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<Original Article>

The cross-sectional study of the relationship between soy isoflavones, equol and the menopausal symptoms in Japanese women

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Summary A study of menopausal symptoms and urinary excretion of isoflavones in peri- and postmenopausal Japanese women was conducted, and the relationship between the two was investigated. The study was conducted by self-rated postal questionnaires sent to 108 women aged 40-60 belonging to the Fukuoka Dietetic Association. Twenty-four hour urine samples were also collected. The self-administered questionnaires inquired about occupation, form of employment, marital history, birth history, menstrual status, past history, physical parameters and menopausal symptoms. The simplified menopausal index (SMI) was calculated, and 24-hr urine samples were measured for daidzein, genistein and equol, an intestinal metabolite of daidzein. The mean urinary excretions of total isoflavones, daidzein, genistein and equol were 38.9±29.2, 19.6±15.1, 10.0±8.9, and 9.3±14.1 μmol/24 hr, and the 50 percentile values were 34.8, 17.0, 8.0, and 1.2 μmol/24 hr, respectively. Daidzein and genistein were detected in all of the subjects, but equol was detected in only 49 out of 95 subjects (51.6%) (equol excretors). The mean SMI score of 59 peri- and post-menopausal women was 19±14, with a 50 percentile value of 15 and a range of 0-53. The incidences of "facial flushing", "perspiration", and "chilliness of the back and extremities" were 20.8%, 41.5%, and 34.0%, respectively. The results of comparing the urinary excretion of daidzein, genistein, and equol in high SMI patients (SMI>15) and low SMI patients (SMI≤15) yielded no significant differences in daidzein and genistein between the groups, but the equol levels in high SMI patients (4.9±9.1 μmol/24 hr) were significantly lower than in low SMI patients (16.3±19.4 μmol/24 hr) (p<0.05). In a comparison of the ratios of equol urinary excretors in high and low SMI patients, the ratio was significantly higher in low SMI patients than in high SMI patients (p<0.05). This study suggests that equol produced in the human intestines contributes to menopausal symptoms. The results

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of quantitative analysis also indicated that menopausal symptoms (SMI) are milder in women with 24-hr urinary equol excretion of at least 5 μmol.

Summary The objective of the study was to investigate the relationship between urinary excretion of isoflavones and menopausal symptoms of Japanese women in peri- and postmenopausal periods.

Hundred and eight dietitians living in Fukuoka prefecture were recruited into the study. Self-rated postal surveies and collections of 24hr urine sample for measurement of urinary total isoflavonoids, genistein, daidzein and equol levels by HPLC were conducted.

The urinary excretions of isoflavonoids, genistein, daidzein and equol were 38.9 ± 29.2, 19.6 ± 15.1, 10.0 ± 8.9, 9.3 ± 14.1μmol/24hr (mean ± SD), respectively. Urinary genistein and daidzein were detected in all samples, but equol was detected only 51.6%. Menopausal symptoms were evaluated using simplified menopausal index (SMI) in 59 peri- and postmenopausal women. The 50 percentile value of SMI score was 15, and the women complaining hot flushes, palpitation and chillness in extremities were 20.8%, 41.5%, 34.0%, respectively.

No significant difference in urinary excretion of genistein and daidzein was detected between the groups of high SMI (greater than 15) and low SMI (less than 15), but the equol levels of higher SMI group were significantly lower than those of lower SMI group ($P<0.05$).

These results suggest that the amount of equol converted from isoflavones by intestinal bacteria mainly contribute to suppress the menopausal symptoms. The quantitative analysis indicated that urinary epuol more than 5μmol/24hr is necessary for reducing the menopausal symptoms of Japanese menopausal women.

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Key words : Soy isoflavone, Equol, Menopausal symptoms, urinary excretion, Japanese peri- and postmenopausal women

Introduction

Isoflavones contained in soy have been reported to have a preventative effect as phytoestrogens against hormone-dependent cancers such as breast cancer and prostate cancer[1]. This is associated with the antiestrogenic action of soy isoflavones.

On the other hand, it has also been reported that soy isoflavones have a weak estrogenic action[2] and an inhibitory effect on postmenopausal bone density loss[3)-7)].

This effect has been supported by intervention studies of soy isoflavones on menopausal disorders – vasomotor symptoms, in particular – performed with attention focused on this estrogenic action[8)9)].

