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A cross-sectional study of the gut microbiota associated with urinary and serum equol production status in a general population of Japanese men

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ABSTRACT

Equol is a metabolite produced by the gut microbiota from the soy isoflavone daidzein. Previous studies identified bacteria capable of converting daidzein to equol. We investigated whether equol producers among Japanese with a high soy intake contained these bacteria. We also examined differences in equol production status between urine and serum and how the gut microbiota differs between these statuses. To minimize the potential confounding effects of hormonal variability in women, this cross-sectional study analyzed 853 Japanese men. Urinary and serum isoflavones were collected in the morning after fasting and were analyzed using LC-MS/MS. By applying a finite mixture model for each $\log_{10}$ equol/daidzein ratio, we defined equol producers and non-producers from urine and serum. Among 669 participants with fecal microbial measurements, the 16S rRNA gene was sequenced on a MiSeq System. The cut-off values for the $\log_{10}$ equol/daidzein ratio were −0.94 for urine and −0.95 for serum. Equol production status in urine and serum matched in 97 %, and equol producers from urine or serum were 42 %. The microbiota was more diverse in producers than in non-producers; the genus *Senegalimassilia* included strains with high sequence identity (>98 %) to daidzein reductase. The family Oscillospiraceae and class *Clostridia* also had approximately 46 %–48 % sequence identity. The equol production status of fasting urine and serum almost matched among a general population of Japanese men. Although we did not detect a microbiota with known daidzein reductase in equol producers, several shared similar sequences; these may include equol-producing bacteria that have not yet been identified.

1. Introduction

Equol, a terminal metabolite of daidzein, a soy isoflavone, has higher affinity for estrogen receptor beta than its precursor daidzein, exhibiting stronger estrogen-like activity. Its health benefits have been extensively documented, including the attenuation of menopausal symptoms, improved bone health, and reductions in the risks of breast and prostate cancers, dyslipidemia, and hypertension (Sekikawa et al., 2019, 2022). However, equol production depends on intestinal bacteria that are capable of metabolizing intermediate products, such as dihydrodaidzein or tetrahydrodaidzein. This capability classifies individuals as equol producers or non-producers. Approximately 50 %–60 % of Asians are equol producers, in contrast to only 20 %–30 % of Europeans and Americans (Messina et al., 2006), which may be attributed to the higher

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intake of soy isoflavones in Asian diets (25–50 mg/day in Japan versus <3 mg/day in the USA) (de Kleijn et al., 2001; Messina, 2016).

Previous studies demonstrated that the distribution of the urinary equol/daidzein ratio is bimodal, even in individuals consuming a typical diet without a soy load (Ideno et al., 2018). While equol is present in both blood and urine, it currently remains unclear whether blood-based measurements yield a similar equol production status. Moreover, limited information is currently available on the concordance between urinary- and blood-based measurements of equol production.

The intestinal bacteria that metabolize daidzein to dihydrodaidzein, tetrahydrodaidzein, and equol continue to be identified in isolation and identification experiments (Mayo et al., 2019). Equol-rich supplements are commercially available. These supplements have been produced by the equol-producing bacteria (Yee et al., 2008). However, it has yet to be established whether these bacteria are present in the intestines of Asians, approximately 50 % of whom are known to be equol producers. Furthermore, it remains unclear whether *Lactococcus garvieae* 20–92 and the genera *Eggerthella*, *Adlercreutzia*, and *Slackia* belonging to the family Eggerthellaceae, which are equol-producing bacteria, are all major inhabitants of the human intestines (Iino et al., 2019; Kodera et al., 2023). Therefore, a more detailed understanding of the gut microbiota in a large human sample will provide insights into the equol-producing bacteria that inhabit the intestines of equol producers.

The present study aims to address critical gaps in our knowledge of equol production. Therefore, we investigated whether the equol production status, assessed from urine and serum, was concordant in a general population of Japanese men. We then examined differences in the composition of the gut microbiota between equol producers and non-producers. To eliminate potential confounders from hormonal fluctuations in women, this study focused on men. We hypothesized that the abundance of specific equol-producing bacteria, such as those identified in previous isolation studies (e.g., *L. garvieae* 20–92 or *Eggerthella*), may be higher in the gut microbiota of equol producers.

2. Methods

2.1. Study participants

Between 2006 and 2008, 1096 randomly selected age-stratified men in their 40s–70s who were residents of Kusatsu, Japan gave their consent to participate in the Shiga Epidemiological Study of Subclinical Atherosclerosis (SESSA) (Kadota et al., 2013). Of these, 853 consented to participate in the 2010–2014 follow-up study (SESSA2). Men with insufficient urine or serum samples were excluded (urine: n = 10, serum: n = 5), and cut-off values for the equol production status were selected. Since there were no duplicates of insufficient samples between urine and serum, the equol production status of all 853 participants was assessed. The gut microbiota between equol production status was analyzed in 669 participants who completed fecal microbial measurements (Fig. 1). The SESSA and SESSA2 studies were approved by the Institutional Review Board of the Shiga University of Medical Science, Otsu, Japan (R2008-061).

2.2. Urinary and serum equol measurements

In SESSA2, urine and serum were collected in a morning visit after a 12-h fast. From collection to equol measurement, samples were stored in a freezer at −80 °C for 8–12 years. Serum separation was performed in tubes with a coagulation-enhancing film, clotted for 60 min, and centrifuged at 1700 g-force, 4 °C, for 15 min. Urine samples were treated without additives prior to freezing. Urine and serum concentrations of equol, daidzein, and genistein were measured at Saga Nutraceuticals Research Institute, Otsuka Pharmaceutical Co., Ltd. The sample preparation procedure was reported elsewhere (Fleck et al., 2016; Zhang et al., 2022). In brief, cryopreserved urine and serum, after a deconjugation treatment, were cleaned using Isolute SLE+ 96-well plates (Biotage, Uppsala, Sweden) and subjected to measurements using liquid chromatography-tandem mass spectrometry (Triple Quad 5500+ mass

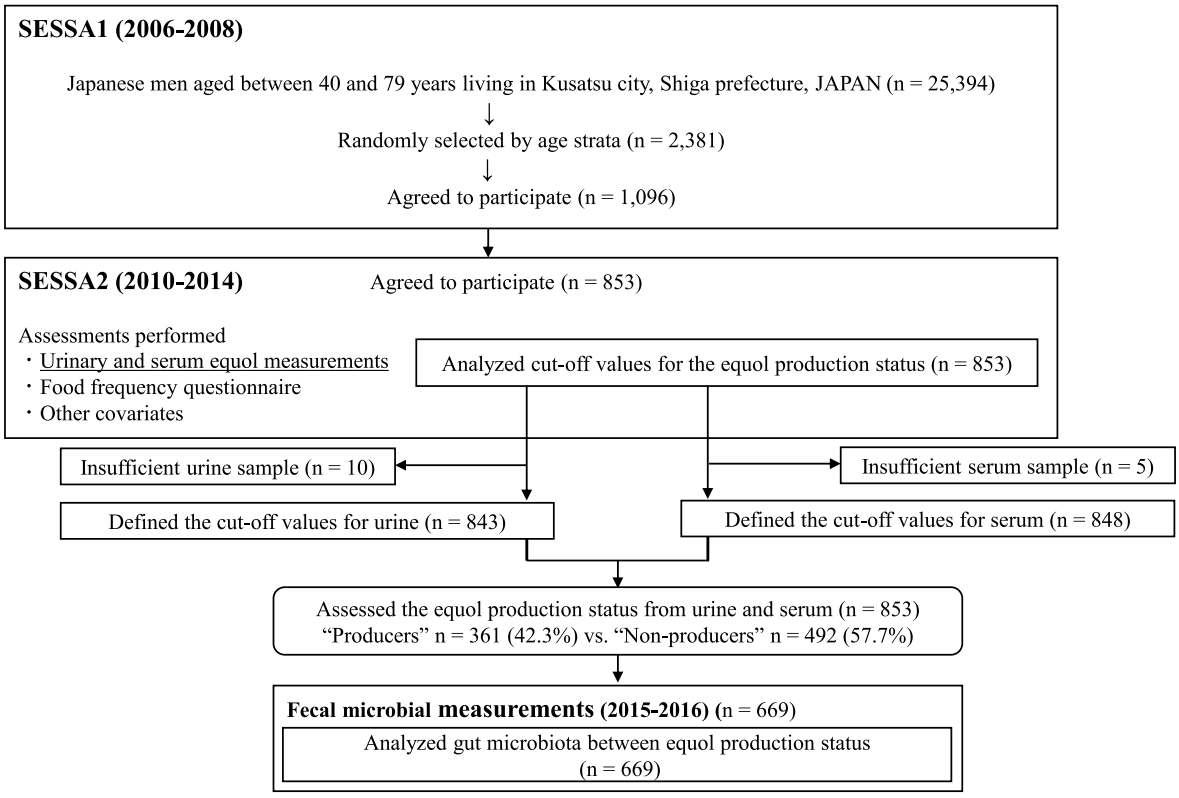


Fig. 1. Flow diagram of participants.

spectrometer, AB SCIEX) with an atmospheric pressure chemical ionization ion source in the negative ion mode and reversed-phase columns on the Ultra High Performance Liquid Chromatography system (Urine: Sunshell C18, 2.6 μm 2.1 mm × 100 mm; Serum: Sunshell C18, 2.6 μm 3.0 mm × 100 mm; ChromaNik Technologies, Inc.; Nexera X2, Shimadzu). The limits of detection (LOD) were 17.59 nmol/L for urinary genistein, 19.67 nmol/L for urinary daidzein, 4.13 nmol/L for urinary equol, 3.52 nmol/L for serum genistein, 3.93 nmol/L for serum daidzein, and 0.413 nmol/L for serum equol.

Coefficient of variations (CV) in the urine analysis were 6.9 %, 2.4 %, and 2.4 % for equol 3 ng/mL, 250 ng/mL, and 7500 ng/mL, respectively; 5.4 %, 2.2 %, and 1.7 % for daidzein 15 ng/mL, 500 ng/mL, and 15000 ng/mL, respectively; 4.6 %, 1.9 %, and 2.0 % for genistein 15 ng/mL, 500 ng/mL, and 15000 ng/mL, respectively. CV in the serum analysis were 12.8 %, 2.4 %, and 3.1 % for equol 0.3 ng/mL, 15 ng/mL, and 375 ng/mL, respectively; 6.0 %, 2.1 %, and 2.8 % for daidzein 3 ng/mL, 30 ng/mL, and 700 ng/mL, respectively; 7.1 %, 1.9 %, and 2.4 % for genistein 3 ng/mL, 30 ng/mL, and 700 ng/mL, respectively. The same standard urine and standard serum samples were used in each analysis, and all were confirmed to be within ±15 % of the standard. Daily CV for standard urine samples were 3.8 % for equol, 4.8 % for daidzein, and 4.2 % for genistein, and those for standard serum samples were 3.4 % for equol, 3.6 % for daidzein, and 4.1 % for genistein.

