Relationships among fecal daidzein metabolites, dietary habit and BMI in healthy volunteers: a preliminary study

Motoi TAMURA1*, Sachiko HORI1, Hiroyuki NAKAGAWA1, Kazuhiro KATADA2, Kazuhiro KAMADA2, Kazuhiko UCHIYAMA2, Osamu HANEDA2, Tomohisa TAKAGI2, Yuji NAITO2 and Toshikazu YOSHIKAWA2

1National Food Research Institute of the National Agriculture and Food Research Organization, 2-1-12, Kannondai, Tsukuba, Ibaraki 305-8642, Japan
2Molecular Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Kawaramachidori Hirokijagaru, Kamigyo-ku, Kyoto 602-8566, Japan

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To investigate the relationships among fecal isoflavone metabolism, dietary habit and Body Mass Index (BMI), 15 healthy men and 15 healthy women were recruited and provided stool samples for analysis of ex vivo anaerobic incubation of fecal suspension with daidzein. A negative correlation was observed between BMI and the dihydrodaidzein (DHD) production in men, and between BMI and the equol production in women. There was a positive correlation between intake of soluble dietary fiber and the DHD production in men. The results suggest that dietary habits and BMI are related to the metabolic activity of isoflavonoids by fecal intestinal microbiota.

Key words: daidzein, dihydrodaidzein, equol, microbiota

Phytoestrogens are found in plants and have weak estrogenic activity. Much attention has focused on the health benefits of phytoestrogens. Daidzin, genistin, daidzein (the aglycone of daidzin), and genistein (the aglycone of genistin) are the most common isoflavones found in soy products. Human gastrointestinal bacteria play important roles in isoflavone metabolism [1, 2]. Equol is a metabolite of daidzein that is produced by intestinal microbiota [1]. The estrogenic activity of equol is stronger than that of daidzein [3]. Equol seems to be an important phytoestrogen produced by intestinal microbiota in the gut. However, individual variations in equol production have been identified. Only 30–50% of humans are equol producers [4, 5]. It has been reported that 59% of vegetarians and 25% of non-vegetarians are equol producers [6].

It has been suggested that equol-producing individuals may have superior cardiovascular health benefits [7], and people excreting equol may receive benefits from soya isoflavones in preventing cardiovascular disease [8]. In a case-control study involving residents in Japan and Korea, it was suggested that the ability to produce S-equol was closely related to a lower prevalence of prostate cancer [9]. S-equol administration to postmenopausal women reduced hot flash frequency [10]. S-equol production by microbiota seems to contribute positively to host health.

In a randomized, placebo-controlled crossover trial of 26 mildly hypercholesterolemic and/or hypertensive participants, soy-based milk and yoghurt improved plasma lipid levels only in the equol-positive group [11]. It has been reported that there are significant differences in the preventive effects of isoflavones on bone loss and fat accumulation observed between equol producers and non-producers in early postmenopausal women, with equol producers showing the greatest preventive effects [12].

Dihydrodaidzein (DHD) is a bacterial metabolite of the daidzein and is proposed as a precursor of equol [13]. Thus, DHD seem to be important bacterial metabolite. DPPH radical-scavenging activity of DHD has been reported. DHD has the ability to antagonize the contractile effects of noradrenaline and is a potential candidate for the cardioprotective therapeutics [14].

Intestinal microbiota appears to affect the efficacy of isoflavonoids in humans. However, few reports have focused on the relationships of isoflavonoids with gender differences, dietary habits, body mass index (BMI), and fecal isoflavone metabolism.

To investigate the role of microbiota in the metabolism of isoflavone, we measured daidzein metabolites...
produced by the anaerobic incubation of fecal suspension in healthy volunteers and investigated the relationship between these metabolites and several parameters determined by habitual dietary intakes.

Daidzein and equol were purchased from LC Laboratories (Woburn, MA, USA). DHD was purchased from Toronto Research Chemicals, Inc. (North York, Canada).