In the investigation of Lock *et al*, a comparison of the frequencies of menopausal symptoms in Japanese and Canadian women demonstrated that menopausal symptoms are much less common in Japan[10]. Aldercreutz *et al* have reported that the reason for this is that Japanese women intake large amounts of soy-related foods such as tofu, soybean paste, and soy sauce, and the soy isoflavones contained in these foods reduce the frequency of menopausal disorders[11].

In epidemiological research on breast cancer and prostate cancer, Aldercreutz *et al* also demonstrated that blood isoflavone concentration and urinary isoflavone excretion are substantially higher in Japanese women, who have a low prevalence of menopausal disorders, than in Finnish women, who have a high prevalence[1)11)12)].

The main isoflavones ingested from ordinary soy processed foods are daidzein and genistein, but it has been reported that daidzein is metabolized by intestinal bacteria in the lower intestinal tract and is transformed into the active metabolite equol[13]. Equol was discovered by Axelson *et al* as an estrogenic substance in human urine, and its physiological action has attracted attention[15] since it bonds with estrogen receptors (ER) – ER-β, in particular – more strongly than soy isoflavones themselves[14]. Equol produced by intestinal bacteria is excreted in urine after it is absorbed by the body[16)17)], but there are individual differences in the production of equol, so Lampe *et al*[18] are performing a quantitative analysis using a 24-hr urine collection method in order to evaluate this production function (categorized by

people who produce and people who do not produce equol) and the amount produced.

Most previous reports of studies related to soy isoflavone intake or urinary isoflavone excretion of peri- and postmenopausal women in Japan have had as few as around a dozen subjects or lack quantitative content due to partial urine, and there have been few quantitative studies of the relationship between menopausal symptoms and soy isoflavones. Moreover, virtually no studies including metabolites with anticipated physiological action, such as equol, have been conducted, so quantitative evaluations including metabolites are necessary in order to evaluate soy isoflavones. It has also been reported that, in epidemiological research, the measurement the 24-hr urinary excretion of soy isoflavones is an extremely effective means for evaluating the relationship between soy isoflavones and human health since it enables a quantitative understanding of the intake of soy isoflavones and the amount present in the body and allows analyses including metabolites[19].

Therefore, we conducted a study of menopausal symptoms and urinary isoflavone and equol excretion in peri- and postmenopausal Japanese women in order to investigate the relationship between them.

Subjects and Methods

1. Subjects

Women aged 40-60 living in urban or suburban areas of Fukuoka Prefecture and belonging to the Fukuoka Dietetic Association were recruited. The gist of the study was adequately explained orally and in writing to 116 applicants, and 108 women who gave written consent by their own free will were used as the subjects of the study.

2. Methods

A self-rated postal survey was conducted using questionnaires in September and October 1996. Urine collection containers were also directly sent at the same time for 24-hr urine collection.

The self-administered questionnaires inquired about occupation, form of employment, marital history, birth history, menstrual status, past history, physical parameters and menopausal symptoms.

Since the questionnaires of this research comprised content involving personal information, ethical considerations were made with emphasis on respecting the protection of privacy.

The questionnaires included questions regarding ten symptoms routinely observed according to methods of menopausal diagnosis[20]: "facial flushing", "perspiration", "chilliness of the back and extremities", "shortness of breath or palpitation", "insomnia", "irritability", "depression", "headache, dizziness, or nausea", "fatigue", and "stiff shoulders, back pain, or pain of the extremities".

The degree of each symptom was evaluated in four stages in accordance with the method of Kawano *et al*[21] (absolutely no symptom observed: 0, the symptom is personally concerning has virtually no effect on everyday life: 1, the symptom is observed both subjectively and objectively and somewhat impedes everyday life and work: 2, the symptom significantly impedes everyday life and work: 3).

Menopausal symptoms were scored in accordance with a calculation method for the simplified menopausal index (SMI)[20].

The 24-hr urine accumulation time was generally from 9:00 am to 9:00 am of the following day, and the subjects were instructed to send the urine in the refrigerated state immediately after collection. No particular urine collection date was specified.

The questionnaires and the collected urine were respectively reviewed or analyzed. The urine was cryopreserved at -40ºC until analysis.