2.3. Assessment of the equol production status

The method is described in detail elsewhere (Ideno et al., 2018). Briefly, we applied finite mixture models to estimate the cut-off point for defining the equol production status for urine and serum. In this model, two normal distributions are assumed, and the cut-off value is the minimum misclassification of the combined false-positive and false-negative probabilities. First, we assigned each value of LOD/2 to participants with no detectable daidzein or equol (9.84 nmol/L for urinary daidzein, 1.97 nmol/L for serum daidzein, 2.07 nmol/L for urinary equol, and 0.21 nmol/L for serum equol). Second, the distribution of the $\log_{10}$ equol/daidzein ratio was drawn for urine and serum using the SAS FMM procedure to form a finite mixture model of two normal distributions. Finally, the cumulative distribution function estimated the value at which the misclassification of the two normal distributions was minimized. This value was used as the cut-off value, and those above and below the cut-off value were considered to be "producers" and "non-producers" respectively.

2.4. Fecal microbial measurements

Methods for gut microbial measurements are described elsewhere (Okami et al., 2024). Briefly, participants collected stool samples at home using a brush-type fecal collection kit (TechnoSuruga Laboratory Co., Ltd., Shizuoka, Japan). The V3-V4 region of the 16S ribosomal RNA gene in feces was amplified using the primer pair 341F/805R in a two-step tailing polymerase chain reaction method and the Illumina MiSeq platform for a sequencing analysis. We used QIIME2 (version 2023.2) software (Bolyen et al., 2019) to perform quality filtering, noise removal with DADA2 (Callahan et al., 2016), and feature table creation from raw Illumina FASTQ files. Taxonomy was estimated based on the SILVA 138 database (Bokulich et al., 2018; Robeson et al., 2020).

2.5. Other covariates

All information on the other covariates described in the present study was collected in SESSA2. The body mass index (BMI) was calculated by measuring height and weight without shoes and wearing light clothing at the time of the visit. Blood pressure was measured twice after 5 min of rest and the average value was used. Fasting blood tests were performed to measure blood glucose and lipid-related markers (Kadota et al., 2013). A self-administered questionnaire was completed on food intake frequencies, the smoking status, alcohol consumption status, and medication use. Responses to questions about food frequencies were categorized as "≤1 day/week", "2–3 days/week", or "≥ 4 days/week". If the total number of missing values was ≤5, they were categorized as "≤1 day/week". Participants who responded "≤1 day/week" to questions on all four soy foods (*natto*, tofu, fried tofu, and soy milk) were classified as non-consumers of soy foods.

2.6. Statistical analysis

The prevalence of equol producers was calculated using the criterion described above. In comparing characteristics between equol production status, the *t*-test was used for numerical variables, the Cochrane-Armitage test for ordinal variables, and the chi-square test for categorical variables. The value of $p < 0.05$ was considered to be statistically significant. Spearman's rank correlations were used to compare soy isoflavones and equol between urine and serum and to obtain an overview of the relationships between the $\log_{10}$ equol/daidzein ratio and genera. In the gut microbiota analysis, we compared producers and non-producers at the phylum level. We then compared the α-diversity and β-diversity of the 10926 minimum number of reads that reached a plateau from the rarefaction curve. In the α-diversity analysis, we used Observed features and the Shannon index as a measure of richness and evenness to compare diversity within individuals. In the β-diversity analysis, the weighted UniFrac distance and unweighted UniFrac distance were used as quantitative and qualitative measures to compare differences between individuals. We then used the linear discriminant analysis (LDA) effect size (LEfSe) (Segata et al., 2011) to extract genera with the greatest differences between producers and non-producers. LEfSe uses the non-parametric factorial Kruskal-Wallis sum-rank test to identify taxa with a significantly different abundance between groups, followed by LDA to estimate the effect size of each feature of the abundance difference (Okami et al., 2024). We herein excluded rare taxa that were found in <10 % of participants. LEfSe has been applied with default alpha values (0.05) and the LDA effect size has been evaluated by setting the absolute value of the logarithmic LDA threshold to 2.0. To establish whether genera found in high abundance in producers had known daidzein reductase sequences, we searched the National Center for Biotechnology Information (NCBI) Protein to identify any similar features. The Basic Local Alignment Search Tool (BLAST) was used for sequence identity with known daidzein-equol reductase-aligned strains (e.g., *L. garvieae* 20–92) (Altschul et al., 1997).

In the stratified analysis, LEfSe-extracted genera differed between producers and non-producers by age (40–60s, 70–80s), BMI (<25, ≥25), and frequency of soy consumption (≤1 day/week, ≥2 days/week). In the sensitivity analysis, we defined high producers as those whose urine or serum $\log_{10}$ equol/daidzein ratio was higher than the mean value of producers, and genera that differed from non-high producers were extracted by LEfSe. To ensure the validity of the LEfSe results, we also checked Analysis of Composition of Microbiomes (ANCOM) (Mandal et al., 2015) for genera that differed between producers and non-producers. In ANCOM, a threshold was set from the cumulative distribution function for all samples, and genera with W above the threshold were considered to have significant differences. The centered log-ratio (CLR) was used as a measure of magnitude for abundance in producers and non-producers. The MetaCyc pathway database in Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) (Douglas et al., 2020) was employed to predict metabolic pathways for producers and non-producers. Significant metabolic pathways were extracted by ANCOM.

All analyses were performed using the QIIME2 version 2023.2 package (Bolyen et al., 2019), MicrobiomeAnalyst version 2.0 (Chong et al., 2020; Lu et al., 2023), and SAS version 9.4 statistical software (SAS Institute, Cary, NC, USA).

3. Results

3.1. Equol production status

Spearman's correlation matrix of urine and serum for genistein, daidzein, equol, and $\log_{10}$ equol/daidzein ratio in 853 participants is shown in Fig. 2. Correlation coefficients between urine and serum were 0.83 for both genistein and daidzein, 0.92 for equol, and 0.97 for the $\log_{10}$ equol/daidzein ratio, indicating a strong correlation between urine and serum for all parameters. In contrast, the correlations between genistein and equol or daidzein and equol were weak.

The distribution of $\log_{10}$ equol/daidzein ratios for urine and serum is shown in Fig. 3. Spearman's correlation coefficient between the urine and serum ratios was 0.97. Cut-off values of −0.94 and −0.95 were the smallest misclassifications for the ratios of urine and serum, respectively (Fig. 4). A total of 99.2 % (482/486) urinary non-producers were also serum non-producers, while 96.1 % (343/357) of urinary producers were also serum producers (Table 1). Urine- and serum-producing statuses matched in 96.7 % (825/853). In subsequent analyses, participants who were producers by urine or serum were referred to as "Producers" of equol; other participants were called "Non-producers" of equol. Therefore, there were 361 producers (42.3 %) and 492 non-producers (57.7 %) (Fig. 1).

3.2. Characteristics of equol production status

Table 2 shows the urinary and serum soy isoflavone and equol concentrations of producers and non-producers. In both urine and serum, producers had lower concentrations of genistein and daidzein and higher concentrations of equol and the $\log_{10}$ equol/daidzein ratio than non-producers. Table 3 shows the backgrounds of producers and non-producers. The mean age of all participants was 69.1 years (46–83 years). Producers were older and had lower body fat, triglycerides, uric acid, and γ-GPT than non-producers. Table 4 shows food intake frequencies in producers and non-producers. Producers consumed soy foods (soybeans) more frequently than non-producers, particularly fermented soybeans ("*natto*"), boiled soybeans ("*nimame*"), or green soybeans ("*edamame*"), Tofu, frozen tofu, or thick deep-fried tofu ("*atsuage*"), and deep-fried tofu with vegetables ("*ganmodoki*"). Producers consumed seafood (fresh fish), fruit, and seaweed more frequently than non-producers, but less frequently consumed staple foods (rice, bread, and noodles).

3.3. Gut microbiota of equol production status

A total of 11,328,217 quality-filtered sequences were obtained from samples, with a mean of 16,933 non-chimeric sequences per sample. Filtered data were assigned to 16,726 features, 379 genera, and 17 phyla. Following the exclusion of 9 phyla present in less than 10 % of the participants and a mean value of 0.0 % in the current sample, the results for the remaining 8 phyla (*Firmicutes*, *Bacteroidota* (*Bacteroidetes*), *Actinobacteriota*, *Proteobacteria*, *Fusobacteriota*, *Verrucomicrobiota*, *Desulfobacterota*, and *Euryarchaeota*) are shown in Fig. 5. Among the 669 participants who completed fecal microbial measurements, 274 (41.0 %) were producers and 395 (59.0 %) were non-producers. The relative abundance of *Firmicutes* was 56.9 % in producers and 50.9 % in non-producers, while that of *Bacteroidota* was 31.4 % in producers and 34.4 % in non-producers. The ratio of *Firmicutes* to *Bacteroidota* was higher in producers than in non-producers. In the α-diversity analysis, observed features and the Shannon index were both significantly higher in producers than in non-producers ($p < 0.001$) (Fig. 6). In the β-diversity analysis, the weighted UniFrac distance and unweighted UniFrac distance were both significantly higher between producers and non-producers ($p < 0.001$) (Fig. 7). Table 5 shows Spearman's correlation with age adjustments between the $\log_{10}$ equol/daidzein ratio and genera. Urine and serum showed similar relationships with genera. In LEfSe, 27 of the 136 genera detected in more than 90 % of participants significantly differed between producers and non-producers. Of these, 22 had significantly more producers than non-producers and 5 had significantly fewer producers than non-producers (Fig. 8, Table S1). Among the 22 genera with producers exceeding non-producers, there were no equol-producing bacteria that had previously been identified in isolation and identification experiments (Mayo et al., 2019; Setchell & Clerici, 2010). However, *Senegalimassilia anaerobia* and *Senegalimassilia (S.) faecalis*, which belong to the genus *Senegalimassilia*, were listed as bacteria with daidzein reductase sequences in NCBI Protein (Table S2). Furthermore, a BLAST search of the sequence of *L. garvieae* 20–92 (Shimada et al., 2010), which is already commercially available as an equol supplement, revealed 98.6 % homology for *S. faecalis* and 98.3 % for *S. anaerobia* (Table S3). The family Oscillospiraceae and class *Clostridia*, which were frequently detected in producers (Table S3), also had moderately high homologies of 48 % and 46.5 %, respectively.

In the stratified analysis, common genera were observed in both age groups (40–60 years and 70–80 years) (Fig. S1); *UCG-005*, *UCG-002*, Christensenellaceae *R-7* group, [*Eubacterium*]_*coprostanoligenes*_group, [*Eubacterium*]_*siraeum*_*group*, *UCG-010*, *NK4A214*_group, and *Clostridia*_*vadinBB60*_group were more abundant in producers, while [*Ruminococcus*] *gnavus* group was more abundant in non-producers.