To estimate the nutritional factors affecting daidzein metabolism by intestinal microbiota we recruited 15 healthy men (mean age 33.6 ± 1.3; range 22–41) and 15 healthy women (mean age 33.9 ± 1.4; range 23–41) who did not have any gastrointestinal disease. Participants agreed to provide stool samples. Participants were asked about their habitual dietary intake in the week prior to stool samples being provided. The study is performed in accordance with the principles of the Declaration of Helsinki and with the approval of the Human Investigations Review Board of the National Food Research Institute, NARO and Kyoto Prefectural University of Medicine. Informed consent was obtained from all participants or their legal guardians.

A food frequency questionnaire based on food groups (FFQg) was answered by all participants and was used to calculate habitual dietary intake for the week prior to the stool sample collection. The FFQg was based on 29 food groups and 10 methods of cookery [15]. Energy and nutrition intake for the week was estimated for each participant from the data provided by the FFQg using FFQg software (Excel Eiyoukun, version 2.0, Yoshimura Y and Takahashi K, Kenpakusha, Tokyo, Japan). This software is based on the 5th revised and enlarged edition of the Standard Tables of Food Composition in Japan. Using the FFQg software, we obtained energy intake per day and measures of protein (g/d), fat (g/d), saturated fatty acid (g/d), monounsaturated fatty acid (g/d), polyunsaturated fatty acid (g/d), n-3 polyunsaturated fatty acid (g/d), n-6 polyunsaturated fatty acid (g/d), cholesterol (mg/d), carbohydrate (g/d), soluble dietary fiber (g/d), insoluble dietary fiber (g/d), total dietary fiber (g/d), retinol (µg/d), α-carotene (µg/d), β-carotene (µg/d), cryptoxanthin (µg/d), retinol equivalent (µg/d), vitamin D (µg/d), α-tocopherol (µg/d), vitamin K (µg/d), vitamin B1 (mg/d), vitamin B2 (mg/d), niacin (mg/d), vitamin B6 (mg/d), vitamin B12 (µg/d), folic acid (µg/d), pantothenic acid (mg/d), vitamin C (mg/d), K (mg/d), Na (mg/d), Ca (mg/d), Mg (mg/d), P (mg/d), Fe (mg/d), Zn (mg/d), Cu (mg/d), and Mn (mg/d).

We estimated the anaerobic equol and DHD production from the fecal microbiota ex vivo. After defecation, feces from participants were collected on paper sheets and quickly transferred into sterilized sampling containers for stool examination (Sarstedt K.K., Tokyo, Japan). Each sampling container was placed in an AnaeroPouch® with a CO2 generator (Mitsubishi Gas Chemical Company, Inc., Tokyo, Japan) and transported to the National Food Research Institute by parcel delivery service at a temperature kept below 10°C.

Approximately 0.1 g of feces was transferred to a sterilized glass homogenizer and 30-fold anaerobic medium was added and homogenized by gassing with O2-free CO2. Daidzein (5 mg) was dissolved in 1 ml dimethyl sulfoxide. The daidzein solution (1 µl) was transferred into 0.2 ml of homogenate. The solution was incubated under an atmosphere of CO2 generated using the AnaeroPack® system (Mitsubishi Gas Chemical Company, Inc., Tokyo, Japan) for 5 days at 37°C.

The anaerobic medium used was prepared as follows: brain heart infusion (37 g), agar (1 g), L-cysteine HCl·H2O (0.5 g) and Na2CO3 (4 g) were dissolved in 1,000 ml distilled water. Aliquots of the broth (9 ml) were placed in test tubes, gassed with O2-free CO2, sealed with a butyl rubber stopper and sterilized by autoclaving. After incubation, 0.3 ml distilled water was added to the reaction mixture, which was then treated with 500 µl of ethyl acetate, vortexed for 30 sec and centrifuged at 5,000 × g for 5 min at 4°C. The supernatant was transferred to an eggplant-type flask. The same volume of ethyl acetate as that used in the first extraction was added to the sediment, and the procedure was repeated. The supernatants from both extractions were pooled in an eggplant-type flask and evaporated completely using a rotary evaporator. The sample was then dissolved in 800 µl of 80% methanol and filtered through a 0.2 µm filter. The filtrates were used for LC-MS/MS analysis. LC-MS/MS analysis was performed using the same method as reported before [16].