The daidzein, genistein, and equol, a metabolite of daidzein, in the collected 24-hr urine samples were assayed using an improved HPLC method of Lundh *et al*[22]. The total quantity of the measured daidzein, genistein, and the daidzein metabolite, equol, was defined as the total isoflavones.

The urine that was cryopreserved at -40ºC was thawed in a water bath. After 0.5 ml of this was collected, 1.5 ml of 0.2 M sodium acetate (pH 5.5) and 10 µl of an enzyme solution containing β-glucuronidase and sulfatase were added. This was reacted for 30 min at 37ºC and promptly iced after the reaction was complete, and it was then extracted twice with 5 ml of ethyl acetate. After it was dried under reduced pressure, it was dissolved in 0.5 ml of methanol and used in HPLC analysis. The urinary isoflavone excretion was calculated from the analytical values and the 24-hr urine.

Authentic samples of daidzein, genistein, and equol with 98-99% purity made by Funakoshi Corporation were used. β-Glucuronidase and sulfatase were prepared by Sigma (Lot No.: G0876), and commercially available special-grade products were used for other reagents. The intraday and interday fluctuation coefficients of the daidzein,

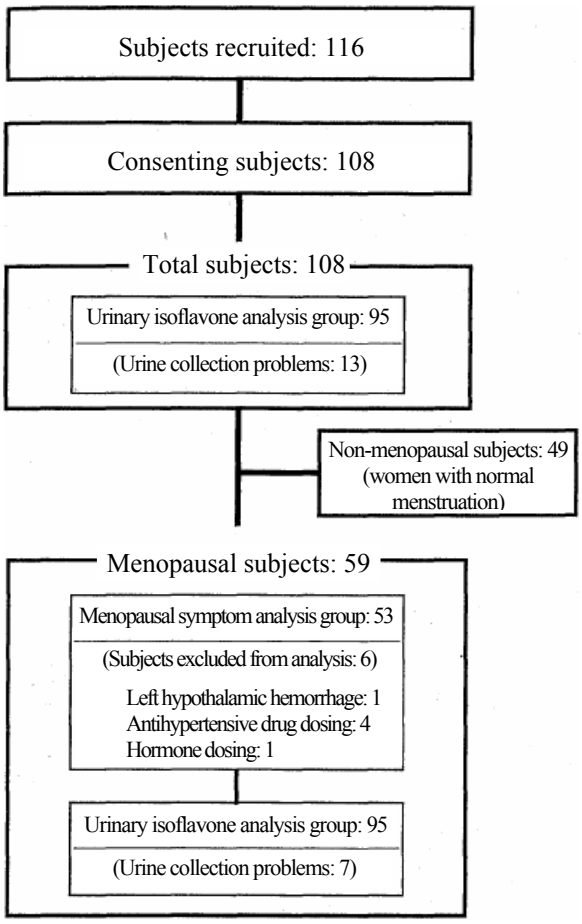

Fig. 1 Analysis subjects

genistein and equol measurements were 5% or less, and the measurement limits were 20, 20, and 50 ng/ml, respectively.

The 95 women from whom 24-hr urine specimens were obtained were used as the urinary isoflavone and equol excretion analysis group (Fig. 1).

In addition, 59 women who were amenorrheic for 3-11 months or 12 or more months were used as menopausal subjects, and 6 patients were excluded from the menopausal symptom analysis group, including 1 subject with left hypothalamic hemorrhage, 4 subjects who had taken antihypertensive drugs, and 1 patient who had taken hormones.

In statistical analysis, menopausal symptoms were compared and analyzed by the Wilcoxon rank sum test using a Statistic Analysis System (SAS). Equol excretor frequency tests were performed by analyzing the data using X^2 tests of a 2×2 contingency table. In each test, $P<0.1$ was defined as a trend difference, and $P<0.05$ was defined as a significant difference.

