Comparison of Isoflavones Among Dietary Intake, Plasma Concentration and Urinary Excretion for Accurate Estimation of Phytoestrogen Intake

Yusuke Arai [1], Mariko Uehara [1], Yuho Sato [1], Mitsuru Kimira [1], Akira Eboshida [2], Herman Adlercreutz [3], and Shaw Watanabe [1]

Biological effects of dietary isoflavones, such as daidzein and genistein are of interest in preventive medicine. We estimated the dietary intake of isoflavones from dietary records and compared the values with the plasma concentrations and urinary excretions in Japanese middle-aged women. The dietary intake of daidzein and genistein was 64.6 and 111.6 μ mol/day/capita (16.4 and 30.1 mg/day/capita), respectively. The isoflavones intake was mostly attributable to tofu, natto and miso. The median of plasma daidzein and genistein concentration was 72.46 and 206.09 nmol/L, respectively. The median of urinary excretion was 20.54 μ mol/day for daidzein, 10.79 for genistein, 15.74 for equol and 1.64 for *O*-desmethylangolensin (*O*-DMA). Equol and *O*-DMA were excreted by 50 % and 84 % of all participants, respectively. Equol metabolizers were significantly lower the plasma and urinary daidzein and urinary *O*-DMA.

The dietary intake of daidzein and genistein after the adjustment for total energy intake was significantly correlated with the urinary excretion (r=0.365 for daidzein and r=0.346 for genistein) and plasma concentration (r=0.335 for daidzein and r=0.429 for genistein). The plasma concentration of isoflavones was also significantly correlated with the urinary excretion. We conclude that in epidemiological studies measurements of plasma concentration or urinary excretion of these isoflavones are useful biomarkers of dietary intake and important for studies on their relation to human health. *J Epidemiol*, 2000 ; 10 : 127-135

daidzein, genistein, Japanese women, dietary record, biomarker

INTRODUCTION

There is an increasing interest in the biological effects of phytoestrogens because of their chemical structures and molecular weights being similar to those of estrogens [1]. They are found in many edible plants [2] and are classified into two main groups, isoflavones and lignans. They occur in plants mainly as glycosides, and the biologically active compounds (aglycones) are produced after hydrolysis and metabolism by intestinal microflora [3].

Phytoestrogens have been shown to influence sex hormone metabolism and biological activity [4]. They also has anticarcinogenic [5] and antiangiogenic activity [6], and induce intercellular enzymes [7]. Furthermore, they are antioxidants and inhibit tumor growth in *in vitro* studies and animal experiments [8,9].

Soybeans are a rich source of isoflavones [10]. Japanese consume daily considerable amounts of soybeans and its fermented products [11]. Such a high intake of soybean foods suggests to exert a cancer protective effect, especially estrogen-related cancers, such as breast, endometrial, ovarian, prostatic and colon cancer among Japanese [12-14].

A number of studies in human have reported high individual variability in isoflavone metabolism. Daidzein and genistein undergo further metabolism by gut flora. Genistein, in sheep, is metabolized primarily to *p*-ethylphenol. However, p-ethylphenol has not been identified in human urine and plasma. The

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[1] Department of Nutritional Science, Faculty of Applied Bioscience, Tokyo University of Agriculture, Tokyo, Japan.
[2] Department of Life Science, The Scienal and Technology Agency of Japan.
[3] Institute for Preventive Medicine, Nutrition and Cancer, Folkhälsan Research Center and Department of Clinical Chemistry, University of Helsinki, Finland.
Address for correspondence : Yusuke Arai, Department of Nutritional Science, Faculty of Applied Bioscience, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya, Tokyo, 156-8502 Japan.

daidzein metabolites, equol, and *O*-desmethylangolensin (*O*-DMA) have identified in human urine and plasma. Equol has strong affinity for estrogen receptor compared with other isoflavones. Preliminary studies have reported that 20-60 % of the population is able to produce equol after a soy challenge. Recent study, Inglam et al. [15] reported that high equol excretion was associated with a substantial reduction in breast cancer risk.