Data are expressed as the mean ± standard error. All data were analyzed using Sigma Plot 11 (Systat Software, Inc., San Jose, CA, USA). When the data exhibited normally distributed populations with the same variances, the data were analyzed using the Mann-Whitney rank sum test. To measure the strength of associations between pairs of the data, the data were analyzed using the Pearson product moment correlation test. A p-value of <0.05 was considered statistically significant.

The age range of the participants was 22–41 for men and 23–41 for women. The means and standard errors of the ages of the men and women were 33.6 ± 1.3 and
The means and standard errors of the body weights of the men and women were 70.9 ± 2.3 and 50.6 ± 1.8, respectively. The means and standard errors of the BMI of the men and women were 23.2 ± 0.8 and 20.3 ± 0.7, respectively. There were significant differences in body weight (p<0.01) and height (p<0.01) between men and women. BMI was significantly greater in men than in women (p<0.01).

The results of the FFQg are shown in Table 1. For food intake, the amounts of calcium, vitamin K, folic acid, soluble dietary fiber, insoluble dietary fiber and total intake of dietary fiber were significantly greater in women than in men. The amounts of polyunsaturated fatty acids and omega-6 fatty acids were significantly greater in men than in women.

Analyses after ex vivo anaerobic incubation of fecal suspensions with daidzein in men and women revealed that the DHD concentrations was significantly greater in samples from men (39.3 ± 10.5 μmol/l) than in samples from women (10.6 ± 4.3 μmol/l) (p<0.05). Average equol concentrations tended to be higher in women (34.4 μmol/l) than in men (19.8 μmol/l), though there was no

| Table 1. Software data (FFQg ver. 2.0 Kenpaku-sha, Tokyo, Japan) calculations of dietary intake per day of 15 women and 15 men from data collected using the food frequency questionnaire based on food groups |
|---------------------------------|------------------|------------------|------------------|------------------|------------------|
|                                | Men (n=15)       | Women (n=15)     | p value          |
| Energy (Kcal/d)                | 1,833.5 ± 93.8   | 1,631.8 ± 78.8   | 0.11             |
| Protein (g/d)                  | 57.7 ± 2.4       | 59.0 ± 2.7       | 0.73             |
| Fat (g/d)                      | 61.2 ± 4.3       | 56.0 ± 3.3       | 0.34             |
| Saturated fatty acid (g/d)     | 18.4 ± 1.3       | 18.7 ± 1.3       | 0.85             |
| Monounsaturated fatty acid (g/d)| 22.8 ± 1.8     | 19.8 ± 1.1       | 0.16             |
| Polysaturated fatty acid (g/d) | 12.6 ± 0.9       | 10.2 ± 0.7       | 0.04             |
| n-3 Polysaturated fatty acid (g/d)| 2.1 ± 0.1     | 1.7 ± 0.2        | 0.05             |
| n-6 Polysaturated fatty acid (g/d)| 10.5 ± 0.8     | 8.4 ± 0.5        | 0.04             |
| Cholesterol (mg/d)             | 261.7 ± 22.6     | 251.8 ± 19.3     | 0.90             |
| Carbohydrate (g/d)             | 234.2 ± 12.7     | 211.5 ± 12.9     | 0.22             |
| Soluble dietary fiber (g/d)    | 2.3 ± 0.2        | 3.0 ± 0.3        | 0.04             |
| Insoluble dietary fiber (g/d)  | 6.8 ± 0.4        | 8.6 ± 0.6        | 0.02             |
| Total dietary fiber (g/d)      | 9.4 ± 0.6        | 11.8 ± 0.8       | 0.03             |
| Retinol (μg/d)                 | 166.5 ± 11.9     | 188.2 ± 17.2     | 0.41             |
| α-Carotene (μg/d)              | 407.0 ± 54.5     | 564.3 ± 81.9     | 0.09             |
| β-Carotene (μg/d)              | 2,424.3 ± 312    | 3,387.2 ± 480.7  | 0.11             |
| Cryptoxanthin (μg/d)           | 277.5 ± 60.3     | 456.0 ± 124.7    | 0.48             |
| Retinol equivalent (μg/d)      | 401.5 ± 28.0     | 515.1 ± 46.7     | 0.08             |
| Vitamin D (μg/d)               | 5.1 ± 0.4        | 5.3 ± 0.7        | 0.78             |
| α-Tocopherol (mg/d)            | 5.8 ± 0.3        | 5.4 ± 0.3        | 0.28             |
| Vitamin K (μg/d)               | 146.8 ± 8.7      | 188.6 ± 15.6     | 0.03             |
| Vitamin B1 (mg/d)              | 0.8 ± 0.04       | 0.8 ± 0.04       | 0.34             |
| Vitamin B2 (mg/d)              | 0.8 ± 0.04       | 1.0 ± 0.05       | 0.07             |
| Niacin (mg/d)                  | 13.8 ± 0.7       | 12.7 ± 0.7       | 0.29             |
| Vitamin B6 (mg/d)              | 0.9 ± 0.05       | 1.0 ± 0.1        | 0.60             |
| Vitamin B12 (μg/d)             | 5.3 ± 0.4        | 5.1 ± 0.6        | 0.78             |
| Folic acid (μg/d)              | 190.4 ± 10.1     | 246.9 ± 18.5     | 0.02             |
| Pantothenic acid (mg/d)        | 4.4 ± 0.2        | 4.8 ± 0.2        | 0.19             |
| Vitamin C                      | 54.4 ± 5.4       | 81.7 ± 10.4      | 0.08             |
| Na (mg/d)                      | 3,450.5 ± 277.4  | 2,868.8 ± 284.2  | 0.15             |
| K (mg/d)                       | 1,715.6 ± 90.9   | 1,989.1 ± 116.1  | 0.07             |
| Ca (mg/d)                      | 359.2 ± 31.4     | 493.9 ± 39.7     | 0.01             |
| Mg (mg/d)                      | 187.0 ± 9.9      | 201.4 ± 10.3     | 0.32             |
| P (mg/d)                       | 807.5 ± 36.7     | 877.3 ± 48.6     | 0.26             |
| Fe (mg/d)                      | 5.7 ± 0.2        | 6.2 ± 0.3        | 0.19             |
| Zn (mg/d)                      | 6.9 ± 0.3        | 7.1 ± 0.3        | 0.76             |
| Cu (mg/d)                      | 0.9 ± 0.04       | 0.9 ± 0.1        | 0.50             |
| Mn (mg/d)                      | 2.1 ± 0.1        | 2.1 ± 0.1        | 0.99             |
significant difference between the genders. There was no significant difference in daidzein concentrations between men (42.3 ± 8.8 μmol/l) and women (53.6 ± 12.8 μmol/l). It has been reported that soy isoflavone metabolism of microbiota is influenced by the food matrix, especially in women [17]. In the current study, there were differences in the food intake between women and men. These dietary differences might influence the production of different soy isoflavone metabolites by microbiota in men and women.