Table 1 Subject background

	Total subjects	Menopausal subjects
No. of subjects (people)	108	59
Age (years)	50±7	54±5
Menstrual status		
Normal	49 (45.4%)	—
Amenorrheic for 3-11 months	13 (12.0%)	13 (22.0%)
Amenorrheic for 12 or more months	46 (42.6%)	46 (78.0%)
BMI* (%)[1)]	22.1±2.6	22.3±2.7
Work		
Employed full-time	68 (63.0%)	39 (66.1%)
Part-time	29 (26.8%)	14 (23.7%)
Not working	9 (8.3%)	4 (6.8%)
Unknown (no response)	2 (1.9%)	2 (3.4%)
Occupation		
Dietitian/national registered dietitian	74 (68.5%)	41 (69.5%)
Other (school, company)	22 (20.3%)	11 (18.6%)
Housewife	9 (8.3%)	4 (6.8%)
Unknown (no response)	3 (2.7%)	3 (5.1%)
Marital status		
Unmarried	11 (10.2%)	6 (10.2%)
Married	97 (89.8%)	53 (89.8%)
Children (for married subjects)		
None	7 (7.2%)	5 (9.4%)
1	15 (15.5%)	11 (20.8%)
2 or more	75 (77.3%)	37 (69.8%)

* BMI: Body mass index (kg/m^2)
1): Data were missing for 2 out of all of the subjects.

Results

1. Subject characteristics

The backgrounds of all of the subjects and the menopausal subjects are shown in Table 1.

The average age of all of the subjects was 50±7 years, and the BMI was 22.1±2.6 kg/m^2.

The makeup by menstrual status included 49 women with normal menstruation, 13 women who were amenorrheic for 3-11 months (irregular subjects), and 46 women who were amenorrheic for 12 or more months (menopausal subjects). Five of the menopausal subjects had undergone total hysterectomy and bilateral oopherectomy for uterine myoma.

Out of all of the subjects, those undergoing treatment included 3 subjects with hypertension, 3 subjects with peptic ulcers, 2 subjects with hyperlipidemia, 2 subjects with heart disease, and 1 subject each with hepatitis C, systemic lupus erythematosus, menopausal disorders, bronchial asthma, cataracts, diabetes, and left hypothalamic hemorrhage.

The average age of the 59 menopausal subjects was 54±5 years, and the BMI was 22.3±2.7 kg/m^2.

Forty-one of the menopausal subjects (66.1% of the total) were dietitians, national registered dietitians, or licensed cooks employed by a hospital or a public organization, which was approximately the same percentage as that of all of the subjects. There were also virtually no differences between the menopausal subjects and the group including all of the subjects with regard to form of employment, marital status, and number of children.

2. Urinary isoflavone excretion examination results

The analysis population included 95 subjects (Fig. 1), and the distribution of daily urinary isoflavone excretion is shown in Fig. 2. The daily urinary excretion of each isoflavone is shown in Table 2. The urinary excretions of total isoflavones, daidzein, genistein, and equol were 38.9±29.2, 19.6±15.1, 10.0±8.9, and 9.3±14.1 μmol/24 hr, respectively, and the 50 percentile values were 34.8, 17.0, 8.0, and 1.2 μmol/24 hr, respectively. Daidzein and genistein were detected in all of the subjects, but equol was detected in only 49 out of 95 subjects (51.6%) (equol excretors).

Moreover, the results of urinary isoflavone excretion indicated that genistein excretion was greater than daidzein excretion in 90 out of 95 subjects (94.7%).

3. SMI distribution and frequency of each symptom

The SMI distribution of the 53 menopausal women is shown in Fig. 3.

The SMI was 25 or greater in 15 subjects, which was 28.3% of the total. Further, the SMI was 50 or greater in 2 subjects, which was 3.8% of the total. The mean SMI score was 19±14, with a 50 percentile value of 15 and a range of 0-53. The distribution was biased, with a maximum frequency of 10-15.

The frequencies of the various menopausal symptoms are shown in Table 3. The frequencies of three symptoms associated with vasomotor nerves, “facial flushing”, “perspiration”, and “chilliness of the back and extremities” were 20.8%, 41.5%, and