Relatively little is known about the relationship in Japanese subjects among the dietary intake, and plasma concentration and urinary excretion of these compounds [14]. The first purpose in the present study, we calculated the dietary intake of daidzein and genistein in 106 Japanese women and compared them with the plasma concentration and the urinary excretion of these isoflavones and studied whether the plasma and urinary isoflavones could be used as appropriate biomarkers of isoflavone intake. The second purpose was to measure the prevalence of urinary daidzein metabolism, equol and *O*-DMA, in Japanese women.

MATERIALS AND METHODS

Subjects

The study was carried out during three years (1996-1998) in September in a small city, in the Iwate prefecture in the northern part of Japan (Tohoku region). The subjects were 106 women volunteers, who gave their written consent to participate in the study. Each year 30 to 40 women were studied. The age of subjects ranged from 29 to 78 years old (mean ± SD; 58.0 ± 10.0).

Each subject completed a 3-day dietary record and on the 4th day of the study they were checked with regard to their health. The subjects recorded all foods and beverages, which they had consumed during the study period. The dietary records were evaluated by trained dietitians and coded for calculation of energy and nutrient intake using the Standard Tables of Food Composition (4th revised edition) [16]. Isoflavone intakes, daidzein and genistein, were separately calculated from our phytochemical composition table of Japanese food [17].

The health check-up program included physical examinations, such as the measurement of height, weight, and blood pressure. Body mass index (BMI, kg/m^2) was calculated from height and weight. 10 mL of peripheral blood after overnight fasting was collected in a vacuum syringe containing heparin powder. The plasma was obtained by centrifugation (2,000 × g, 10 min, 4 ℃), and used for blood routine biochemistry and an aliquot was stored at a -80 ℃ until analysed for isoflavones.

The 24h-urine sample (from the second urination on the 3rd day of the nutrition survey to the first urination on the 4th day) was collected using the U-mate (24h-urine measuring device with proportional sampling system) (Sumitomo Bakelite, Akita, Japan) [18]. 56 μ mol/L Vitamin C and 3 μ mol/L NaN_3 (Kanto Chemical, Tokyo, Japan) were added to the storage bottle of the U-mate to prevent degradation of isoflavones by oxidation and by bacterial contamination. The volume of the urine samples was measured and the samples stored at -80 ℃ until analysis of isoflavones and their metabolites.

Analysis of isoflavones in the plasma

A sensitive and convenient time-resolved fluorescence immunoassay (TR-FIA) method was used for analysis of plasma isoflavones [19].

For the recovery calculation 20 μ L of ^{3}H-estradiol glucuronide was added to the tubes containing 200 μ L of plasma. After mixing and equilibrating for 30 min at room temperature, 200 μ L of acetate buffer 0.1 mol/L (pH 5.0) containing 0.2 U/mL glucronidase and 2 U/mL sulfatase was added to the tubes. After mixing using a Vortex mixer and incubation overnight at 37 ℃, 2.0 mL of diethyl ether was added and the phytoestrogens extracted equilibrating the phases with a Vortex mixer. The phase was transferred into disposable glass tubes. After thawing, the water phase was re-extracted with the same amount of ether, and the ether phases were combined and evaporated to dryness (preferably with nitrogen) in 45 ℃ water bath. Then 200 μ L of 50 mM Tris-HCl buffer containing 0.5% BSA (pH 7.8) (assay buffer) was added to the tubes containing the dry residues, and after thorough mixing 20 μ L (in duplicate) of the solution was taken for TR-FIA of each compound. This volume corresponds to 20 μ L of the original plasma sample. The samples giving a value outside the range of the standard curve were diluted with assay buffer. Another 20 μ L of the solution was taken for liquid scintillation counting for determination of recovery. Based on the results the final values were corrected for losses during hydrolysis and extraction.

Twenty μ L of standard or hydrolyzed plasma was pippeted into prewashed goat anti rabbit IgG microtitration wells. To each well was added 100 μ L of antiserum (dilution of 1:40,000 for daidzein and 1:50,000 for genistein) in assay buffer and 100 μ L of europium-labelled daidzein or genistein (dilution 1:40,000 and 1:400,000 for daidzein and genistein, respectively). After incubation and shaking the strips slowly on DELFIA plate shaker at room temperature for 90 min, the strips were washed using DELFIA plate washer (using the no. 29 T3 program), enhancement solution (200 μ L) was added to each well and the strips were shaken slowly for an additional 5 min. The enhanced fluorescence were in a VICTOR 1420 multilabel counter wallacoy Finland. Calculation of the final result was done according to the formula:

Final result = Concentration (read) × 1/recovery × dilution factor (nmol/L).