Analyses after ex vivo anaerobic incubation of fecal suspensions with daidzein in men and women revealed that there were some differences in the daidzein metabolism between men and women, so we analyzed the relationships of BMI, metabolites of daidzein, and data from the FFQg. The results suggested there are some gender differences in the correlation coefficients of between data from the FFQg items and DHD, and between those of the FFQg items and equol. The results also suggested there are some gender differences in the correlation coefficients of BMI and DHD, and of BMI and equol (Table 2, the correlation between results for the FFQg items and DHD production, and the correlation between results of the FFQg items and equol production; Table 2. Correlation between results for the FFQg items and DHD production, and the correlation between results of the FFQg items and equol production

|                      | Equol (men) | p value | Equol (women) | p value | DHD (men) | p value | DHD (women) | p value |
|----------------------|------------|---------|---------------|---------|-----------|---------|-------------|---------|
| Energy (Kcal/d)      | 0.35       | 0.20    | -0.13         | 0.64    | 0.16      | 0.56    | -0.40       | 0.14    |
| Protein (g/d)        | 0.53       | 0.04    | -0.17         | 0.56    | -0.02     | 0.94    | -0.05       | 0.85    |
| Fat (g/d)            | 0.38       | 0.17    | -0.43         | 0.11    | 0.05      | 0.87    | -0.19       | 0.50    |
| Saturated fatty acid (g/d) | 0.36   | 0.19    | -0.46         | 0.08    | 0.11      | 0.71    | -0.06       | 0.83    |
| Monounsaturated fatty acid (g/d) | 0.37  | 0.18    | -0.42         | 0.12    | 0.02      | 0.93    | -0.27       | 0.33    |
| Polyunsaturated fatty acid (g/d) | 0.38  | 0.16    | -0.23         | 0.40    | 0.02      | 0.94    | -0.30       | 0.28    |
| n-3 Polyunsaturated fatty acid (g/d) | 0.39  | 0.15    | 0.01          | 0.98    | 0.02      | 0.94    | -0.28       | 0.32    |
| n-6 Polyunsaturated fatty acid (g/d) | 0.37  | 0.17    | -0.30         | 0.28    | 0.02      | 0.95    | -0.29       | 0.29    |
| Cholesterol (mg/d)   | 0.21       | 0.46    | -0.50         | 0.06    | -0.33     | 0.22    | 0.15        | 0.61    |
| Carbohydrate (g/d)   | 0.07       | 0.80    | 0.15          | 0.60    | 0.36      | 0.19    | -0.49       | 0.06    |
| Soluble dietary fiber (g/d) | 0.01  | 0.96    | 0.38          | 0.17    | 0.57      | 0.03    | -0.06       | 0.84    |
| Insoluble dietary fiber (g/d) | 0.19  | 0.51    | 0.37          | 0.18    | 0.42      | 0.12    | -0.04       | 0.89    |
| Total dietary fiber (g/d) | 0.12  | 0.66    | 0.36          | 0.19    | 0.49      | 0.06    | -0.06       | 0.84    |
| Retinol (μg/d)       | 0.42       | 0.12    | -0.53         | 0.04    | -0.29     | 0.29    | 0.17        | 0.55    |
| α-Carotene (μg/d)    | -0.13      | 0.65    | 0.24          | 0.40    | -0.09     | 0.74    | 0.24        | 0.39    |