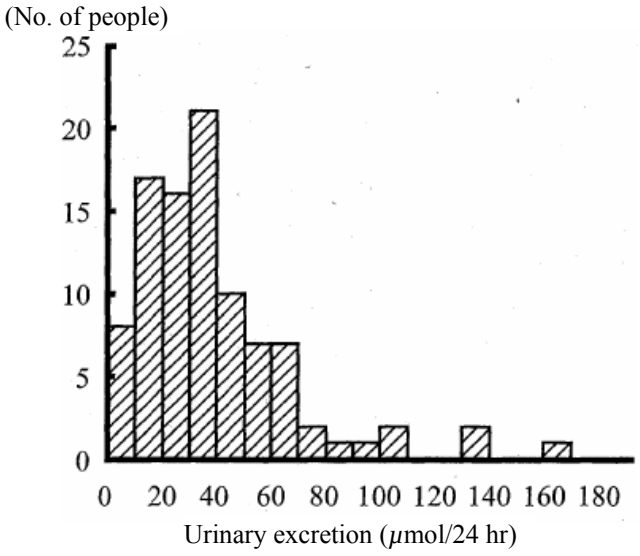

Fig. 2 Distribution of total isoflavone excretion in 24-hr urine (n = 95)

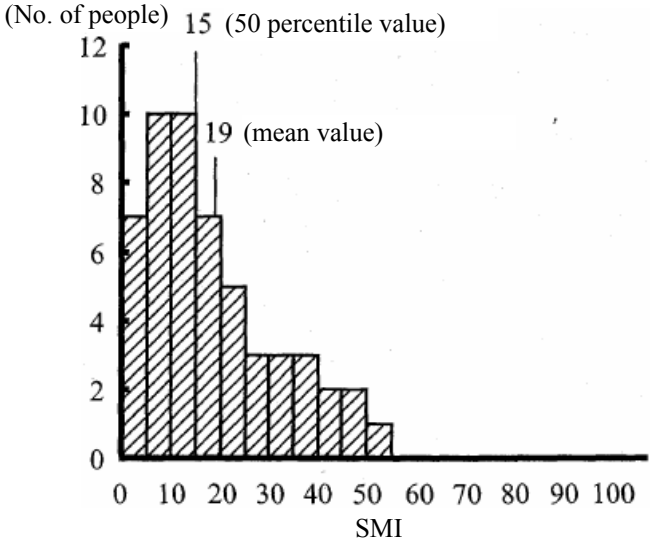

Fig. 3 SMI distribution of menopausal women (n = 53)

Table 2 Urinary isoflavone and equol excretion

Isoflavones	Detected cases (detection rate %)	Mean ± S.D.	Percentile value		Range (minimum-maximum)
			50%	95%	
			(μmol/day)		
Total isoflavone	95 (100.0%)	38.9 ± 29.2	34.8	100.3	1.7 ～ 160.2
Daidzein	95 (100.0%)	19.6 ± 15.1	17.0	47.1	1.0 ～ 87.2
Genistein	95 (100.0%)	10.0 ± 8.9	8.0	22.7	0.4 ～ 68.4
Equol	49 (51.6%)	9.3 ± 14.1	1.2	37.7	0.0 ～ 73.2

34.0%, respectively. The frequencies of mental symptoms of "irritability" and "depression" were both 41.5%. The frequencies of "fatigue" and "stiff shoulders, back pain, or pain of the extremities" tended to be higher than the frequencies of other symptoms at 71.7% and 77.4%, respectively.

4. Relationship between the degree of menopausal symptoms and the urinary excretion of each isoflavone

Analysis was performed after the subjects were divided into high and low SMI groups based on the 50 percentile value of 15 calculated from the SMI distribution of the menopausal subjects of this study (53 subjects). The results of comparing the urinary excretion of daidzein, genistein, and equol in high SMI patients (SMI>15) and low SMI patients (SMI≤15) are shown in Fig. 4.

Table 3 Frequency of each menopausal symptom

Symptom	Frequency (people)	Incidence* (%)
Facial flushing	11	20.8
Perspiration	22	41.5
Chilliness of the back and extremities	18	34.0
Palpitation or shortness of breath	16	30.2
Insomnia	20	37.7
Irritability	22	41.5
Depression	22	41.5
Headache, dizziness, or nausea	23	43.4
Fatigue	38	71.7
Stiff shoulders, back pain, or pain of the extremities	41	77.4

*: Incidence relative to the 53 analysis subjects

No significant differences were observed in the urinary excretion of daidzein and genistein between the groups. On the other hand, the urinary equol excretion in high SMI subjects (4.9±9.1 μmol/24 hr, 50 percentile value: 0.0 μmol/24 hr) was significantly lower than in low SMI subjects (16.3±19.4 μmol/24hr, 50 percentile value: 11.8 μmol/24 hr) (P<0.05).

