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Obesity prevalence in relation to gut microbial environments capable of producing equol or *O*-desmethylangolensin from the isoflavone daidzein

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Abstract

Background—Studies have observed associations between the gut microbiome and obesity. *O*-desmethylangolensin (ODMA) and equol are gut bacterial metabolites of daidzein, a compound found in high amounts in soy foods. Approximately 80–95% and 25–60% of individuals harbor gut microbial communities capable of producing ODMA or equol, respectively. Given that other phenotypes of gut bacterial metabolism of dietary compounds have been associated with obesity, we hypothesized that daidzein-metabolizing phenotypes would be associated with obesity.

Objective—To compare the prevalence of ODMA-producer and equol-producer phenotypes in obese, overweight, and normal weight individuals.

Methods—Adults aged 18 to 95 years (n=297) provided a first-void urine sample after a three-day soy challenge, and urinary ODMA and equol concentrations were used to classify individuals as producers or non-producers. Body mass index was calculated from self-reported weight and height.

Results—There were 60 ODMA non-producers and 173 equol non-producers. Obese individuals were 2.8-times more likely to be ODMA non-producers (OR=2.8, 95% CI: 1.2, 6.2) compared to normal weight individuals, when adjusted for age, race (white vs. non-white), and gender and menopausal status (male, premenopausal female, and postmenopausal female). Obesity was not associated with equol-producer phenotype (OR=1.1, 95% CI: 0.5, 2.2). Stronger associations with obesity were observed in the ODMA non-producers who were also equol producers than equol non-producers.

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CONFLICT OF INTEREST

The authors have no competing financial interests in relation to this work.

AUTHOR CONTRIBUTIONS

CLF, CA, KW, and JWL contributed to the data acquisition. CLF drafted the article. All authors contributed to the concept and design, interpretation of data, revision of the article for important intellectual content, and approved the final version to be published.

Conclusions—Results from this analysis suggest that the ODMA-producer phenotype, but not equol-producer phenotype, is associated with obesity in adults. These results support further work to replicate these findings and evaluate mechanisms of the observed associations.

Keywords

gut; obesity; microbiome; daidzein; equol; O-desmethylangolensin

INTRODUCTION

The host gut microbiome is an extensive community of microorganisms, including bacteria, fungi, and viruses ¹. The overall effect of this microbiome is a complex system of microorganism-microorganism and host-microorganism interactions. Interindividual differences in gut microbial profiles exist ², and these differences translate to differences in functional capabilities of the microbiome across individuals and have the potential to influence host health. Evidence from both human and animal studies supports that the microbiome is related to obesity ².

Daidzein, an isoflavone present in soy foods ³, can be metabolized by gut bacteria to O-desmethylangolensin (ODMA) and equol (Figure 1). Daidzein and equol are structurally similar to mammalian estrogens. Many studies have evaluated the estrogenic and anti-estrogenic effects of equol; *in vitro* and *in vivo* anti-estrogenic effects of equol include binding to estrogen receptors, inhibition of stimulatory effects of dihydrotestosterone, and scavenging free radicals ⁴. The structure of ODMA is less similar to estrogen than daidzein and equol because one of the phenolic rings is cleaved ^{5, 6}. However, *in vitro* evidence suggests that ODMA exerts androgen receptor antagonistic activity and agonistic activity towards estrogen receptor (ER)- α and ER β .

The presence of ODMA or equol in urine after daidzein consumption serves as a marker of a gut microbial environment that supports ODMA- or equol-producing bacteria ⁷. The prevalence of equol producers in the population ranges from approximately 25–60%, with a higher prevalence observed in studies conducted in Asian populations, such as Japan and China ^{7, 8}. Studies suggest that the prevalence of ODMA producers in the population is more than 80% ^{5, 7, 9, 10}. Some evidence suggests that risk factor profiles for breast cancer, prostate cancer, and cardiovascular disease differ across producers and non-producers. Associations of health effects and each of the phenotypes have been comprehensively reviewed elsewhere ^{4, 5, 11, 12}.

Gut bacteria also metabolize lignans, compounds found in plant foods such as grains, legumes, and seeds, to enterolignans (enterolactone and enterodiol). High urinary concentrations of enterolactone or enterodiol, adjusted for diet, suggest gut microbial environments capable of high levels of lignan biotransformation. A difference in obesity prevalence in relation to these lignan-metabolizing phenotypes was observed in a recent study of US adults and children ¹³. Obese individuals were 42% less likely to have high urinary enterodiol concentrations. Overweight and obese individuals were also 34% and 64% less likely to have high urinary enterolactone concentrations, respectively. Given this

observation, we hypothesized that other microbial phenotypes would be associated with obesity.