| β-Carotene (μg/d)    | -0.12      | 0.67    | 0.24          | 0.39    | -0.09     | 0.76    | 0.23        | 0.41    |
| Cryptoxanthin (μg/d) | 0.16       | 0.58    | 0.12          | 0.68    | -0.04     | 0.90    | -0.19       | 0.51    |
| Retinol equivalent (μg/d) | 0.06  | 0.82    | 0.04          | 0.90    | -0.22     | 0.43    | 0.26        | 0.35    |
| Vitamin D (μg/d)     | 0.03       | 0.92    | 0.09          | 0.74    | 0.08      | 0.79    | -0.12       | 0.68    |
| α-Tocopherol (mg/d)  | 0.19       | 0.51    | 0.12          | 0.68    | 0.12      | 0.66    | -0.26       | 0.35    |
| Vitamin K (μg/d)     | 0.33       | 0.23    | 0.16          | 0.56    | -0.08     | 0.77    | 0.27        | 0.33    |
| Vitamin B₁ (mg/d)    | 0.49       | 0.06    | -0.08         | 0.77    | 0.07      | 0.81    | -0.04       | 0.88    |
| Vitamin B₂ (mg/d)    | 0.40       | 0.14    | -0.31         | 0.27    | -0.06     | 0.82    | 0.19        | 0.50    |
| Niacin (mg/d)        | 0.50       | 0.06    | 0.07          | 0.79    | -0.03     | 0.92    | -0.14       | 0.63    |
| Vitamin B₆ (mg/d)    | 0.67       | 0.01    | 0.06          | 0.85    | -0.09     | 0.74    | 0.02        | 0.93    |
| Vitamin B₁₂ (μg/d)   | 0.24       | 0.38    | 0.06          | 0.83    | -0.11     | 0.69    | -0.09       | 0.76    |
| Folic acid (μg/d)    | 0.33       | 0.23    | 0.25          | 0.36    | 0.10      | 0.71    | 0.19        | 0.50    |
| Pantothenic acid (mg/d) | 0.52  | 0.05    | -0.22         | 0.44    | 0.002     | 0.99    | 0.01        | 0.98    |
| Vitamin C            | 0.24       | 0.39    | 0.27          | 0.32    | 0.07      | 0.82    | -0.01       | 0.97    |
| Na (mg/d)            | 0.11       | 0.70    | 0.32          | 0.25    | 0.52      | 0.05    | -0.14       | 0.61    |
| K (mg/d)             | 0.26       | 0.35    | 0.14          | 0.62    | 0.22      | 0.44    | 0.02        | 0.93    |
| Ca (mg/d)            | 0.21       | 0.45    | -0.23         | 0.42    | 0.11      | 0.69    | 0.06        | 0.51    |
| Mg (mg/d)            | 0.24       | 0.38    | 0.03          | 0.90    | 0.27      | 0.33    | -0.05       | 0.85    |
| P (mg/d)             | 0.51       | 0.05    | -0.20         | 0.47    | 0.03      | 0.92    | 0.03        | 0.91    |
| Fe (mg/d)            | 0.26       | 0.34    | -0.004        | 0.99    | 0.31      | 0.27    | 0.02        | 0.93    |
| Zn (mg/d)            | 0.63       | 0.01    | -0.25         | 0.36    | -0.07     | 0.81    | -0.06       | 0.82    |
| Cu (mg/d)            | 0.28       | 0.32    | 0.05          | 0.87    | 0.40      | 0.14    | -0.23       | 0.40    |
| Mn (mg/d)            | 0.23       | 0.42    | 0.19          | 0.51    | 0.29      | 0.29    | -0.38       | 0.17    |
Table 3. Correlation result for BMI and results of the FFQg items, the correlation between BMI and the DHD production, and the correlation between BMI and the equol production

