using observed distributions for producers and non-producers (1:3 and 9:1 for equol and ODMA, respectively) and a standard deviation of 0.12 gm/cm^3 for spine BMD showed that sample sizes of 64 and 130 were needed to detect a difference of 0.1 gm/cm^3 between producers and non-producers of

equol and ODMA, with two-sided alpha 0.05 and 80% power. This suggests our sample size was sufficient for detecting differences, had they existed, in these women.

We observed no differences in hip, spine, femoral neck, or head BMD or body composition between producers and non-producers of either daidzein metabolite. Because few participants reported regular soy consumption our findings provide information on daidzein-metabolizing phenotypes per se, rather than combined effects of soy and phenotypes, and suggested no association. Future studies in high-soy consuming premenopausal women should assess whether interactions exist between phenotypes and soy consumption in relation to bone density and body composition.

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List of abbreviations

BMC	bone mineral content
BMD	bone mineral density
ENP	equol non-producer
EP	equol producer
ODMA	O-desmethylangolensin
ONP	ODMA non-producer
OP	ODMA producer

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Table 1

Study participant demographics and lifestyle factors by equol-producer and ODMA-producer status

	Equol producers (n=55)	**Equol non-producers (n=144)**	**p**	**ODMA producers (n=181)**	**ODMA non-producers (n=18)**	**p**
Age (years), mean (SD)	42.6 (1.2)	42.3 (1.4)	*0.15*	42.5 (1.4)	42.1 (1.1)	*0.23*
Height (m), mean (SD)	1.65 (0.07)	1.65 (0.07)	*0.82*	1.66 (0.07)	1.61 (0.06)	*0.003*
Weight (kg), mean (SD)	70.5 (14.7)	71.1 (14.2)	*0.78*	71.3 (14.2)	67.1 (15.6)	*0.23*
BMI (kg/m^2), mean (SD)	25.8 (5.1)	26.0 (4.9)	*0.83*	26.0 (4.9)	25.8 (5.0)	*0.90*
Race, n (%)						
White	49 (91)	125 (87)		163 (91)	11 (65)	
Asian	3 (6)	10 (7)		9 (5)	4 (24)	
Other	2 (4)	8 (6)	*0.93*	8 (4)	2 (12)	*0.006*
Ethnicity, n (%)						
Hispanic or Latino	5 (9)	1 (1)		5 (3)	1 (6)	
Not Hispanic or Latino	50 (91)	142 (99)	*0.007*	176 (97)	16 (94)	*0.42*
Education, n (%)						
≤12 years	4 (7)	8 (6)		12 (7)	0 (0)	
13–15 years	12 (22)	40 (28)		45 (25)	7 (39)	
16 years	8 (15)	53 (37)		56 (31)	5 (28)	
≥ 17 years	31 (56)	43 (30)	*0.002*	68 (38)	6 (33)	*0.58*
Smoker, n (%)						
Current	1 (2)	7 (5)		7 (4)	1 (6)	
Past	18 (33)	38 (26)		52 (29)	4 (22)	
Never	36 (65)	99 (69)	*0.51*	122 (67)	13 (72)	*0.65*
Ever used hormones, n (%)						
Yes	42 (76)	100 (69)		129 (71)	13 (72)	
No	13 (24)	44 (31)	*0.33*[a]	52 (29)	5 (28)	*0.93*[a]

Data shown are means and standard deviations (continuous data) and frequencies and percentages (categorical data; questionnaire data were unavailable for one individual (who was an equol non-producer and an ODMA producer); percentages may not add to 100 % due to rounding);

P values calculated using t-tests (continuous data), or chi square test (categorical data; denoted by [a]) and Fishers exact test (categorical data).