In earlier work on daidzein-metabolizing phenotypes, we collected data on weight and height, but did not specifically analyze anthropometry in relation to the phenotypes¹⁴. At the time of the parent study, evaluating obesity in relation to the microbiome was in its infancy as a line of research, and evaluating obesity *per se* in relation to the phenotypes was not part of the original study objectives. The size of the study and manner in which the daidzein-metabolizing phenotypes were measured provides an excellent source of data for evaluating whether daidzein-metabolizing phenotypes are associated with obesity. The objective of this work was to build on our earlier observation and evaluate daidzein-metabolizing phenotypes in relation to categories of overweight and obese in adults.

MATERIALS AND METHODS

Participants were from a study that evaluated familial aggregation and segregation of daidzein-metabolizing phenotypes, and details are published elsewhere¹⁴. Briefly, for this study we analyzed data from adults aged 18 to 95 years who had provided self-reported weight and height, and who had information on ODMA-producer and equol-producer phenotypes (see below). Taking antibiotics in the three months prior to the study was an exclusion criterion.

In order to classify individuals as ODMA producers or equol producers, each individual consumed a commercial soy bar (Revival, Kernersville, NC) or one-third of a bag of soy nuts (Genisoy, San Francisco, CA) once per day for three days, and collected a spot urine sample on the morning of the fourth day. Information provided from the manufacturers indicated that the soy bars contained ~83 mg daidzein and the package of soy nuts contained ~10 mg daidzein. The difference in daidzein dose between the two foods did not bias phenotype determination because producers were identified based on the presence of equol or ODMA in urine, not a specific concentration. Urine samples were shipped to the laboratory for analysis. Prior testing demonstrated that daidzein and metabolite compounds were stable in urine for a two-week testing period (the stability was not tested longer than two weeks), as detailed elsewhere¹⁴. Urine was stored at -20°C until analysis, and compounds were measured using gas chromatography-mass spectrometry, as detailed elsewhere¹⁵.

Male and female adults ages 18 to 95 (mean=48, SD=15) were included in this study, and analyses were adjusted for age. Age was considered as a continuous variable in regression models. BMI (kg/m²) was categorized as normal weight (BMI 18.5 to 24.9), overweight (BMI 25.0 to 29.9), and obese (BMI ≥30.0), according to World Health Organization criteria¹⁶. The number of individuals who were underweight (BMI<18.5) was too small to make meaningful comparisons (n=7), and underweight individuals were excluded from our analyses. Associations between phenotypes and BMI were modeled with logistic regression. Unadjusted models and models adjusted for gender and menopausal status (males, premenopausal females, and postmenopausal females), race (white vs. non-white), and age (years) were evaluated. Generalized estimating equations (GEE) were used to account for

the correlative nature of the familial data. In regression analyses, the producer phenotype was considered as the reference category because of its higher frequency than the non-producer phenotype. Odds ratios are presented with 95% confidence interval (CI). Data analysis was conducted with Stata (StataCorp, version MP 11.2).

RESULTS

Twenty percent of participants were ODMA non-producers and 58% of participants were equol non-producers. Age and race did not differ across non-producers and producers for either phenotype (Table 1). A smaller proportion of males than females were ODMA producers. A smaller proportion of premenopausal females were equol non-producers, compared to postmenopausal females. Being an ODMA non-producer was positively, but not significantly, associated with being above average height (OR=1.90, 95% CI: 0.94, 3.83) and above average weight (OR=1.82, 95% CI: 0.98, 3.35). Equol-producer phenotype was not significantly associated with being above average height or weight ($p>0.05$). Equol-producer phenotype and ODMA-producer phenotype were not significantly associated with each other ($\chi^2=2.40$, $p=0.12$).

Obese individuals were approximately three-times more likely to be ODMA non-producers than normal weight individuals (OR=2.8, 95% CI: 1.2, 6.2), adjusted for age, race, and gender and menopausal status (Table 2). Obesity was not associated with the equol-producer phenotype (OR=1.1, 95% CI: 0.5, 2.2).