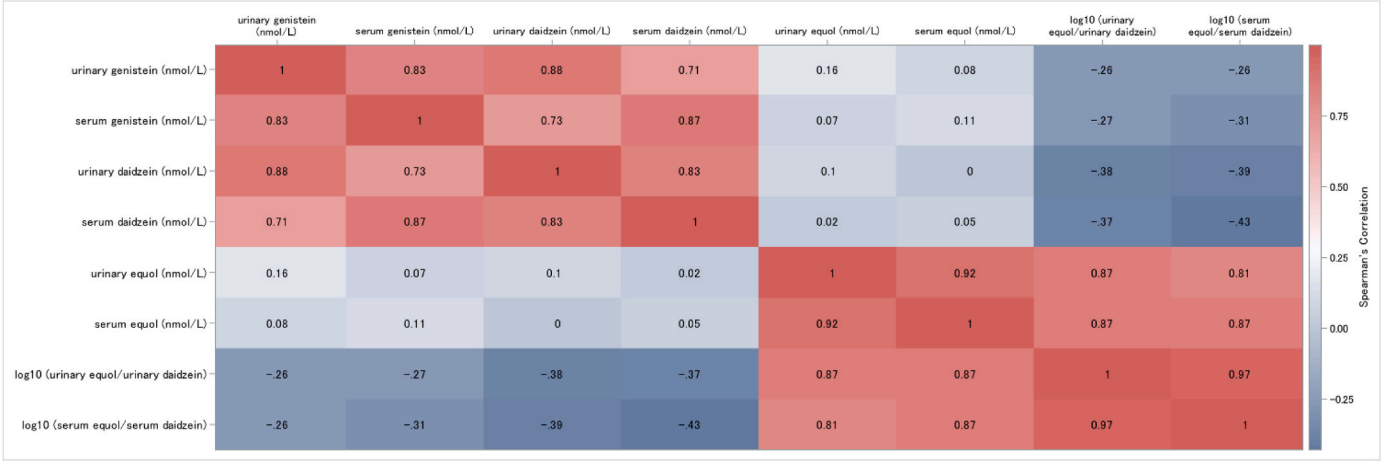
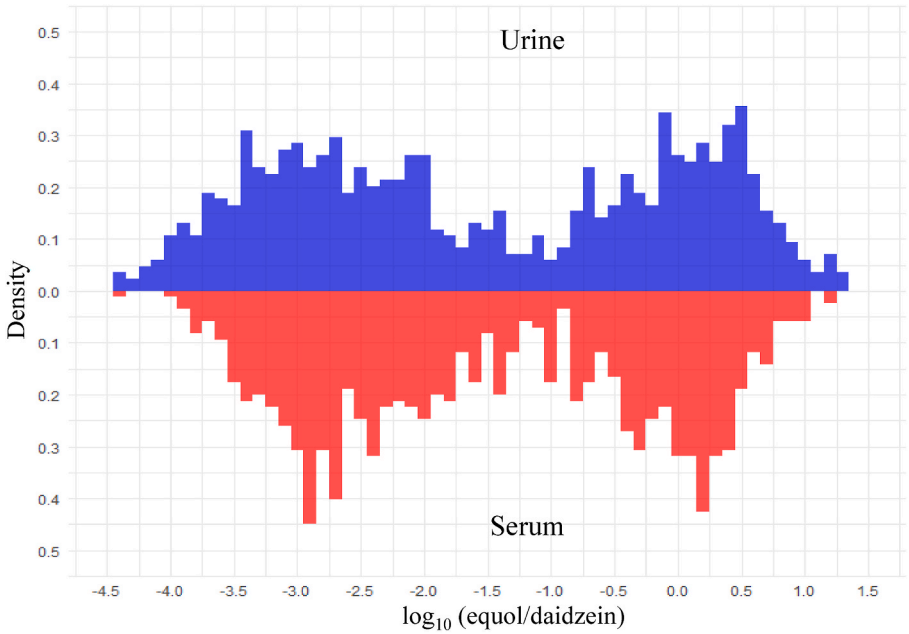


Fig. 2. Spearman's correlation matrix of soy isoflavones between urine and serum in SESSA2 (n = 853). Numbers in the matrix indicate unstandardized regression coefficients (red: positive association, blue: inverse association).



	mean (SD)	median (IQR)	min, max
Urine	-1.52 (1.54)	-1.71 (-2.89, -0.03)	-4.42, 1.34
Serum	-1.47 (1.38)	-1.66 (-2.72, -0.13)	-4.38, 1.21

Fig. 3. Distributions of the $\log_{10}$ (equol/daidzein) ratio in urine and serum in SESSA2 (n = 853). Spearman's Correlation Coefficient = 0.97.

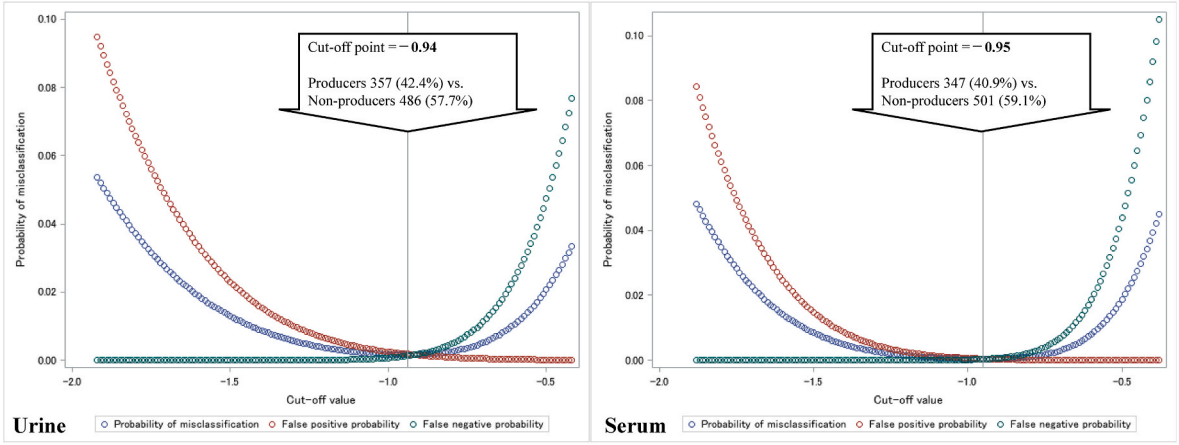


Fig. 4. Probability of misclassification by cut-off values of $\log_{10}$ (equol/daidzein) in SESSA2 (n = 853).

Common genera were observed in both BMI groups (<25 and ≥ 25) (Fig. S2); *UCG-005*, *UCG-002*, Christensenellaceae *R-7* group, [*Eubacterium*]*_coprostanoligenes_*group, *UCG-010*, *Clostridia_vadinBB60_*group, *Subdoligranulum*, *Family_XIII_UCG-001*, *Family_XIII_AD3011_*group, *Senegalimassilia*, and *Butyricimonas* were more abundant in producers, while [*Ruminococcus*] *gnavus* group was more abundant in non-producers. Common genera were also detected in both soy consumption groups (≤1 day/week, ≥2 days/week) (Fig. S3); *UCG-005*, Christensenellaceae *R-7* group, [*Eubacterium*]*_coprostanoligenes_group*, *Subdoligranulum*, and *NK4A214_*group were more abundant in producers, while [*Ruminococcus*] *gnavus* group and *Bacteroides* were more abundant in non-producers. However, the low soy consumption group had a lower number of genera that were abundant in producers than the high soy consumption group. In the sensitivity analysis, 20 genera were significantly more prevalent in high producers (n = 152) than in non-high producers (n = 517), while 6 genera were significantly less prevalent (Fig. S4). Most of these genera were also identified in the main analysis as being associated with either producers or non-producers. The genera that significantly differed between producers and non-producers by ANCOM were nearly identical to those of LEfSe (Fig. S5).

In PICRUSt2, 21 metabolic pathways were extracted (Fig. 9). Of these, 9 metabolic pathways were involved in Cofactor, Carrier, and Vitamin Biosynthesis, 3 each in Degradation/Utilization/Assimilation, and Fatty Acid and Lipid Biosynthesis, 2 each in Generation of Precursor Metabolites and Energy, and Secondary Metabolite Biosynthesis (Terpenoid Biosynthesis), 1 in Nucleic Acid Processing, and 1 in Cell Structure Biosynthesis (Table S4). It is important to note that several metabolic pathways involved in the vitamin K_2 production pathway (PWY-7374, PWY-6263, and PWY-7371) were detected, as well as several metabolic pathways involved in the vitamin B group and

Table 1
Equol production status between urine and serum in SESSA2 (n = 853).

		SESSA2_serum criterion			
		< −0.95 (Non-producers)	≥ −0.95 (Producers)	Missing	
SESSA2_urine criterion	< −0.94 (Non-producers)	482[b]	2[a]	2[b]	486
	≥-0.94 (Producers)	11[a]	343[a]	3[a]	357
	Missing	8[b]	2[a]	0	10
		501	347	5	853

Data are expressed as counts.
Participants who were producers by urine or serum were referred to as "Producers"[a] of equol; other participants were named "Non-producers"[b] of equol.

Table 2
Urinary and serum concentrations of soy isoflavones between equol production status in SESSA2 (n = 853).

	Non-producers n = 492	Producers n = 361	P-value [a]
Urinary genistein, nmol/L	8859 ± 14579	6089 ± 8220	<0.01
Urinary daizein, nmol/L	14587 ± 26005	7470 ± 11674	<0.01
Urinary equol, nmol/L	92 ± 443	11206 ± 18036	<0.01
Log_{10} (urinary equol/ urinary daizein)	−2.70 ± 0.79	0.08 ± 0.54	<0.01
Serum genistein, nmol/L	606 ± 889	429 ± 483	<0.01
Serum daizein, nmol/L	263 ± 428	139 ± 156	<0.01
Serum equol, nmol/L	2 ± 10	159 ± 202	<0.01
Log_{10} (serum equol/serum daizein)	−2.52 ± 0.70	−0.03 ± 0.51	<0.01

Data are expressed as the mean ± standard deviation.
[a] T-test.

methanogenesis pathways.

4. Discussion

In a cross-sectional study on 669 middle-aged and older Japanese men, the equol production status derived from urine and serum were almost identical under daily life without a soy load. Therefore, future epidemiological studies to assess the equol production status may not require the collection of both urine and serum samples from participants. The present study confirmed significant bacterial differences between producers and non-producers. Bacteria capable of converting daidzein to equol are present in the intestines of actual producers. However, bacteria that were significantly more abundant in the microbiota of producers did not include those with a daidzein-reducing function in previous culture experiments (Mayo et al., 2019; Setchell et al., 2009). On the other hand, bacteria with sequences that were very close to the known daidzein reductase sequence were detected, suggesting that these unidentified bacteria may be responsible for metabolism in equol producers.