| Energy (Kcal/d) | BMI (men) p value | BMI (women) p value |
|----------------|------------------|---------------------|
| 0.07           | 0.80             | –0.33               | 0.24            |
| Protein (g/d)  | 0.28             | 0.31                | –0.22            | 0.42            |
| Fat (g/d)      | 0.25             | 0.37                | –0.23            | 0.41            |
| Saturated fatty acid (g/d) | 0.20 | 0.48                | –0.09            | 0.75            |
| Monounsaturated fatty acid (g/d) | 0.32 | 0.25                | –0.22            | 0.44            |
| Polysaturated fatty acid (g/d) | 0.19 | 0.50                | –0.51            | 0.06            |
| n-3 Polysaturated fatty acid (g/d) | 0.11 | 0.70                | –0.65            | 0.01            |
| n-6 Polysaturated fatty acid (g/d) | 0.20 | 0.48                | –0.43            | 0.11            |
| Cholesterol (mg/d) | 0.44 | 0.10                | 0.20             | 0.48            |
| Carbohydrate (g/d) | –0.27          | 0.32                | –0.41            | 0.13            |
| Soluble dietary fiber (g/d) | –0.52          | 0.05                | –0.21            | 0.45            |
| Insoluble dietary fiber (g/d) | –0.43          | 0.11                | –0.37            | 0.18            |
| Total dietary fiber (g/d) | –0.47          | 0.08                | –0.33            | 0.24            |
| Retinol (μg/d) | 0.49             | 0.06                | 0.16             | 0.57            |
| α-Carotene (μg/d) | –0.06          | 0.82                | –0.08            | 0.77            |
| β-Carotene (μg/d) | –0.08          | 0.78                | –0.09            | 0.74            |
| Cryptoxanthin (μg/d) | –0.33          | 0.23                | –0.34            | 0.21            |
| Retinol equivalent (μg/d) | 0.10         | 0.73                | –0.06            | 0.82            |
| Vitamin D (μg/d) | –0.16          | 0.56                | –0.48            | 0.07            |
| α-Tocopherol (mg/d) | –0.07         | 0.79                | –0.68            | 0.01            |
| Vitamin K (μg/d) | 0.10            | 0.73                | –0.15            | 0.59            |
| Vitamin B₁ (mg/d) | 0.28           | 0.32                | –0.13            | 0.64            |
| Vitamin B₂ (mg/d) | 0.11           | 0.70                | –0.04            | 0.88            |
| Niacin (mg/d)   | 0.32            | 0.25                | –0.29            | 0.30            |
| Vitamin B₆ (mg/d) | 0.31         | 0.26                | –0.33            | 0.23            |
| Vitamin B₁₂ (μg/d) | 0.09       | 0.75                | –0.42            | 0.12            |
| Folic acid (μg/d) | –0.18         | 0.53                | –0.18            | 0.51            |
| Pantothenic acid (mg/d) | 0.18       | 0.53                | –0.17            | 0.54            |
| Vitamin C       | –0.30           | 0.28                | –0.34            | 0.22            |
| Na (mg/d)       | –0.41           | 0.13                | –0.47            | 0.08            |
| K (mg/d)        | –0.31           | 0.26                | –0.44            | 0.10            |
| Ca (mg/d)       | –0.30           | 0.28                | –0.22            | 0.43            |
| Mg (mg/d)       | –0.29           | 0.30                | –0.48            | 0.07            |
| P (mg/d)        | 0.08            | 0.77                | –0.28            | 0.32            |
| Fe (mg/d)       | –0.22           | 0.44                | –0.408           | 0.13            |
| Zn (mg/d)       | 0.44            | 0.11                | –0.20            | 0.47            |
| Cu (mg/d)       | –0.24           | 0.39                | –0.52            | 0.05            |
| Mn (mg/d)       | –0.24           | 0.40                | –0.53            | 0.04            |
| DHD (μmol/l)    | –0.66           | 0.007               | 0.31             | 0.26            |
| Equol (μmol/l)  | 0.73            | 0.002               | –0.40            | 0.14            |