In addition, the percentages of urinary equol excretors in the high and low SMI groups were compared (Table 4).

Fifteen out of 23 of the low SMI subjects were equol excretors (65.2%), which was significantly higher (P<0.05) than the 8 out of 23 excretors of the high SMI group (34.8%).

The SMI distributions in urinary equol excretors and non-excretors are shown in Fig. 5. The 50 percentile value of equol excretors was 12, while that of non-excretors was 23, which indicated that the SMI tended to be higher in equol non-excretors.

Therefore, quantitative analysis was performed to determine (1) whether the contribution of equol to the reduction of menopausal symptoms simply depends on whether or not it is produced and (2) whether a certain amount of equol is necessary (Fig. 5).

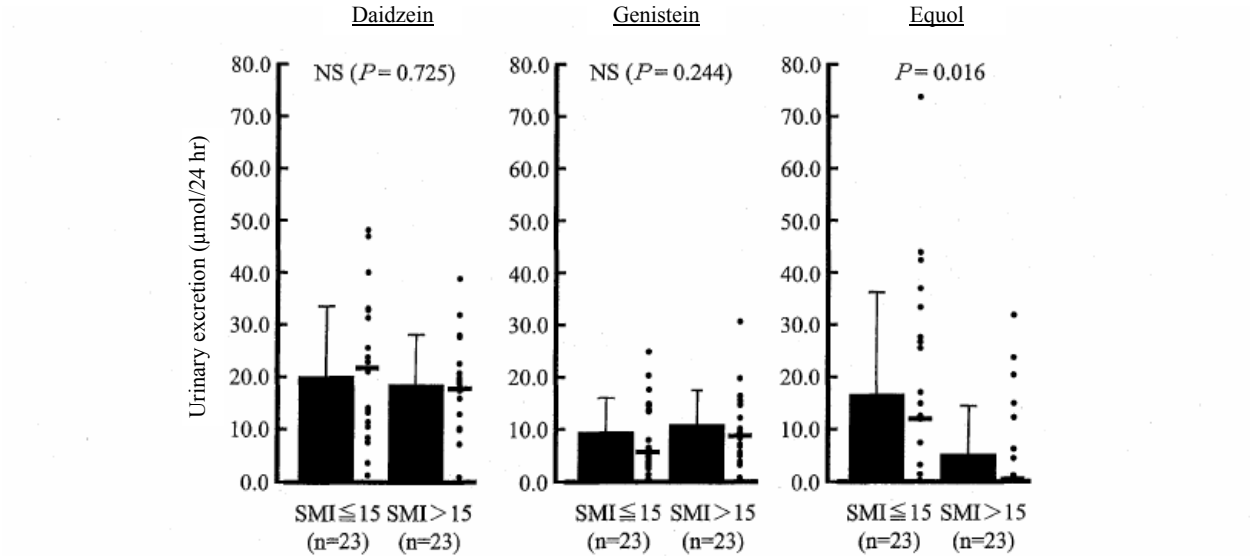

Fig. 4 Comparison of the degrees of menopausal symptoms and urinary isoflavone excretion
NS: Not Significant; P-values indicate the results of analysis using the Wilcoxon rank sum test. The results are expressed as the mean ± S.D. and the 50 percentile (bars in the plots).

Classifying the data based on whether or not equol was excreted (excretors and non-excretors), the SMI tended to be lower in equol excretors (P=0.062). Dividing the data into classes based on the urinary equol excretion level of 5 μmol/24 hr (≥5 μmol/24 hr and <5 μmol/24 hr), the SMI was significantly lower (P=0.038) in subjects with excretion of at least 5 μmol/24 hr than in subjects with excretion of less than 5 μmol/24 hr.

Table 4 Comparison of the percentages of urinary equol excretors in high and low SMI subjects

	Excretors	Non-excretors	Total	P-value
SMI ≦ 15	15	8	23	
SMI > 15	8	15	23	P = 0.039
Total	23	23	46	

P-values represent the results of analysis by X^2 tests.