Analysis of urinary isoflavones and their metabolites

The urinary isoflavones and metabolites were analyzed using the extraction method of Adlercreutz et al. [20] combined

with the modified HPLC method described by Gamache et al. [21]. For the recovery calculation 20 μL of ^{3}H-estradiol glucuronide was added to the tubes containing 1 mL of urine. After mixing and equilibrating for 30 min at room temperature, 0.5 ml enzyme solution (0.5 mL Helix pomatia juice (Type HP-2S, Sigma, St. Louis, Missouri, USA) in 10ml 0.2M acetate buffer (PH 4.0) containing 0.15g ascorbicacid) was added to the tubes. After mixing the sample was hydrolysed overnight at 37 ℃. The hydrolysed sample was extracted twice with 5 mL diethyl ether, and the ether fraction was evaporated to dryness under a flow nitrogen gas. The residue was dissolved in 0.2 mL methanol, and 20 μL sample was injected to HPLC with diode-array UV detection, scanning from 250 to 400 nm (Beckman Coulter K.K., Tokyo, Japan). Another 20 μL of the solution was taken for liquid scintillation counting for determination of recovery. Peaks were detected at 254 nm for daidzein and genistein and at 280 nm for equol and *O*-DMA. The HPLC column was ODS-80Ts-Qa (150×4.6 mmI.D., 5 μm particle size) (Tosoh, Tokyo, Japan) with a guard column (TSKguardgel ODS-80Ts, 1.5×3.2 mmI.D. 5 μm particle size) (Tosoh, Tokyo, Japan), and kept temperature at 25 ℃ using a column oven. HPLC analysis was carried out by linear gradient, from 1.5/0.5/8.0 (methanol, acetonitrile, 0.2 M acetate buffer (pH 4.0), v/v/v) to 6.0/3.0/1.0 for 45 min, and resumed to initial condition for 5 min. The flow rate was 1.0 mL/min.

Quantification was done by measuring peak areas based on calibration plots of the peak area of standards at various concentrations (from 4 to 160 μmol/L) and corrected for losses during hydrolysis and extraction based on the recovery data. All solvent and chemicals were of HPLC grade or analytical grade.

Statistical analysis

All statistical analyses were conducted with the SPSS package ver. 8.0J (SPSS Japan Inc., Tokyo, Japan). Because nutrient intake containing isoflavones is correlated with total energy intake, we used the method of energy adjustment proposed by Willet [22]. Estimation of the adjusted levels of different factors was carried out by the multiple regression analysis (residual model). Because residuals have a mean of zero and include negative values, the energy adjusted values is added the predicted intake for the mean energy intake of the study population. Pearson's correlation coefficients were calculated among the isoflavone of dietary intake, plasma concentration and urinary excretion. Dietary intakes after adjusted total energy intake, plasma concentrations and urinary excretions of isoflavones and metabolites were used after logarithmic transformation values for statistical analysis of correlation coefficients. Comparisons of isoflavones of dietary intake, plasma concentration and urinary excretion between equol metabolizers and non-metabolizers were used Mann-Whitney test. Two-sided *P* values below 0.05 were considered statistically significant.

RESULTS

Physical characteristics of the subjects were as follows; height: 151.1±5.6 (mean±SD) cm, body weight; 54.0±7.0 kg, BMI; 23.7±3.1, systolic blood pressure (SBP); 124±16 mmHg and diastolic blood pressure (DBP); 75±9 mmHg. The results of dietary record are shown in Table 1. The average intake of energy and nutrients exceeded the level of Japanese Recommend Dietary Allowance [23]. Dietary intake of pulses in this study was more than that of the National Nutrition Survey (70.9 g/day/capita) [11].