4.1. Pharmacokinetics of equol in urine and blood

The pharmacokinetics of the oral administration of equol have been clarified at the experimental level (Setchell et al., 2002). Ingested soy isoflavones are present in high concentrations in blood, particularly in portal blood and bile, in the form of conjugates in the liver. They are excreted into the intestinal tract via bile, deconjugated by intestinal bacterial metabolism, and absorbed again for enterohepatic circulation. The blood concentration of equol peaks twice 4–6 h after ingestion, showing the characteristic kinetics of enterohepatic circulation, and decreases by 50 % after 8–9 h (Setchell et al., 2002). Unnecessary soy

Table 3
Characteristics of participants between equol production status in SESSA2 (n = 853).

	Non-producers n = 492	Producers n = 361	P-value
Age, y	68.2 ± 8.6	70.3 ± 8.0	<0.01 [a]
Age group, n (%)			
40–50s	76 (15.5)	34 (9.4)	<0.01 [b]
60s	196 (39.8)	124 (34.4)	
70–80s	220 (44.7)	203 (56.2)	
Body mass index (BMI), kg/ m^2	23.4 ± 2.9	23.3 ± 2.9	0.43 [a]
BMI group, n (%)			
<18.5	22 (4.5)	14 (3.9)	0.75 [b]
18.5–24.9	343 (69.7)	252 (69.8)	
≥25.0	127 (25.8)	95 (26.3)	
Body fat, %	21.7 ± 5.4	20.7 ± 6.0	0.01 [a]
Smoking status, n (%)			
Non	83 (16.9)	79 (21.9)	0.27 [b]
Ex	305 (62.0)	209 (57.9)	
Current 1–20 cigarettes/ day	94 (19.1)	63 (17.5)	
Current >20 cigarettes/ day	10 (2.0)	10 (2.8)	
Drinking status, n (%)			
Non	108 (22.0)	88 (24.4)	0.49 [b]
Ex	25 (5.1)	21 (5.8)	
Current 1–3 days/week	103 (20.9)	65 (18.0)	
Current >3 days/week	256 (52.0)	187 (51.8)	
Systolic blood pressure, mmHg	131.8 ± 16.9	132.6 ± 16.9	0.50 [a]
Diastolic blood pressure, mmHg	77.1 ± 10.9	76.6 ± 9.9	0.48 [a]
Medication for hypertension, n (%)	216 (43.9)	153 (42.4)	0.66 [c]
Total cholesterol, mg/dL	203.3 ± 34.6	198.6 ± 33.5	0.05 [a]
Triglycerides, mg/dL	129.3 ± 88.1	112.1 ± 61.9	<0.01 [a]
HDL cholesterol, mg/dL	59.1 ± 16.8	58.5 ± 16.1	0.59 [a]
Medication for dyslipidemia, n (%)	118 (24.0)	90 (24.9)	0.75 [c]
HbA1c (NGSP), %	6.0 ± 0.8	5.9 ± 0.5	0.31 [a]
Fasting glucose, mg/dL	103.0 ± 24.6	101.1 ± 19.7	0.21 [a]
Medication for diabetes, n (%)	82 (16.7)	60 (16.6)	0.99 [c]
Uric Acid, mg/dL	6.1 ± 1.3	5.8 ± 1.1	<0.01 [a]
Creatinine, mg/dL	0.9 ± 0.3	0.9 ± 0.2	0.99 [a]
Albumin, g/dL	4.4 ± 0.3	4.4 ± 0.3	0.35 [a]
γ-GPT, IU	52.7 ± 75.5	41.8 ± 34.1	<0.01 [a]

Data are expressed as the mean ± SD or counts (percentages). Missing data were excluded from calculations.
BMI: body mass index; γ-GTP: gamma-glutamyl transpeptidase; SD; standard deviation.
[a] T-test.
[b] Cochran-Armitage test.
[c] Chi-squared test.

isoflavones and the metabolite equol are also excreted in urine and partially in feces. The present study revealed that the distribution of log values of the equol to daidzein ratio and the classification of producers and non-producers derived from this distribution were almost identical regardless of urine or blood. It is important to note that participants in the present study were a general population of Japanese men without a soy load.

4.2. Cut-off values for equol production status and the percentage of producers

The cut-off values of the log_{10}-transformed equol/daidzein ratio for equol production in daily life without a soy load in a general population of Japanese men were −0.94 for urine and −0.95 for serum, and 41 %–42 % of participants were estimated to produce equol for both indices. The methodology for calculating cut-offs in the present study was the same as that used by Ideno et al. (the urinary cut-off value was −1.42

Table 4
Food frequency questionnaires between equol production status in SESSA2 (n = 853).

	Non-producers n = 492	Producers n = 361	P-value
Soy food eating habits [a]			
Non-consumers of soy foods	123 (25.0)	71 (19.7)	0.07 [b]
Consumers of soy foods	369 (75.0)	290 (80.3)	
Fermented soybeans (natto), boiled soybeans (nimame), or green soybeans (edamame)			
≤1 day/week	284 (57.7)	182 (50.4)	0.03 [c]
2–3 days/week	103 (20.9)	84 (23.3)	
≥4 days/week	105 (21.3)	95 (26.3)	
Tofu, frozen tofu, thick deep-fried tofu (atsuage), or deep-fried tofu with vegetables (ganmodoki)			
≤1 day/week	183 (37.2)	123 (34.1)	0.04 [c]
2–3 days/week	183 (37.2)	115 (31.9)	
≥4 days/week	126 (25.6)	123 (34.1)	
Deep-fried tofu (aburaage)			
≤1 day/week	331 (67.3)	237 (65.7)	0.74 [c]
2–3 days/week	105 (21.3)	83 (23.0)	
≥4 days/week	56 (11.4)	41 (11.4)	
Soy milk			
≤1 day/week	442 (89.8)	325 (90.0)	0.82 [c]
2–3 days/week	17 (3.5)	14 (3.9)	
≥4 days/week	33 (6.7)	22 (6.1)	
Rice			
None	186 (37.8)	180 (49.9)	<0.01 [c]
1–6 times/week	10 (2.0)	6 (1.7)	
≥7 times/week	296 (60.2)	175 (48.5)	
Bread			
None	255 (51.8)	219 (60.7)	0.02 [c]
1–6 times/week	106 (21.5)	63 (17.5)	
≥7 times/week	131 (26.6)	79 (21.9)	
Noodles			
None	263 (53.5)	232 (64.3)	<0.01 [c]
1–6 times/week	217 (44.1)	123 (34.1)	
≥7 times/week	12 (2.4)	6 (1.7)	
Seafood - fresh fish (blue, red, white)			
≤1 day/week	213 (43.3)	136 (37.7)	0.04 [c]
2–3 days/week	192 (39.0)	143 (39.6)	
≥4 days/week	87 (17.7)	82 (22.7)	
Seafood - salted and dried fish (salted salmon, salted mackerel, horse mackerel openings)			
≤1 day/week	355 (72.2)	250 (69.3)	0.12 [c]
2–3 days/week	110 (22.4)	78 (21.6)	
≥4 days/week	27 (5.5)	33 (9.1)	
Seafood - squid, octopus, shrimp, crab, shellfish			
≤1 day/week	416 (84.6)	309 (85.6)	0.48 [c]
2–3 days/week	61 (12.4)	45 (12.5)	
≥4 days/week	15 (3.1)	7 (1.9)	
Meat - Beef and pork (including liver and offal)			
≤1 day/week	298 (60.6)	216 (59.8)	0.48 [c]
2–3 days/week	162 (32.9)	113 (31.3)	
≥4 days/week	32 (6.5)	32 (8.9)	
Meat - poultry (including liver and entrails)			
≤1 day/week	338 (68.7)	233 (64.5)	0.13 [c]
2–3 days/week	130 (26.4)	103 (28.5)	
≥4 days/week	24 (4.9)	25 (6.9)	
Meat - processed products such as ham, sausages, bacon			
≤1 day/week	345 (70.1)	251 (69.5)	0.45 [c]
2–3 days/week	104 (21.1)	68 (18.8)	
≥4 days/week	43 (8.7)	42 (11.6)	
Eggs			
≤1 day/week	156 (31.7)	108 (29.9)	0.07 [c]
2–3 days/week	183 (37.2)	110 (30.5)	
≥4 days/week	153 (31.1)	143 (39.6)	
Vegetables Morning			
≤1 day/week	198 (40.2)	130 (36.0)	0.10 [c]
2–3 days/week	69 (14.0)	43 (11.9)	
≥4 days/week	225 (45.7)	188 (52.1)	
Vegetables Lunch			
≤1 day/week	153 (31.1)	108 (29.9)	0.32 [c]
2–3 days/week	120 (24.4)	75 (20.8)	
≥4 days/week	219 (44.5)	178 (49.3)	
Vegetables Evening			
≤1 day/week	27 (5.5)	21 (5.8)	0.72 [c]
2–3 days/week	92 (18.7)	60 (16.6)	
≥4 days/week	373 (75.8)	280 (77.6)	
Fruit			
≤1 day/week	151 (30.7)	90 (24.9)	0.03 [c]
2–3 days/week	110 (22.4)	76 (21.1)	
≥4 days/week	231 (47.0)	195 (54.0)	
Milk - High-fat and regular			
≤1 day/week	287 (58.3)	188 (52.1)	0.05 [c]
2–3 days/week	48 (9.8)	36 (10.0)	
≥4 days/week	157 (31.9)	137 (38.0)	
Milk - Low fat			
≤1 day/week	422 (85.8)	290 (80.3)	0.05 [c]
2–3 days/week	26 (5.3)	27 (7.5)	
≥4 days/week	44 (8.9)	44 (12.2)	
Dairy products - Yogurt			
≤1 day/week	317 (64.4)	222 (61.5)	0.66 [c]
2–3 days/week	35 (7.1)	37 (10.3)	
≥4 days/week	140 (28.5)	102 (28.3)	
Dairy Products - Cheese			
≤1 day/week	416 (84.6)	291 (80.6)	0.07 [c]
2–3 days/week	45 (9.2)	35 (9.7)	
≥4 days/week	31 (6.3)	35 (9.7)	
Dairy products - Ice Cream			
≤1 day/week	448 (91.1)	321 (88.9)	0.14 [c]
2–3 days/week	31 (6.3)	22 (6.1)	
≥4 days/week	13 (2.6)	18 (5.0)	
Seaweed - seaweed (wakame, kelp, mozuku, mekabu, hijiki)			
≤1 day/week	248 (50.4)	136 (37.7)	<0.01 [c]
2–3 days/week	134 (27.2)	110 (30.5)	
≥4 days/week	110 (22.4)	115 (31.9)	
Seaweed - Nori (grilled seaweed, seasoned seaweed)			
≤1 day/week	291 (59.2)	170 (47.1)	<0.01 [c]
2–3 days/week	125 (25.4)	106 (29.4)	
≥4 days/week	76 (15.5)	85 (23.6)	
Beverages - Green tea and Genmaicha (brown rice tea)			
≤2 times/week	177 (36.0)	116 (32.1)	0.23 [c]
3–6 times/week	57 (11.6)	42 (11.6)	
≥1 time/day	258 (52.4)	203 (56.2)	
Beverages - Oolong tea			
≤2 times/week	402 (81.7)	296 (82.0)	0.74 [c]
3–6 times/week	35 (7.1)	29 (8.0)	
≥1 time/day	55 (11.2)	36 (10.0)	
Beverages - Mugi-cha (barley tea), hojicha (roasted green tea), bancha (green tea)			
≤2 times/week	165 (33.5)	108 (29.9)	0.30 [c]
3–6 times/week	55 (11.2)	43 (11.9)	
≥1 time/day	272 (55.3)	210 (58.2)	
Beverages - Black tea			
≤2 times/week	447 (90.9)	320 (88.6)	0.11 [c]
3–6 times/week	25 (5.1)	15 (4.2)	
≥1 time/day	20 (4.1)	26 (7.2)	
Beverages - Coffee			
≤2 times/week	108 (22.0)	70 (19.4)	0.10 [c]
3–6 times/week	72 (14.6)	38 (10.5)	
≥1 time/day	312 (63.4)	253 (70.1)	