The association of obesity and being an ODMA non-producer was stronger in the equol producers than in the equol non-producers, adjusted for age, race, and gender and menopausal status (Table 3). Individuals who were equol producers and obese had approximately 9-fold higher odds of being ODMA non-producers than ODMA producers (OR=8.7, 95% CI: 2.5, 30.8). Whereas, ODMA-producer phenotype was not associated with obesity within the equol non-producers (OR=1.5, 95% CI: 0.5, 4.7).

DISCUSSION

The gut microbiome is an important factor in food and nutrient metabolism, and interindividual differences in the composition of the gut microbiome influence nutrient exposure in the host. Interindividual differences in the microbiome are proposed to influence host factors such as obesity through, for example, an increase in energy extraction from non-digestible food components, alterations in gut permeability, gut hormone release in response to gut microbial metabolites, and an impact on host metabolism signaling molecules^{2, 17}. In other work, high urinary concentrations of enterolignans (gut microbial metabolites of phytoestrogen lignans) were associated with obesity¹³. Specifically, after adjustment for demographics and intakes of energy, grain, vegetable, fruit, meat, and dairy, high urinary enterodiol concentration was associated with 18% and 42% lower likelihood of being overweight and obese, respectively. High urinary enterolactone concentration was associated with 24% and 64% lower likelihood of being overweight and obese, respectively. Daidzein is also a phytoestrogen that is metabolized to different extents between individuals, and we hypothesized that daidzein-metabolizing phenotypes (characterized by the ability to produce

equol and / or ODMA) would be associated with obesity in adults. We observed that obese adults were more likely to be ODMA non-producers than normal weight adults, but there was no difference across equol-producer phenotype in obese, overweight, and normal weight individuals.

Associations between daidzein-metabolizing phenotypes and health may be related to microbial profiles associated with the ability or inability to produce daidzein metabolites. In the study sample used for this analysis, weekly servings of soy foods was less than two servings/week (previously published in Frankenfeld et al 2004¹⁸). Given this low exposure to soy, the likely mechanism of influence on obesity is through microbial profiles associated with daidzein metabolism. Not all individuals harbor gut microbial profiles capable of metabolizing daidzein to equol or ODMA. The prevalence of equol producers in the population ranges from approximately 25–60%, with a higher prevalence observed in studies conducted in Asian populations, such as Japan and China^{7, 8}. The ODMA-producer phenotype has been less studied than the equol-producer phenotype, but studies suggest that the prevalence of ODMA producers in the general population is more than 80%^{5, 7, 9, 10}. There are several possible reasons for geographic variations in equol-producer phenotype prevalence, including study design differences (e.g., serum vs. urine measurements, absolute vs. relative amount of compounds) or the physical location influencing the gut microbial environment. Geographic variation in microbiota was observed in Japanese residents, both comparing urban and rural elderly Japanese¹⁹ and comparing elderly Japanese with their Japanese-American counterparts²⁰. More recently, researchers have observed differences between residents of France, Germany, Italy and Sweden²¹ as well as rural and urban areas of China²².

The ODMA-producer phenotype has been associated with disease risk factors in some studies. For example, in a low-soy consuming population of overweight, postmenopausal women, ODMA non-producers, compared with ODMA producers, had lower bone mineral density (BMD), lower mammographic density, and lower 2-hydroxyestrone concentrations^{15, 23, 24}. However, in similar work with an also low-soy consuming normal weight, premenopausal women, associations with the ODMA-producer phenotype were null for BMD, urinary estrogen metabolites, and mammographic density^{25–27}. Here, we observed that obese individuals were approximately three-times more likely to be ODMA non-producers in a population that included a wide age range and males, premenopausal females, and postmenopausal females.

The combination of the ODMA-producer and equol-producer phenotypes may represent particular bacterial compositions. Bolca *et al* (2012) reviewed the role of polyphenol-metabolizing phenotypes (metabotypes), which includes the metabolism of isoflavones, flavonols, and other compounds, and highlighted that an overall metabotype may be an important contributor to the health effects of polyphenols²⁸. We observed that the relationship between obesity and ODMA non-producer phenotype was stronger in the equol producers. However, the number of individuals in some groups after stratification by equol-producer phenotype was small and these results should be interpreted cautiously.