The dietary intake of isoflavones (sum of daidzein and genistein) was 176.2±82.8 (mean±SD) μmol/day/capita, ranged from 48.7 to 450.0 μmol/day/capita (Table 2 and Fig. 1). Genistein represented two-thirds of total isoflavone intake (111.6±53.2 μmol/day/capita, 30.1±14.4 mg/day/capita), and daidzein the rest (1/3rd) (64.6±29.7 μmol/day/capita, 16.4±7.6 mg/day/capita). Table 3 showed the attribute of the isoflavone intake. Tofu (sum of various types), natto and miso covered 85.3 % of pulses intake by weight.

The plasma concentration and urinary excretion of isoflavones and metabolites are shown in Table 2 and Fig. 1. There was a 600-fold variation between maximum and minimum values for plasma daidzein and 100-fold variation for plasma genistein between the subjects. Genistein was main component of the plasma isoflavone and its concentration was 3 times higher than that of daidzein. The recovery rate was calculated according to the formula: Recovery rate = urinary excretion / 3-days mean intake × 100. The recovery rate of isoflavone intake from the urinary excretion was 38.0 % for daidzein and 13.0 % for genistein. Daidzein was the main component of urine and identified in all women (n=106), and genistein was found in all but women (n=105). Excretion of equol was found in 53 subjects (50.0 %) and of *O*-DMA in 89 subjects (84.0 %).

Table 4 shows the isoflavone status among equol metabolizers and non-metabolizers in Japanese women. Equol metabolizers were lower the isoflavones of plasma concentrations and urinary excretions compared with non-metabolizers. There was no significant difference in nutrition and food group intake between equol metabolizers and non-metabolizers.

The correlation matrix of isoflavones intake, plasma concentration and urinary excretion is shown in Table 5. Dietary intake of daidzein and genistein showed a very good correlation with their plasma concentration and urinary excretion. The plasma concentration of daidzein and genistein showed a good correlation with the urinary excretion. Urinary equol excretion showed a good correlation with urinary daidzein, but not with plasma daidzein concentration. Urinary *O*-DMA correlated with daidzein intake and plasma daidzein concentration.

Table 1. Intake of nutrients and consumption of foods by food groups by Japanese women.

Various	Mean	SD	Median	Min	Max	Percentile	
						25	75
Energy (kcal/d)	1816	364	1795	1070	3244	1571	2045
(kJ/d)	7597	1522	7509	4478	13571	6570	8555
Protein (%)[1]	16.6	2.4	16.5	10.8	23.4	14.7	18.2
Fat (%)[1]	23.4	4.9	23.9	6.9	33.0	19.6	26.8
Carbohydrate (%)[1]	60.1	6.0	59.9	47.2	82.3	56.2	63.2
Animal protein (%)[2]	47.5	9.5	48.0	18.5	67.1	42.9	54.8
Cereals (g/d)	326.5	190.2	256.5	52.0	926.0	119.5	377.7
Pulses (g/d)	94.3	46.8	85.1	28.9	254.1	59.5	117.8
Tofu (g/d)	48.7	36.2	36.7	0.0	177.0	21.6	66.7
Miso (g/d)	17.0	9.8	15.4	0.0	46.5	9.7	24.2
Natto (g/d)	14.8	11.8	13.3	0.0	50.0	6.7	22.3
Soysource (g/d)	18.6	10.7	17.1	1.3	51.4	10.9	24.0
Fruits (g/d)	135.8	96.2	110.3	0.0	433.7	71.6	181.1
Vegetables (g/d)	376.5	147.1	345.7	145.3	956.0	274.7	443.5
Green yellow (g/d)	160.6	103.6	137.0	19.0	608.3	84.1	207.0
Others (g/d)	215.9	92.5	201.6	50.0	552.3	150.4	269.4
Fishs (g/d)	92.7	46.1	83.9	18.4	202.1	59.2	123.9
Meats (g/d)	35.4	29.4	29.0	0.0	191.7	16.7	48.3

[1] Percent of total energy intake.
[2] Percent of total protein intake.

Table 2. Isoflavone status in Japanese women.