Data are expressed as the mean ± standard deviation.
[a] Participants who responded "≤1 day/week" to questions on all four soy foods (natto, tofu, fried tofu, and soy milk) were classified as non-consumers of soy foods.
[b] Chi-squared test.
[c] Cochran-Armitage test.

and 41.5 % of Japanese women were producers) (Ideno et al., 2018). By using appropriate cut-off values for each target population, 41 %–42 % of both Japanese men and women were equol producers under the conditions of daily life without a soy load based on a fasting morning urine sample. In a study by Yoshikata et al. on 466 Japanese men and women, 39 % and 47 %, respectively, were identified as equol producers (Yoshikata et al., 2024). These findings support a previous study showing that approximately 40 %–50 % Japanese men and women were producers and also that Asians were higher producers than Western populations (Messina et al., 2006).

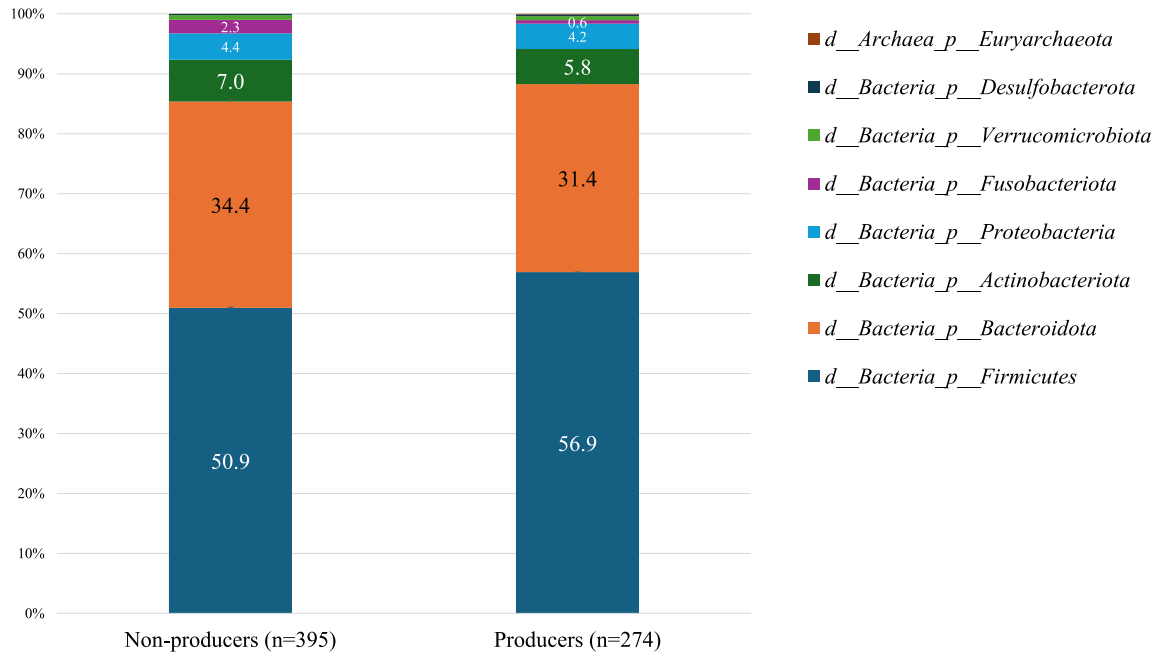


Fig. 5. Relative abundance of phyla between equol production status in SESSA2 (n = 669).

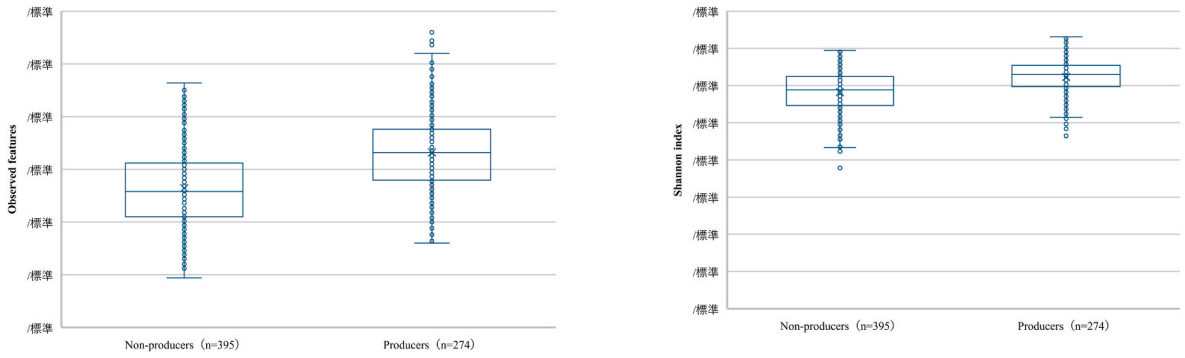


Fig. 6. Alpha diversities between equol production status in SESSA2 (n = 669). The results of the Kruskal-Wallis test showed that the p values for both Observed features and the Shannon index were less than 0.001.

4.3. Food intake frequency, natto, and metabolic pathways

In the present study, producers consumed soy products, particularly the categories including *natto* (fermented soybeans) and *tofu*, more frequently than non-producers. Vitamin K_2 (menaquinone, n = 4–14) synthesis pathways were significantly detected. Menaquinones are produced by intestinal bacteria. *Natto* contains menaquinones 4–8, green laver contains menaquinones 6 and 7, cheese contains menaquinones 4, 8, and 9, and meat and butter contain menaquinone 4. *Natto* contains large amounts of vitamin K_2 due to the high K_2-producing capacity of *Bacillus natto*. Therefore, *natto* consumption appears to be mainly responsible for the significant detection of these metabolic pathways in producers. The direct relationship between K_2 pathway and *natto* consumption cannot be verified in this study and is therefore speculative. However, it is well known that *Bacillus subtilis natto* produces vitamin K_2 in the intestines (Zhang et al., 2021). The serum vitamin K_2 (menaquinone 7) concentration increases on the day after *natto* consumption, halves after 3 days, and almost disappears after 7 days, but the *Bacillus subtilis natto* remains in the feces even after 7 days (Kaneki et al., 2001). It has been epidemiologically shown that eating *natto* 2 days a week maintains serum vitamin K_2 levels and reduces the hip-fracture risk (Kaneki et al., 2001). In this study, since producers also consumed seaweed, cheese, and meat frequently, these effects may be included. In addition, vitamin B metabolic pathways were enriched in producers. The relatively high vitamin B_2 and B_6 contents of *natto* as well as B vitamins in dairy products and meat may also have contributed to these results.

Equol is not typically present at levels higher than trace amounts in the urine of most healthy adults unless they consume soy (Setchell et al., 2002). Moreover, 30 %–50 % of adults do not excrete equol in their urine, even if they consume soy daily. However, a high daily soy intake may contribute to the development or maintenance of an equol production capacity. This is supported by the present results showing that fewer genera were detected in producers with a low soy consumption. It is hypothesized that in the absence of daidzein, the precursor of equol, equol cannot be produced, leading to a loss of function by equol-producing bacteria as well as their loss. However, it is unclear how long and how often non-producers need to consume soy foods to become producers, and what percentage of non-producers may become producers; therefore, further research is warranted.

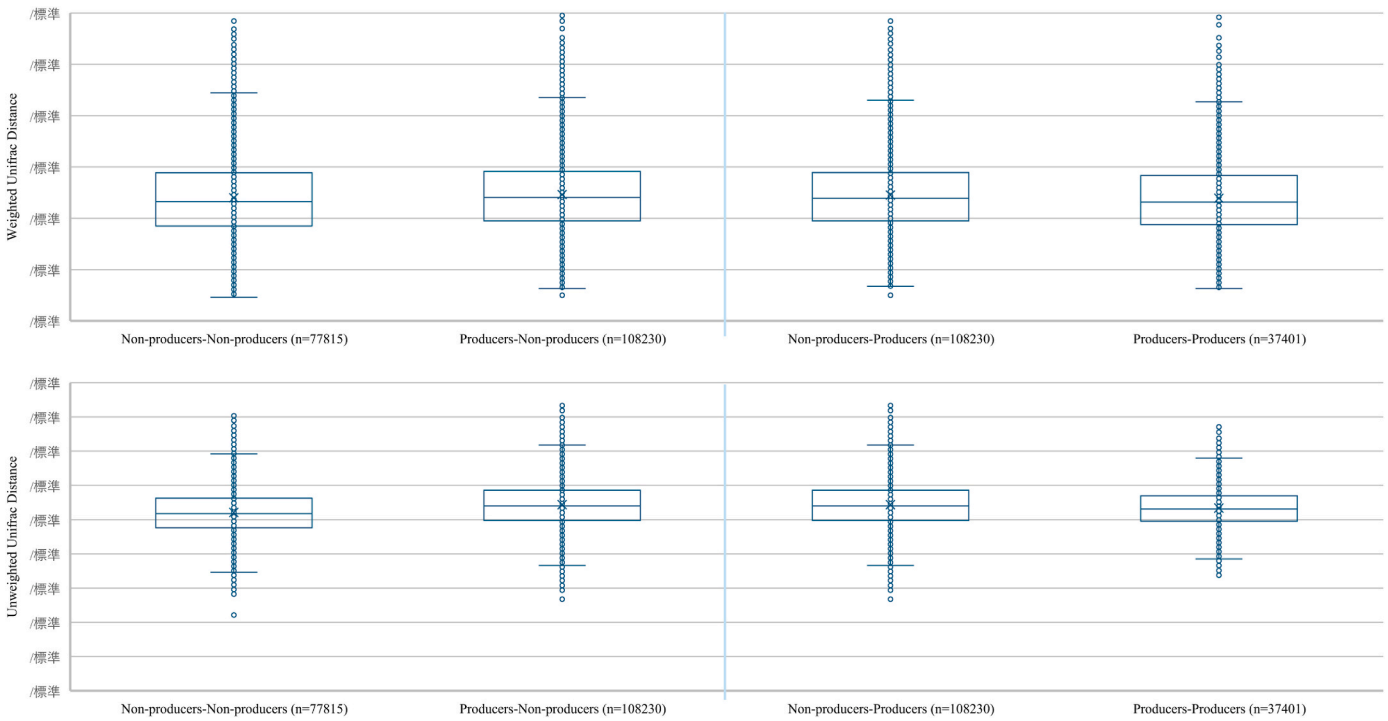


Fig. 7. β-diversities between equol production status in SESSA2 (n = 669). The results of the Kruskal-Wallis test showed that the p values for both weighted and unweighted UniFrac distances were less than 0.001.