The ODMA-producer phenotype (or, conversely, ODMA non-producer phenotype) may be a marker of harboring bacteria with particular functional capacity, and the particular bacterium or consortia of bacteria may be important for disease risk. Daidzein metabolism to ODMA or equol requires several steps^{5, 7}. For example, a C-ring cleavage step is required to biotransform daidzein to ODMA, but not to transform daidzein to equol. Bacteria that have been observed to biotransform daidzein to ODMA *in vitro* include *Eubacterium ramulus*²⁹ and *Clostridium sp* HGH 136³⁰. *E. ramulus* has also been observed to metabolize other polyphenolic compounds to phenolic acids *in vitro*³¹. The presence of ODMA-producing bacteria may be relevant in the biotransformation of other polyphenolic compounds associated with human disease risk or in the production of other metabolic signaling molecules that may influence adiposity; this hypothesis could be evaluated in future studies. Microbial diversity has been associated with obesity in other studies³². It is possible that the ODMA-producer phenotype reflects differences in microbial diversity across producers and non-producers, and it is this diversity that is related to obesity. Differences in microbial diversity across producers and non-producers have not been studied, but this could be considered in future work.

ODMA and equol are of interest in cancer and cardiovascular research because of their structural similarity to mammalian estrogens^{5, 7}. Many studies have evaluated the estrogenic and anti-estrogenic effects of equol, and several reviews detail *in vitro* and *in vivo* effects^{4, 33-41}. Compared to equol, the structure of ODMA is less similar to estrogen because one of the phenolic rings is cleaved. *In vitro* evidence suggests the ODMA exerts androgen receptor antagonistic activity, but does not exert agonistic or antagonistic activity toward glucocorticoid receptive, thyroid hormone (TR)- α_1 , or TR β ⁴². Results of *in vitro* studies also suggests the ODMA exerts agonistic activity towards estrogen receptor (ER)- α and ER β , as well as effects on cancer cell growth and integrity⁴³. However, evidence suggests that ODMA itself does not exert sufficient biological action *in vivo* to influence disease risk, due to low circulating concentrations or low activity⁵. These observations, along with the low soy exposure in this population, support that associations of ODMA-producer phenotype and obesity are more likely related to gut microbial environments than circulating ODMA concentrations.

There are some limitations to the analysis. One, the study relied on self-reported height and weight, and a reporting bias may be present. However, because there is no clinical test for ODMA or equol producer status, individuals are unaware of their phenotypes and any reporting bias is unlikely to be related to their phenotype. The effect of such non-differential bias is that odds ratios tend to be biased towards the null and our observed point estimates may be conservative. Two, the study was cross-sectional in design, and temporal sequence cannot be determined. It is unknown whether the ODMA-producer phenotype preceded obesity or vice versa; however, evidence suggests that daidzein-metabolizing phenotypes may be established early in life and that the phenotype remains relatively stable in adults^{9, 44}. The study was also a family-study design, and the possibility of intrafamily correlations exists. This analysis statistically accounted for intrafamily correlations. The prevalence of ODMA and equol producer is similar to other published studies, suggesting the family study design did not bias selection based on phenotypes. If ODMA-producer

phenotype precedes obesity, this study provides a foundation for evaluating if lack of ODMA-producing bacteria influences the onset of obesity. Another limitation of the study was the small sample size after creation of subgroups, and the association between the combined equol-producer and ODMA-producer metabolites could be further evaluated in larger studies.

There are also strengths to the analysis. Evidence regarding health conditions in relation to daidzein-metabolizing phenotypes is limited. One critical reason for limited evidence that the phenotypes needs to be determined in the presence of daidzein consumption, which can be done in populations that regularly consume soy or in study samples that have undergone a soy challenge. Secondary analyses of studies in low-soy consuming populations may be prone to false-negative non-producer phenotype classification. A strength of the present study is that daidzein-metabolizing phenotypes were measured after a soy challenge. Another reason is that the study was designed to evaluate the phenotypes, and individuals who were taking antibiotics were excluded. Antibiotics would alter the gut microbial environment and could lead to misclassification of producer phenotype. Three, the prevalence of ODMA producers in the general population is high (80–90%)⁷, which creates a need for a large sample in order to obtain a sufficient number of ODMA non-producers. Despite that the sample size was too small to adequately evaluate subgroups of combined equol- and ODMA-producer phenotypes, the analysis was a relatively large sample for the individual phenotypes. This study population also included a broad distribution of age and representation from both genders and menopausal status. It is unlikely that sources of bias fully explain our observed relationship between the ODMA-producer phenotype and obesity.