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Table 2

Spine, hip, femoral neck, and head bone mineral density (BMD) and body composition by daidzein-metabolizing phenotypes

	Unadjusted, mean (95 % CI)			Adjusted, mean (95 % CI)a		
Measure	**Equol-producers**	**Equol non-producers**	**p**	**Equol-producers**	**Equol non-producers**	**p**
BMD (g/cm^2)						
Spine (L1-L4)	1.04 (1.04,1.07)	1.04 (1.02,1.06)	*0.90*	1.01 (0.96,1.06)	0.99 (0.94,1.05)	*0.38*
Total hip	0.95 (0.92,0.98)	0.94 (0.93,0.96)	*0.64*	0.93 (0.89,0.97)	0.91 (0.87,0.96)	*0.22*
Femoral neck	0.84 (0.81,0.87)	0.83 (0.81,0.84)	*0.59*	0.83 (0.79,0.88)	0.82 (0.77,0.87)	*0.33*
Head	2.39 (2.31,2.47)	2.33 (2.28,2.38)	*0.22*	2.41 (2.26,2.57)	2.36 (2.20,2.52)	*0.31*
Body composition						
Whole body fat (kg)	24.23 (21.61,26.84)	24.28 (22.76,25.80)	*0.97*	23.72 (21.73,25.72)	22.78 (20.63,24.94)	*0.21*
Whole body lean (kg)	45.38 (43.68,47.01)	45.98 (44.97,46.98)	*0.54*	44.84 (42.55,47.14)	45.05 (42.59,47.51)	*0.80*
Whole body % fat	33.62 (31.62,35.62)	33.54 (32.41,34.67)	*0.94*	33.19 (30.78,35.59)	32.24 (29.65,34.82)	*0.29*
	ODMA-producers	ODMA non-producers	p	ODMA-producers	ODMA non-producers	p
BMD (g/cm^2)						
Spine (L1-L4)	1.04 (1.03,1.06)	1.02 (0.95,1.08)	*0.38*	1.07 (1.03,1.10)	1.06 (1.00,1.12)	*0.77*
Total hip	0.95 (0.93,0.96)	0.92 (0.87, 0.98)	*0.35*	0.94 (0.92,0.97)	0.94 (0.90,0.99)	*0.99*
Femoral neck	0.83 (0.82,0.85)	0.80 (0.76,0.85)	*0.27*	0.86 (0.83,0.89)	0.85 (0.80,0.90)	*0.69*
Head	2.36 (2.31,2.4)	2.27 (2.07,2.46)	*0.24*	2.31 (2.22,2.40)	2.32 (2.16,2.47)	*0.97*
Body composition						
Whole body fat (kg)	24.34 (22.97,25.72)	23.47 (18.96,27.98)	*0.71*	25.20 (23.87,26.53)	25.93 (23.89,27.97)	*0.45*
Whole body lean (kg)	46.12 (45.24,47.00)	42.73 (39.31,46.16)	*0.03*	46.17 (45.48,46.86)	45.67 (44.52,46.83)	*0.39*
Whole body % fat	33.47 (32.43,34.51)	34.49 (31.62,37.36)	*0.56*	34.92 (33.03,36.80)	35.16 (32.23,38.08)	*0.87*

T-tests and multiple regression analyses were conducted to assess relationships between daidzein-metabolizing phenotypes bone and body composition measures; Data shown as mean (95 % confidence interval); Adjusted data were adjusted for covariates significantly associated with either bone mineral density or daidzein-metabolizing phenotype, excluding hormone measures [all equol-producer phenotype models adjusted for BMI, Hispanic or Latino, and education, and all ODMA-producer phenotype models adjusted for BMI, height, and race. Individual models adjusted as follows: spine (waist to hip ratio (WHR), ever used hormones (yes/no), age at menarche, number of months breastfed a child/children, equol-producer (EP) n=52, equol non-producer (ENP) n=137 and ODMA-producer (OP) n=172, ODMA non-producer (ONP) n=18); hip (WHR, age at menarche, EP n=53, ENP n=140 and OP n=176, ONP n=118); femoral neck (WHR, age at menarche, income, EP n=53, ENP n=140 and OP n=176, ONP n=18); head (age at menarche, ever pregnant (yes/no), number of induced abortions (EP n=55, ENP n=139 and OP n=177, ONP n=18); total fat (WHR, income, number of induced abortions, age at end of first pregnancy, EP n= 31, ENP n=89 and OP n= 111, ONP n=9); total lean (WHR, number of induced abortions, day of cycle, EP n=53, ENP n=137 and OP n=173, ONP n=18); % fat (WHR, income, age at end of first pregnancy, EP n=31, ENP n=90 and OP n=112, ONP n=9)]