4.4. Gut microbiota in producers and comparison with known daidzein reductase

Producers exhibited higher α-diversity, and β-diversity comparisons showed greater distances between producers and non-producers than the distances within producers or within non-producers. Producers had a more diverse gut microbiota than non-producers, particularly the phylum *Firmicutes* - class *Clostridia* - order *Oscillospirales*, and the genera *UCG-002*, *UCG-005* and Christensenellaceae *R-7* group were frequently detected in producers, even in stratified and sensitivity analyses. Previous studies showed that the genera *UCG-002*, *UCG-005*, and Christensenellaceae *R-7* group frequently coexist, are responsible for degrading cellulose, and function in the energy conversion of vegetable fiber in monkeys (Xi et al., 2023). In humans, these genera correlated with amino acids, such as ornithine and arginine in feces (Jiang et al., 2024; Liang et al., 2022), were low in depressed patients (Liang et al., 2022), diabetics, irritable bowel syndrome patients (Kamp et al., 2024), and Crohn's disease patients, and were common in elderly Chinese individuals (Ai et al., 2024). Therefore, these genera are not specialized for daidzein degradation, but may contribute to the degradation of plant fibers. In addition, butyrate-producing bacteria, such as the genera *Coprococcus* and *Faecalibacterium*, and short-chain fatty acid-producing bacteria, including the genus *Prevotella*, were abundant in producers, and were commonly found in individuals who frequently consume Japanese food in the Kyotango area, which is known as a longevity region in Japan (Takagi et al., 2022). Many other butyrate-producing bacteria, such as the genera *Subdoligranulum* and family Lachnospiraceae, were detected in producers. These butyrate-producing bacteria are known to increase with the frequency of consumption of Japanese food, decrease depressive tendencies, and improve quality of life (Valles-Colomer et al., 2019).

The amino acid sequence of daidzein reductase was elucidated in culture experiments (Mayo et al., 2019). Bacteria with high homology to this sequence and with a significantly higher abundance in producers in the present study belonged to the genus *Senegalimassilia*, family Eggerthellaceae. The genera *Eggerthella*, *Adlercreutzia*, and *Slackia*, which belong to the family Eggerthellaceae, are responsible for equol production (Kawada et al., 2016). Therefore, the genus *Senegalimassilia*, belonging to the family Eggerthellaceae and detected in the present study, has high homology to the known daidzein reductase sequence and may be equol-producing bacteria in many Japanese producers. Regarding the genus *Senegalimassilia*, it has been reported that *S. faecalis* strain KGMB04484^{T} (GCF_004135645.1) possesses the equol biosynthesis gene cluster (Han et al., 2020) and is capable of metabolizing daidzein (Soukup et al., 2021). On the other hand, comparative genomics analyses have revealed the existence of *S. faecalis* strains that do not harbor the equol biosynthesis gene cluster (Dufault-Thompson et al., 2022). These findings indicate that the ability to produce equol within the genus *Senegalimassilia* is strain-dependent rather than a universally conserved trait. Furthermore, in metagenomic analyses of human fecal samples, *S. faecalis* has been identified as a bacterium carrying the equol biosynthesis gene cluster (Dufault-Thompson et al., 2022). The family Oscillospiraceae and class *Clostridia* also had highly homologous sequences and, thus, may also contain equol-producing bacteria. On the other hand, it is unclear why the previously reported equol-producing bacteria themselves (Mayo et al., 2019) were not frequently observed in the equol-producers in the present study. We cannot rule out the possibility that variations in soy consumption may have made statistical differences less detectable.

4.5. Gut microbiota in non-producers

Fewer bacteria in the gut microbiota were abundant in non-producers, suggesting that the microbiota of non-producers is less diverse than that of producers. The genera *Fusobacterium* and [*Ruminococcus*] *gnavus* group were more abundant in non-producers than in producers, even in stratified and sensitivity analyses. *Fusobacterium nucleatum* was found to grow in colon cancer cells (Castellarin et al., 2012), and has also been associated with ulcerative colitis; therefore, it is commonly treated as a pathogenic organism (Brennan & Garrett, 2019). [*Ruminococcus*] *gnavus group* was previously reported to be common in Japanese patients with Kawasaki disease, although limited

evidence is available to support this finding (Teramoto et al., 2023). The abundance of [*Ruminococcus*]_*gnavus*_group was found to increases with the consumption of inflammatory diets and intestinal diseases (Henke et al., 2019; Lozano et al., 2022). The genera [*Ruminococcus*] *gnavus* group and *Fusobacterium* have also been associated with obesity (Andoh et al., 2016). These genera, which were more abundant in non-producers, may be linked to inflammatory factors.

4.6. Strengths and limitations

A major strength of this study is that three types of samples were collected: urine, blood, and feces. There has never been a large-scale epidemiological study on equol that has collected these three sample types. This protocol allowed us to reveal that the percentage of producers did not change with urine or blood. It also allowed us to identify the gut microbiota of actual equol producers. However, there are some limitations that need to be addressed. First, the present study only included Japanese men; therefore, it may be difficult to generalize the present results to women or other populations. However, the similarity in the equol production status between urine and serum and the apparent difference in the gut microbiota between producers and non-producers may be generalizable to Japanese individuals who consume large amounts of soybeans. Because equol exhibits estrogen-like activity, its health benefits are great in women (Setchell, 2017). Estrogen changes over the life course (e.g., menopause) may also need to be considered in women. Second, although we investigated the frequency of consumption of the soy foods, *natto* and tofu, which are commonly consumed in Japan, they were not regarded as single items. Therefore, *nimame* or *edamame*, not *natto*, may have been more common among producers, which were in the same response category. However, in consideration of

Table 5
Age-adjusted Spearman's correlation matrix of genera with urinary and serum $\log_{10}$ equol/daidzein ratios in SESSA2 (n = 669).

Genera	$\log_{10}$ (urinary equol/urinary daidzein)	$\log_{10}$ (serum equol/serum daidzein)
g_UCG-005	0.422	0.404
g_Christensenellaceae_R-7_group	0.417	0.407
g_UCG-002	0.393	0.384
g_[Eubacterium]_coprostanoligenes_group	0.376	0.356
f_Oscillospiraceae|g__uncultured	0.342	0.315
g_NK4A214_group	0.325	0.306
g_Peptococcus	0.318	0.321
g_[Eubacterium]_siraeum_group	0.308	0.298
f_Ruminococcaceae|g__uncultured	0.296	0.280
g_Alistipes	0.294	0.274
g_Clostridia_vadinBB60_group	0.293	0.282
g_Subdoligranulum	0.288	0.289
g_Desulfovibrio	0.284	0.280
g_UCG-010	0.276	0.272
g_Butyricimonas	0.272	0.264
g_Clostridia_UCG-014	0.271	0.253
g_Family_XIII_UCG-001	0.264	0.257
f_Coriobacteriales_Incertae_Sedis|g__uncultured	0.257	0.246
g_Coprococcus	0.255	0.247
g_RF39	0.249	0.237
g_Methanobrevibacter	0.249	0.236
g_Catenibacterium	0.245	0.243
g_Odoribacter	0.240	0.230
g_Family_XIII_AD3011_group	0.238	0.229
g_Barnesiella	0.236	0.226
f_Prevotellaceae|g__uncultured	0.235	0.232
g_Senegalimassilia	0.226	0.237
g_Marvinbryantia	0.226	0.211
g_Ruminococcus	0.225	0.214
g_UCG-003	0.223	0.219

Genera	log_{10} (urinary equol/urinary daidzein)	log_{10} (serum equol/serum daidzein)
g_Lachnospiraceae_FCS020_group	0.222	0.211
g_Incertae_Sedis	0.216	0.195
g_Lachnospiraceae_NK4A136_group	0.215	0.219
g_Holdemanella	0.211	0.205
g_[Eubacterium]_ruminantium_group	0.208	0.191
g_Dorea	0.207	0.210
f_Oscillospiraceae|__	0.204	0.180
g_Paraprevotella	0.191	0.180
g_[Ruminococcus]_gauvreauii_group	0.191	0.169
g_Alloprevotella	0.190	0.197
f_Victivallaceae|g__Victivallis	0.186	0.189
g_Prevotella	0.182	0.197
f_Ruminococcaceae|__	0.179	0.156
g_Negativibacillus	0.177	0.172
g_[Eubacterium]_ventriosum_group	0.176	0.157
g_[Ruminococcus]_torques_group	0.176	0.170
g_Lachnospiraceae_ND3007_group	0.173	0.157
g_Slackia	0.173	0.182
g_Muribaculaceae	0.168	0.175
g_Olsenella	0.167	0.171
f_Barnesiellaceae|g__uncultured	0.166	0.142
c_Clostridia|__|__|__	0.166	0.160
f_Eggerthellaceae|g__uncultured	0.165	0.165
g_CAG-56	0.160	0.144
g_Coprobacter	0.157	0.144
g_Faecalibacterium	0.156	0.140
g_[Eubacterium]_brachy_group	0.152	0.141
g_Prevotellaceae_NK3B31_group	0.146	0.154
g_[Eubacterium]_eligens_group	0.138	0.124
g_[Eubacterium]_oxidoreducens_group	0.131	0.124
g_CAG-352	0.129	0.113
f_uncultured|g__uncultured	0.114	0.104
g_Collinsella	0.113	0.125

Genera	log_{10} (urinary equol/urinary daidzein)	log_{10} (serum equol/serum daidzein)
g_Oscillibacter	0.113	0.092
g_Monoglobus	0.107	0.088
g_Oscillospira	0.106	0.110
f_Christensenellaceae|g__uncultured	0.106	0.093
g_Lachnospiraceae_NC2004_group	0.105	0.101
g_Lachnospiraceae_UCG-001	0.102	0.094
g_Akkermansia	0.100	0.089
g_[Eubacterium]_hallii_group	0.100	0.083
g_Colidextribacter	0.099	0.100
g_DTU089	0.098	0.081
g_Romboutsia	0.098	0.098
g_Erysipelotrichaceae_UCG-003	0.088	0.089
g_Terrisporobacter	0.085	0.085
g_Lactobacillus	0.082	0.078
g_Clostridium_sensu_stricto_1	0.081	0.078
g_UCG-009	0.080	0.070
g_Frisingicoccus	0.077	0.071
g_Adlercreutzia	0.075	0.046
g_Acidaminococcus	0.071	0.068
g_Megamonas	0.071	0.085
g_Phascolarctobacterium	0.067	0.070
g_Lachnospiraceae_UCG-010	0.056	0.048
d_Bacteria|__|__|__|__|__	0.054	0.059
g_Rothia	0.050	0.055
g_Turicibacter	0.048	0.030
g_Parasutterella	0.042	0.023
g_Lachnospiraceae_UCG-008	0.042	0.037
g_Streptococcus	0.042	0.041
g_Agathobacter	0.037	0.035
g_UBA1819	0.037	0.019
g_Anaerotruncus	0.037	0.024
g_Parabacteroides	0.037	0.034
g_Roseburia	0.036	0.042