As an extension of other work that observed obesity prevalence to differ across individuals with high and low urinary enterolignan concentrations¹³, the hypothesis for this study was that daidzein-metabolizing phenotypes are related to obesity. We observed that obese individuals were more likely to be ODMA non-producers. However, a significant association of obesity and equol-producer phenotype was not observed. A hypothesis generated from this analysis is that there may specific components of the ODMA-producer phenotype or that ODMA non-producers are lacking related to underlying gut microbial community that are associated with obesity. The results of this study support further evaluation of obesity and other health outcomes in relation to ODMA-producer phenotype, as an overall phenotype or stratified by other phenotypes.

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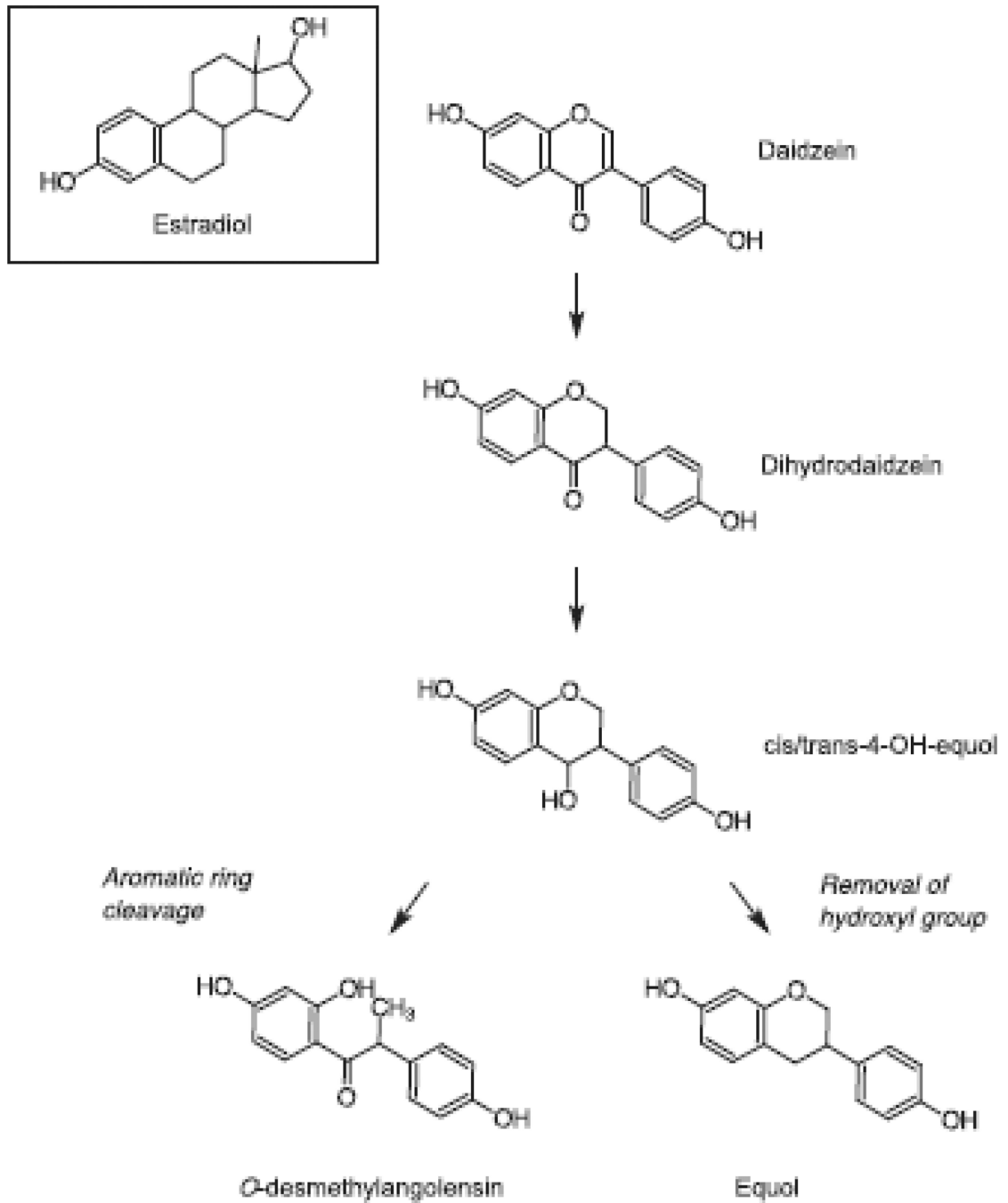