Table 3, the correlation result for BMI and results of the FFQg items, the correlation between BMI and the DHD production, and the correlation between BMI and the equol production.

A negative correlation (r=–0.66) was observed between BMI and the DHD concentration of the fecal incubation solutions from men. In men in the current study, a moderate positive correlation (r=0.57) was observed between soluble dietary fiber intake and the DHD concentration of the fecal incubation solutions from men. It has been reported that bacterial numbers in fecal matter are affected by the intake of soluble dietary fiber [18]. Soluble dietary fiber seems to have an impact on the metabolic activity of human intestinal microbiota. These results suggest that some bacterial groups in the microbiota involved in the DHD production might affect BMI in men. This is a novel result, as there are few reports of a relationship between BMI and the DHD-producing activity of fecal microbiota.

A weak negative correlation (r=–0.40) was observed from men. It has been reported that bacterial numbers in fecal matter are affected by the intake of soluble dietary fiber [18]. Soluble dietary fiber seems to have an impact on the metabolic activity of human intestinal microbiota. These results suggest that some bacterial groups in the microbiota involved in the DHD production might affect BMI in men. This is a novel result, as there are few reports of a relationship between BMI and the DHD-producing activity of fecal microbiota.
between BMI and the equol concentration of the fecal incubation solutions from women. It has been reported that overweight or obese individuals show a higher proportion of non-producers of equol than in the rate of non-producers in the general population [19]. In a 1 year isoflavone intervention study in early postmenopausal Japanese women, there were significant differences between the equol producers and non-producers in the isoflavone group in terms of fat accumulation [12]. In women, intestinal bacterium related to equol production might affect BMI. However, a positive correlation (r=0.73) was observed between BMI and the equol concentration of the fecal incubation solutions from men. Recently, sex-characteristic-microbiota correlations have been reported [20]. Our different results show sex differences in the relationship between the fecal metabolic activities of isoflavonoids and BMI which might be due to the sex-characteristic-microbiota between men and women.

Like the relationship of soluble dietary fiber with BMI, there was also a weak positive correlation between the amount of total dietary fiber intake and equol production in women (r=0.36). It has been reported that diet containing inulin significantly increased the cecal and colonic concentration of butyrate [21]. Resistant starch feeding increased the cecal concentration of butyric acid [22]. Human colonic butyrate-producing strains exhibited growth on a substrate including short-chain fructooligosaccharides [23]. However, a combination of dietary fructooligosaccharides and isoflavone conjugates increased equol production in ovariectomized mice [24]. Resistant starch promotes equol production [25]. It has been reported that butyric acid increases the conversion ratio of daidzein to equol in equol-producing bacterium [26]. Butyrate stimulated the equol production in a culture of mixed bacteria [27]. These indicate that some dietary fiber intake increases the butyrate production by intestinal microbiota. It is possible that some dietary fiber and oligosaccharides might promote the equol production by intestinal microbiota in women.

This is the first report to explore the relationship between the fecal DHD activity and BMI in men. A limitation of this study was that we could not identify what kinds of intestinal bacterium affect BMI and what diet components increase the equol production in the human gut. Further studies are needed to clarify these points.

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