Genera	log_{10} (urinary equol/urinary daidzein)	log_{10} (serum equol/serum daidzein)
g_Megasphaera	0.032	0.040
g_Intestinibacter	0.027	0.000
g_Eubacterium	0.020	0.027
g_Coprobacillus	0.020	0.016
g_Bacillus	0.016	0.008
g_Dialister	0.013	0.013
g_Lachnospira	0.005	0.009
g_Holdemania	0.005	0.006
g_Allisonella	0.002	0.004
g_Actinomyces	0.000	0.007
g_Butyricicoccus	-0.017	-0.006
g_Bilophila	-0.019	-0.016
g_Fusicatenibacter	-0.021	-0.027
g_Klebsiella	-0.024	-0.014
f_Enterobacteriaceae|__	-0.027	-0.036
g_Sutterella	-0.030	-0.021
g_Haemophilus	-0.031	-0.042
g_Enterococcus	-0.036	-0.027
g_Blautia	-0.040	-0.039
g_Fournierella	-0.042	-0.061
g_Lachnospiraceae_UCG-004	-0.042	-0.052
g_Faecalitalea	-0.043	-0.062
g_Granulicatella	-0.060	-0.054
f_Lachnospiraceae|__	-0.065	-0.082
g_Hungatella	-0.065	-0.080
g_Escherichia-Shigella	-0.081	-0.070
g_Sellimonas	-0.103	-0.116
g_Anaerostipes	-0.106	-0.110
g_Bifidobacterium	-0.113	-0.132
g_Lachnoclostridium	-0.126	-0.123
f_Lachnospiraceae|g__uncultured	-0.127	-0.126
f_Veillonellaceae|g__Veillonella	-0.163	-0.162
g_Tyzzerella	-0.183	-0.178

Genera	$\log_{10}$ (urinary equol/urinary daidzein)	$\log_{10}$ (serum equol/serum daidzein)
g_[Clostridium]_innocuum_group	-0.213	-0.217
g_Erysipelatoclostridium	-0.213	-0.221
g_Flavonifractor	-0.238	-0.252
g_Eggerthella	-0.241	-0.249
g_Fusobacterium	-0.287	-0.252
g_Bacteroides	-0.305	-0.308
g_[Ruminococcus]_gnavus_group	-0.336	-0.331

Numbers in the matrix indicate age-adjusted unstandardized partial regression coefficients (red: positive association, blue: inverse association).

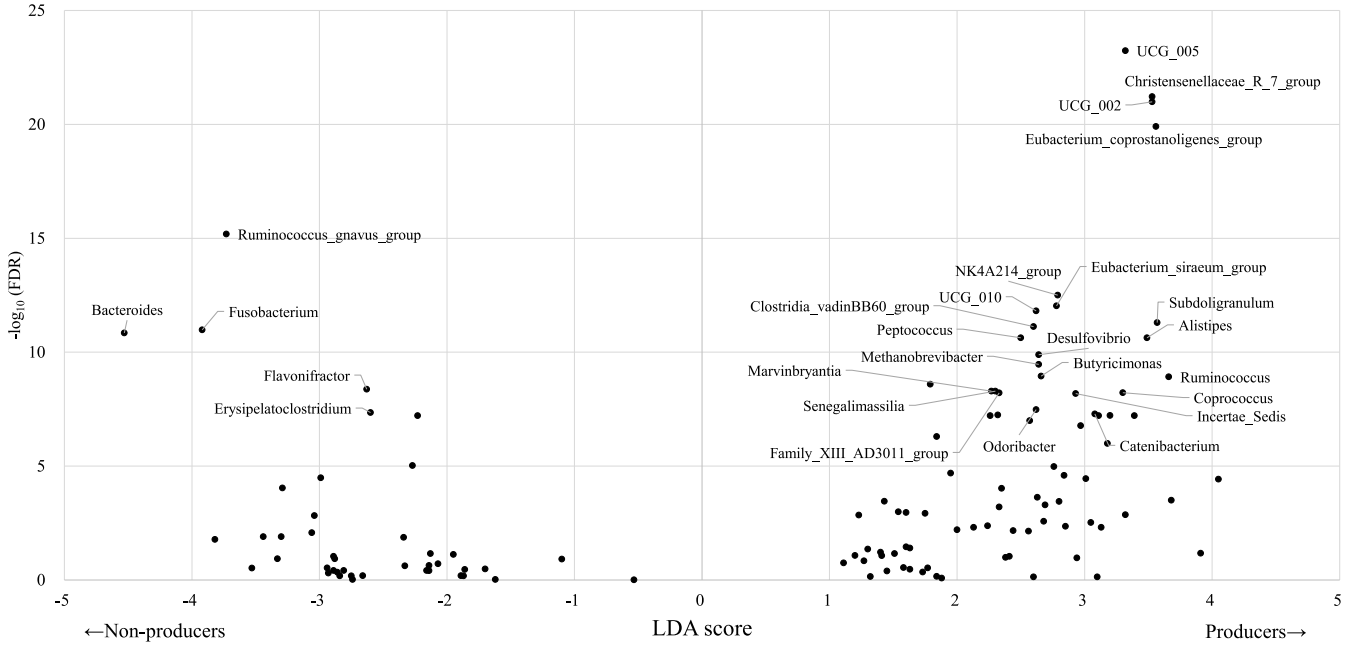


Fig. 8. Significantly differentiated genera between equol production status in SESSA2 (n = 669). The positive direction of the LDA score indicates genera that were more abundant in producers, and the inverse direction indicates genera that were more abundant in non-producers. We denoted by name the genera that showed significant differences in the equol production status by LEfSe. FDR: false discovery rate; LDA: linear discriminant analysis; LEfSe: linear discriminant analysis effect size.

the Japanese lifestyle, tofu and *natto* appear to be the most representative sources of soy intake. Third, due to the cross-sectional nature of the present study, it is not possible to provide evidence of causal or sequential relationships or mechanisms. However, a follow-up study of SESSA2 (=SESSA3) is already underway. In the future, we plan to use these data to validate these health outcomes. Finally, because the present study was based on a 16S ribosomal RNA gene sequence-based analysis of the microbiota, it was not possible to specify detailed strains or species. In the present study, the genus *Senegalimassilia* detected in producers may contain a strain that is capable of producing equol. This will be clarified in isolation and identification experiments in the future.

Despite these limitations, the clinical implications of the present study include the following: since the equol production capacities of urine and blood were identical, only one may be required to assess the equol production status. Furthermore, culture experiments have yet to isolate the equol-producing bacteria present in many equol producers.

5. Conclusion

In this cross-sectional study on middle-aged and older Japanese men, the equol production status of fasting urine and serum almost matched. The gut microbiota of equol producers was highly diversified and functional. Although we did not detect the exact microbiota with known daidzein reductase in equol producers, the sequences of several were close, suggesting the presence of equol-producing bacteria that have yet to be identified.

CRediT authorship contribution statement

Yukiko Okami: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal

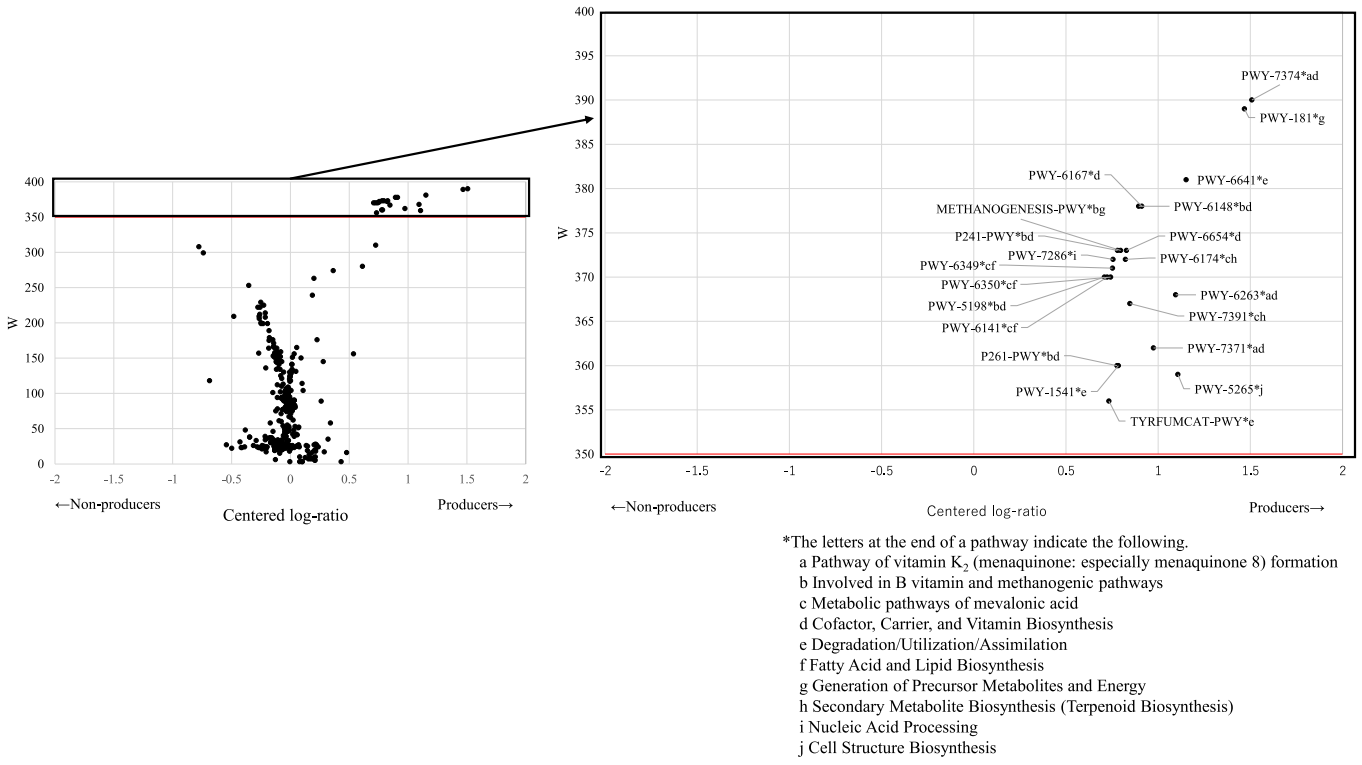


Fig. 9. Significantly differentiated pathways between equol production status in SESSA2 (n = 669). The positive direction of the centered log-ratio indicates pathways that were more abundant in producers, and the inverse direction indicates genera that were more abundant in non-producers. ANCOM W values greater than 350 indicate a significant difference in equol production status. The right panel shows the 21 significant pathways enlarged from the left panel.
*The letters at the end of a pathway indicate the following.
a Pathway of vitamin K_2 (menaquinone: especially menaquinone 8) formation
b Involved in B vitamin and methanogenic pathways
c Metabolic pathways of mevalonic acid
d Cofactor, Carrier, and Vitamin Biosynthesis
e Degradation/Utilization/Assimilation
f Fatty Acid and Lipid Biosynthesis
g Generation of Precursor Metabolites and Energy
h Secondary Metabolite Biosynthesis (Terpenoid Biosynthesis)
i Nucleic Acid Processing
j Cell Structure Biosynthesis.