Figure 1. Selected metabolic steps and notations for the biotransformation of daidzein to equol and *O*-desmethylangolensin. Adapted from Heinonen *et al* (1999)⁶

TABLE 1

Mean and percentage¹ of age, sex, race, height, and weight, in relation to ODMA-producer and equal-producer phenotypes in 297 adults

Characteristics	ODMA producers (n=237)	ODMA non-producers (n=60)	Equal producers (n=124)	Equal non-producers (n=173)
Sex				
Male	78 (32.9)	26 (43.3)	42 (33.9)	62 (35.8)
Pre-menopausal female	101 (42.6)	17 (28.3)	54 (43.6)	64 (37.0)
Post-menopausal female	58 (24.5)	17 (28.3)	28 (22.6)	47 (27.2)
Race				
White	215 (90.7)	50 (83.3)	111 (89.5)	154 (89.0)
Non-white	22 (9.3)	10 (16.7)	13 (10.5)	19 (11.0)
Height ²				
Below or at population average	72 (30.4)	10 (16.7) *	30 (24.2)	52 (30.1)
Above population average	165 (69.6)	50 (83.3) *	94 (75.8)	121 (69.9)
Weight				
Below or at population average	195 (82.3)	41 (68.3) *	103 (83.1)	133 (76.9)
Above population average	42 (17.7)	19 (31.7) *	21 (16.9)	40 (23.1)

¹ Column percentages

² Height and weight were divided into two categories. Below or above average height and weight were calculated separately for females (average height=63.8 inches, average weight=166.2 lbs) and males (average height=69.3 inches and average weight=195.5 lbs)(22)

* p<0.05 compared to producers

TABLE 2

Relationship of body mass index to the ODMA-producer phenotype and equol-producer phenotype in 297 adults

Phenotype	18 to <25 kg/m ²	25 to 29.9 kg/m ²	30+ kg/m ²	p-trend
All				
ODMA producers, n (%)	142 (59.9)	71 (30.0)	24 (10.1)	
ODMA non-producers, n (%)	29 (48.3)	17 (28.3)	14 (23.3)	
OR ¹	Reference	1.1 (0.6, 2.2)	3.0 (1.4, 6.5)	0.014
OR ²	Reference	1.0 (0.5, 2.1)	2.8 (1.2, 6.2)	0.032
All				
Equol producers, n (%)	77 (62.1)	32 (25.8)	15 (12.1)	
Equol non-producers, n (%)	94 (54.3)	56 (32.4)	23 (13.3)	
OR ¹	Reference	1.4 (0.8, 2.4)	1.1 (0.6, 2.3)	0.400
OR ²	Reference	1.3 (0.7, 2.2)	1.1 (0.5, 2.2)	0.629

¹Unadjusted odds ratios for the comparison of being a non-producer to a producer

²Odds ratios adjusted for age (in years), race (non-white or white), and gender and menopausal status (male, premenopausal female, postmenopausal female)

TABLE 3

Relationship of body mass index to the ODMA-producer phenotype stratified by equol-producer phenotype in 297 adults

Phenotype	18 to <25 kg/m ²	25 to 29.9 kg/m ²	30+ kg/m ²	p-trend
Equol producers				
ODMA producers, n (%)	63 (67.0)	25 (26.6)	6 (6.4)	
ODMA non-producers, n (%)	14 (46.7)	7 (23.3)	9 (30.0)	
OR ¹	Reference	1.3 (0.5, 3.6)	7.2 (2.2, 23.6)	0.003
OR ²	Reference	1.1 (0.33, 3.3)	8.7 (2.5, 30.8)	0.001
Equol non-producers				
ODMA producers, n (%)	79 (55.2)	46 (32.2)	18 (12.6)	
ODMA non-producers, n (%)	15 (50.0)	10 (33.3)	5 (16.7)	
OR ¹	Reference	1.1 (0.4, 2.6)	1.6 (0.5, 4.9)	0.455
OR ²	Reference	1.0 (0.4, 2.5)	1.5 (0.5, 4.7)	0.585

¹Unadjusted odds ratios for the comparison of being a non-producer to a producer

²Odds ratios adjusted for age (in years), race (non-white or white), and gender and menopausal status (male, premenopausal female, postmenopausal female)