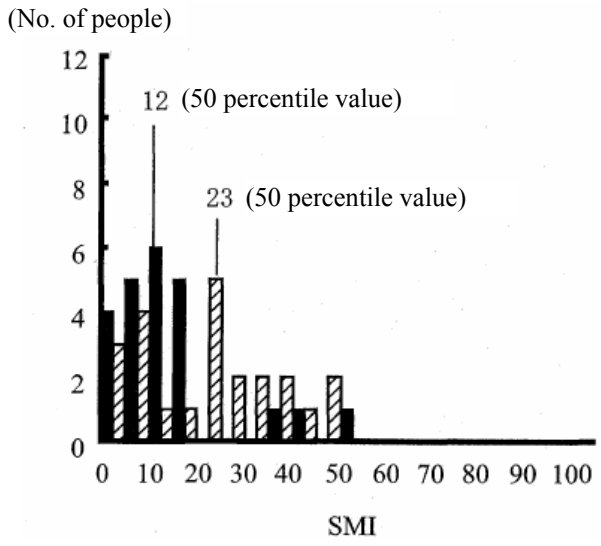

Fig. 5 SMI distributions in equol excretors and non-excretors
■ Equol excretors (n = 23)
▨ Equol non-excretors (n = 23)

Discussion

We conducted a quantitative analysis on the relationship between soy isoflavones and menopausal symptoms in Japanese women using 24-hr urine samples. A 24-hr urine sample is an index which quantitatively reflects the amount of soy isoflavones present in the body, and this method is advantageous in that metabolites can also be analyzed[19].

The 24-hr urinary excretions of isoflavones and the metabolite equol were studied in dietitians from Fukuoka Prefecture. Daidzein and genistein, which are present in soy, were observed as soy isoflavones in the urine of all of the subjects, and individual differences proved to be large, with ranges of 1.0-87.2 μmol/24 hr and 0.4-68.4 μmol/24 hr, respectively. Equol is not ordinarily present in soy processed foods, but it is an active metabolite that is produced when daidzein is metabolized by intestinal bacteria. Therefore, there are individual differences in its production, and it has been reported that approximately 30% of Europeans and Americans and approximately 50% of Japanese are equol excretors, [15)23)]. In this study, 51.6% of the subjects were equol excretors, which was consistent with the results for Japanese reported previously. The maximum 24-hr urinary equol excretion was 73.2 μmol/24 hr, and it was produced in approximately the same quantities as daidzein and genistein in people with high metabolism. The report of Herman *et al*[1] compares the urinary isoflavone excretion of Finnish and Japanese (agricultural community of Nagano Prefecture) women. The urinary excretions of daidzein, genistein, and equol in Finnish women were 0.15, 0.07, and 0.06 μmol/24 hr, respectively, and those in Japanese women were 7.01, 4.98, and 1.01 μmol/24

Table 5 Relationship between urinary equol excretion and SMI

Category		SMI	P-value
Equol non-excretors	(n = 23)	22.52 ± 14.65	
Equol excretors	(n = 23)	15.17 ± 13.06	P = 0.062
<5 μmol/24 hr	(n = 27)	22.52 ± 15.27	
≥5 μmol/24 hr	(n = 19)	13.63 ± 10.93	P = 0.038

Wilcoxson rank sum test

hr, respectively. The urinary excretions of daidzein, genistein, and equol in the peri- and postmenopausal women of Fukuoka Prefecture studied here were 19.6, 10.0, and 9.3 μmol/24 hr, respectively. These values were higher than those reported by Herman *et al*, which suggested a large intake of soy and soy processed foods.

The menopausal symptoms of a menopausal group consisting of women who were amenorrheic for 3-11 months and women who were amenorrheic for 12 or more months were evaluated by SMI. Menopausal patients examined on an outpatient basis typically have an SMI score of approximately 50-70[20], and the menopausal patient group studied here was relatively mild with a maximum SMI score of 53. The frequencies of the symptoms of "facial flushing", "perspiration", and "chilliness of the back and extremities" were 20.8%, 41.5%, and 34.0%, respectively, which tended to be higher than the subjective observations of "facial flushing" in 10% of patients and "perspiration" in 4% of patients reported by Lock *et al*[10] in a previous study of Japanese women (1983-1984). Kudo *et al* have reported "facial flushing" in 36.9% of patients and "perspiration" in 45.6% of patients in urban areas in a study of urban areas (Kanagawa Prefecture) and agricultural communities (Nagano Prefecture) conducted in 1998[24], which demonstrates frequencies similar to those observed in our study. This suggested that the frequencies of "facial flushing" and "perspiration", which were previously considered infrequent in Japanese women, tend to be higher recently.