analysis, Data curation, Conceptualization. **Hisatomi Arima:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation. **Shigeki Bamba:** Writing – review & editing, Software, Methodology, Formal analysis. **Fu Namai:** Writing – review & editing, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Keiko Kondo:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Yuki Ideno:** Writing – review & editing, Software, Methodology, Conceptualization. **Ayumi Soejima:** Writing – review & editing, Resources, Data curation. **Haruna Miyakawa:** Writing – review & editing, Resources, Data curation. **Sayuki Torii:** Writing – review & editing, Resources, Project administration, Investigation. **Hiroyoshi Segawa:** Writing – review & editing, Resources, Project administration, Investigation. **Mizuki Ohashi:** Writing – review & editing, Resources, Investigation. **Megumi Kawashima:** Writing – review & editing, Resources, Investigation. **Takashi Hisamatsu:** Writing – review & editing, Resources, Project administration, Investigation, Data curation. **Aya Kadota:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Data curation. **Akira Sekikawa:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition. **Akira Fujiyoshi:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Katsuyuki Miura:** Writing – review & editing, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: SESSA (Shiga Epidemiological Study of Atherosclerosis) has been supported by the National Institute of Health (US) (R01HL068200), Glaxo-Smith Kline GB (281–2149), the Uehara Memorial Foundation (202210038), and JSPS KAKENHI Grant Numbers JP13307016, JP15H02528, JP15H04773, JP17209023, JP18H03048, JP18H04074, JP18K11127, JP18K19716, JP21249043, JP23249036, and JP25253046 from the Ministry of Education, Culture, Sports, Science and Technology Japan. The present study was initiated and analyzed by the authors. The funding sources listed above have no role in the study design, collection, analyses, or interpretation of results. Techno Suruga Laboratory Co., Ltd. and Bioengineering Lab. Co., Ltd. performed gut microbial measurements. Otsuka Pharmaceutical Co., Ltd. provided free measurements of isoflavone metabolites in urine and blood and technical advice on measurements. Note that Otsuka Pharmaceutical Co., Ltd. was not involved in data analyses using test results or in the process of presenting results in any way.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2025.107048.

Data availability

The data that has been used is confidential.

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Corrigendum to "A cross-sectional study of the gut microbiota associated with urinary and serum equol production status in a general population of Japanese men" [Food Bioscience 71 (2025) 107048]

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The authors regret not noticing that the vertical axis labels in Figs. 6 and 7were incorrect. The correct figures are attached below.

The authors would like to apologise for any inconvenience caused.

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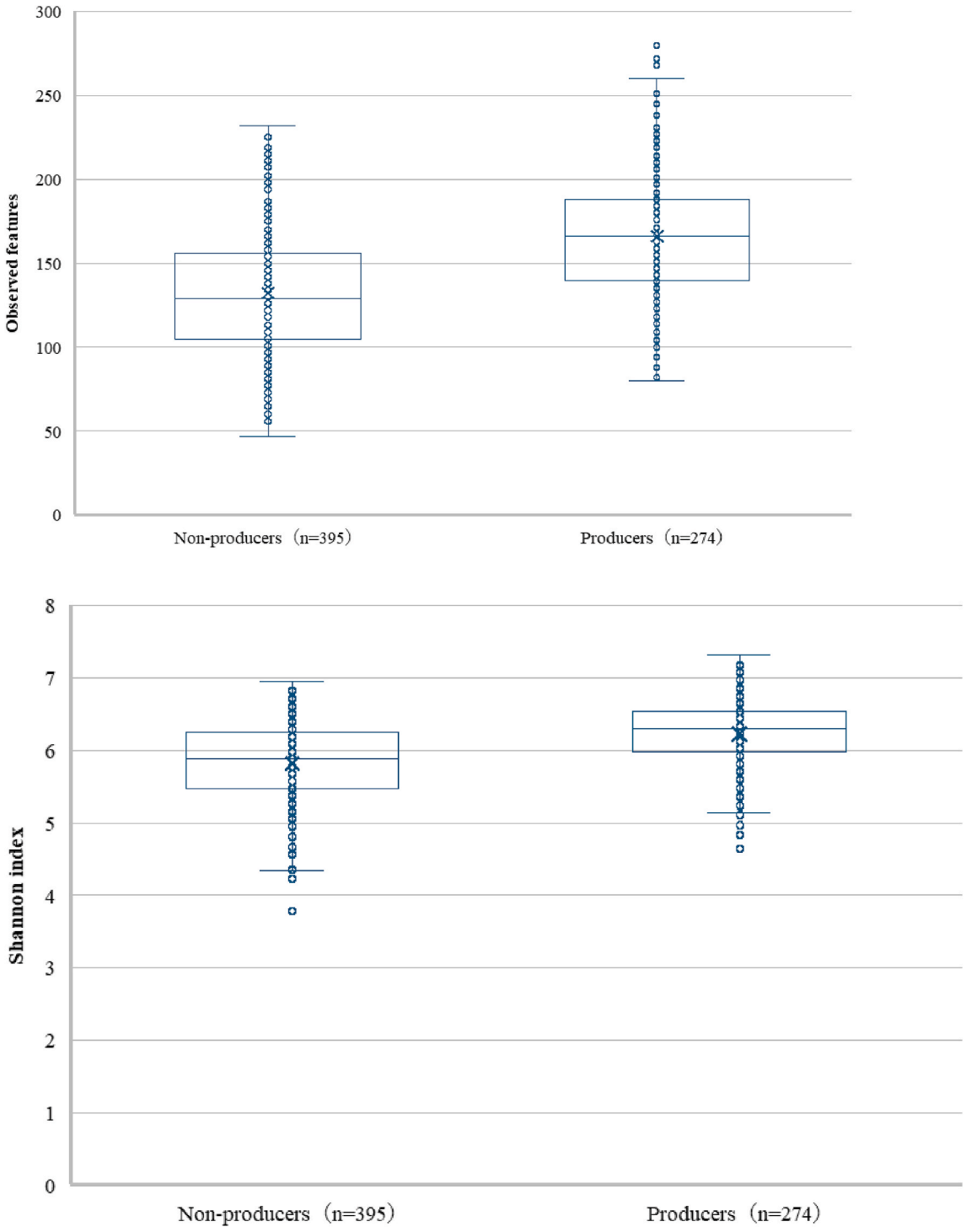

Fig. 6. Alpha diversities between equol production status in SESSA2 (n = 669).

The results of the Kruskal-Wallis test showed that the p values for both Observed features and the Shannon index were less than 0.001.

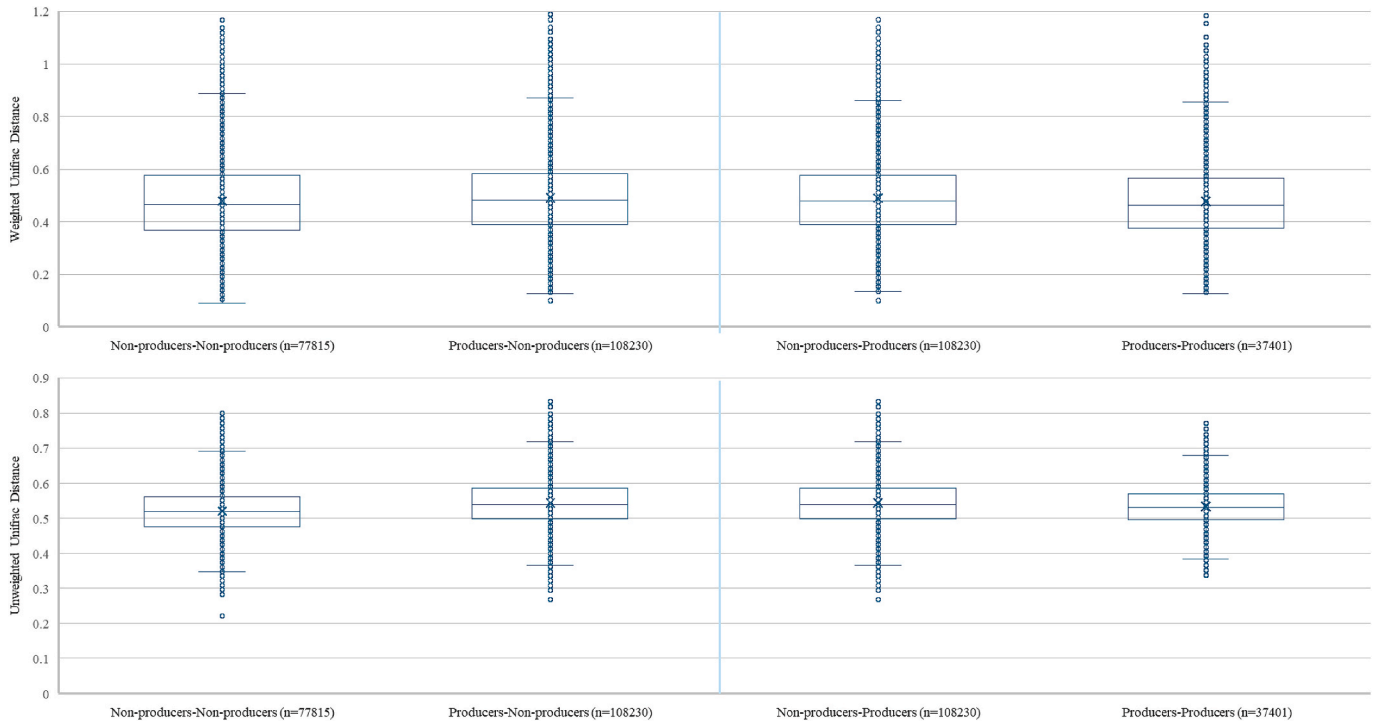

Fig. 7. β-diversities between equol production status in SESSA2 (n = 669).

The results of the Kruskal-Wallis test showed that the p values for both weighted and unweighted UniFrac distances were less than 0.001.