Various		n	Mean	SD	Median	Min	Max	Percentile	
								25	75
Intake	Daidzein	106	64.6	29.7	57.9	18.2	167.2	41.1	82.4
(μ mol/day)	Genistein	106	111.6	53.2	103.2	30.6	282.8	71.5	139.1
Plasma	Daidzein	106	111.7	187.8	72.5	3.0	1766.2	23.9	221.0
(nmol/L)	Genistein	106	307.5	325.4	206.1	25.3	2459.7	113.7	393.0
Urine	Daidzein	106	23.3	20.4	20.5	0.2	155.2	9.1	31.3
(μ mol/day)	Genistein	105	13.2	10.4	10.8	0.2	62.4	5.2	18.5
	Equol	[a] 53	0.0	-	-	-	-	-	-
		[b] 53	23.4	26.7	15.7	0.4	123.6	5.6	29.4
	O-DMA [1]	[a] 17	0.0	-	-	-	-	-	-
		[b] 89	3.3	6.4	1.6	0.2	56.3	0.7	3.4

[1] *O*-DMA : *O*-Desmethylangolensin.
[a] Non-metabolizer
[b] Metabolizer

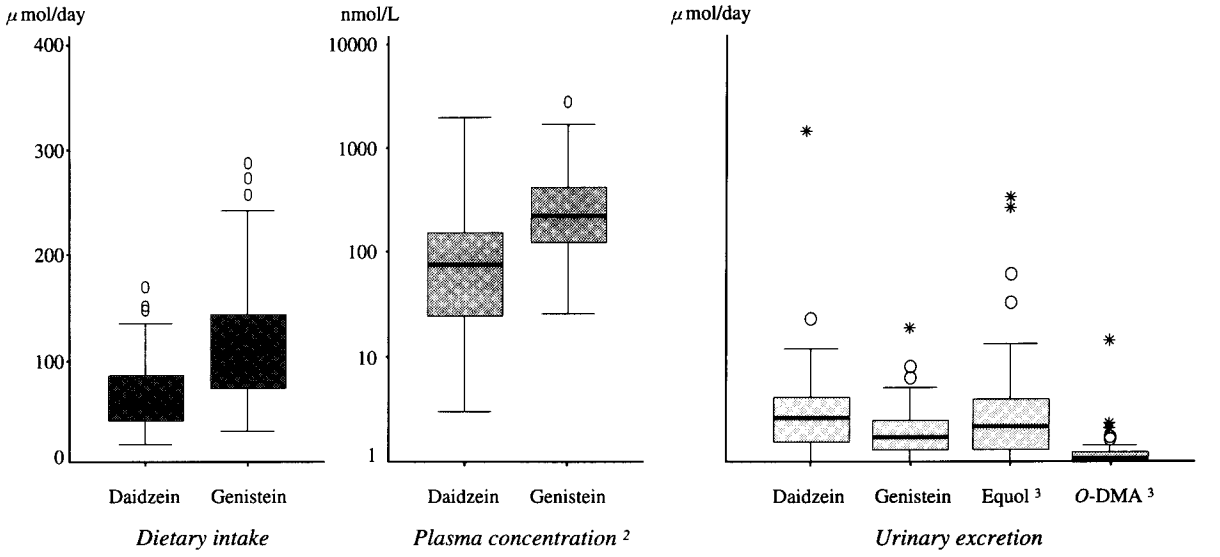

Figure 1. Distribution of isoflavones of dietary intake, plasma concentration and urinary excretion [1].

[1] n=106, n=105 for urinary genistein

[2] Plasma concentrations are logarithm transferred values.

[3] Metabolizers only: equol (n=53) and *O*-DMA (n=87)

This figure is called box-and-whisker plot.

The length of the box corresponds to the interquartile range. The median of the data values fall within the range of the box. The whiskers show the range values that fall within 1.5 length of the box. An open circle (○) shows values between 1.5 and 3 lengths of the box. An asterisk (*) shows over 3 lengths of the box.

Table 3. Attributable percent of soy foods to isoflavone intake.