As a result of investigating the relationship between soy isoflavones and equol in high SMI patients (SMI≤15) and low SMI patients (SMI>15) based on the SMI 50 percentile value of 15 of menopausal subjects in this study, no relationship with daidzein or genistein contained in soy was observed, and significant difference differences were only observed in the levels of the metabolite equol. The fact that there is a relationship between equol and menopausal symptoms indicates that equol produced in the human intestines contributes to menopausal symptoms to a greater degree than constituents ingested from food such as daidzein and genistein. Moreover, the percentage of people capable of excreting equol in urine in the high menopausal symptom group of the study population was low, which suggested that the degree of menopausal symptoms is closely related to whether equol is produced in the intestines. Furthermore, a comparison of the SMI distributions in urinary equol excretors and non-excretors revealed that SMI clearly tends to be lower in equol excretors (Fig. 5).

The urinary excretions of daidzein and genistein reflect the amounts of daidzein and genistein ingested from food, but the amount of equol produced by intestinal bacteria is influenced by the productive ability of each patient. In other words, whereas the urinary excretions of daidzein and genistein are affected by food content – the amount of soy processed food ingested, in particular – equol is not simply regulated by the amount of its substrate daidzein alone, and an important factor is whether or not equol is produced.

People who are capable of producing equol may be able to effectively use equol in the body by maintaining their intestinal environment. However, although many researchers have investigated ways to improve equol production by dietary fiber[25], soy protein[26)-31)], and probiotics such as oligosaccharides and lactic acid bacteria[32)33)] in people who are unable to produce equol, a means of improvement has not yet been discovered.

Possible factors of equol productive ability include racial differences, dietary differences, and aging, but these have not yet been clarified. Differences due to aging were investigated in this study by comparing urinary isoflavone excretion in the menopausal subjects of the study group and women with normal menstruation (excluded from the menopausal group), but no differences were observed in the urinary excretions of total isoflavones, daidzein, genistein and equol. Moreover, there were no differences in the percentages of equol excretors. Since the subjects of this study were in the age bracket from 40 to 60 years of age, the data do not reflect generational differences in dietary habits. Furthermore, the dietary habits of younger generations differ substantially from middle aged and elderly people due to the westernization of their meals. This yields the possibility that generational differences in urinary isoflavone excretion may emerge, so future studies will likely be necessary.

It was previously thought that menopausal disorders could be improved by the estrogenic/antiestrogenic action of daidzein and genistein, which are isoflavones found in soy[8)9)11)]. However, the results of this study do not support this, but rather suggest the possibility that equol, a metabolite of daidzein, is deeply involved. Moreover, it was inferred from the results of quantitative

analysis that urinary equol excretion of at least 5 μmol/24 hr is required to reduce everyday menopausal symptoms.

On the other hand, when we attempted to analyze the relationship between vasomotor disorder-like symptoms ("facial flushing", "perspiration", and "chilliness of the back and extremities"), which are closely related to estrogen imbalance, and equol based on whether or not symptoms were observed, the percentage of women with symptoms whose urinary equol excretion was at least 5 μmol/24 hr was 37.5%. Although this was somewhat lower than in women without symptoms (50.0%), no statistically significant difference was observed.

It has been reported that equol has stronger affinity to estrogen receptors than daidzein and genistein in *in vitro* experiments[34] and that its tissue distribution into the breasts[35] and prostate[36] is high. In studies in which soy isoflavones are introduced, it has been reported that their effects are more prominent in equol producers than in non-producers with regard to their inhibitory effect on postmenopausal bone loss and lipid metabolism[6)15)]. These results also suggest that equol, a metabolite of daidzein produced in the intestines, plays a significant role in health benefits after ingesting soy and soy processed foods.

In this study, equol was considered a constituent with a promising preventative effect against menopausal symptoms. The results also indicated that menopausal symptoms (SMI) are milder in women with 24-hr urinary excretion of at least 5 μmol, but it will be necessary to accumulate additional data or conduct empirical research on this quantity in the future.

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