Rank	Food	Intake (g/day)		% of isoflavone intake	Cumulative % of isoflavone intake
		mean ± SD	median		
1	Tofu	48.6±36.7	43.8	36.8	36.8
2	Natto	14.8±11.8	13.3	32.0	68.8
3	Miso	17.0±9.8	15.4	18.7	87.5
4	Deep fried tofu (Thin type)	3.9±6.1	1.5	3.6	91.1
5	Ganmodoki	1.4±5.5	0.0	1.8	92.9
5	Soybean powder (kinako)	0.4±2.2	0.0	1.8	94.7
7	Deep fried tofu (Thick type)	0.9±3.8	0.0	1.2	95.9
8	Soybean (immature)	3.8±14.4	0.0	1.1	97.0
9	Soysouce	18.6±10.7	17.1	0.8	97.8
9	Soymilk	2.0±21.0	0.0	0.8	98.6
11	Soybean	0.4±2.0	0.0	0.7	99.3
12	Bean sprout	1.2±5.0	0.0	0.4	99.7
13	Tofu (Dried type)	0.1±0.5	0.0	0.3	100.0
14	Okara (Soybean pulp)	0.2±1.2	0.0	0.0	100.0

Table 4. Isoflavones data among equol metabolizers and non-metabolizers in Japanese women.

Various		Non-Metabolizers n=53	Metbolizers n=53	p for difference [2]
Intake (μ mol/day)	Daidzein	61.4	56.7	p=0.892
	Genistein	106.2	93.0	p=0.852
Plasma (nmol/L)	Daidzein	96.9	42.6	**p=0.001**
	Genistein	278.3	181.4	p=0.281
Urine (μ mol/day)	Daidzein	24.5	12.9	**p=0.007**
	Genistein	11.3	10.6	p=0.446
	O-DMA [1]	2.1	1.1	**p=0.008**

[1] *O*-DMA : *O*-Desmethylangolensin.
[2] Mann-Whitney test

Table 5. Pearson correlation coefficients between various isoflavones and their metabolites in Japanese women [1].

		Intake		*Plasma*		*Urine*		
		Daidzein	Genistein	Daidzein	Genistein	Daidzein	Genistein	Equol
Intake [2]	Genistein	0.992***						
Plasma	Daidzein	0.335***	0.334***					
	Genistein	0.418***	0.429***	0.880***				
Urine	Daidzein	0.365***	0.377***	0.449***	0.485***			
	Genistein	0.340***	0.346***	0.347***	0.499***	0.879***		
	Equol [3]	0.395***	0.396***	0.035	0.217	0.719***	0.755***	
	O-DMA [3,4]	0.272*	0.250*	0.311**	0.225*	0.548***	0.412***	0.336*

* p<0.05 * p<0.01 *** p<0.001
[1] All values are logarithmic. n=106, n=105 for urinary genistein
[2] Energy adjusted values (residual model).
[3] Metabolizers only
[4] *O*-DMA : *O*-Desmethylangolensin.

DISCUSSION

Epidemiological studies have shown that the mortality of estrogen dependent cancers among Japanese was lower than that of Caucasian [13, 24]. Japanese consume a lot of soybean products rich in phytoestrogenic isoflavones. We have previously reported the concentrations of flavonoids and isoflavones in various foods and estimated intakes of isoflavones in 50 Japanese women to be 39.5 mg/day/capita [15]. It is necessary to find good biomarkers in epidemiological studies to get information on the possible effects of phytoestrogens in the population. We studied whether the plasma concentration and the urinary excretion of isoflavones could be used as appropriate biomarkers of isoflavone intake.

According to the National Nutrition Survey [11], the consumption of pulses (mainly soybean products) by Japanese is 70.9 g/day/capita. It is 94.6 g/day/capita in Tohoku region, where the largest consumption of soybean products in Japan. In our study area, the consumption of pulses was 94.3 g/day/capita, which showed similar level as the National Nutrition Survey in Tohoku. The isoflavones intake (sum of daidzein and genistein) was about 176.2 μ mol/day/capita (46.5 mg/day/ capita). Adlercreutz et al. [14] reported 19 residents (9 men and 10 women) in a village outside Kyoto whose intake of pulses was 40.9 g/day in males and 56.5 g/day in women and the urinary excretion of daidzein 2.6 μ mol/day, equol 2.6 μ mol/day and

O-DMA 0.7 μ mol/day. They estimated that the isoflavone intake was 24 mg/day in women (19 mg of genistein and 5 mg of daidzein) [4]. Wilcox et al. [25] reported that the average of daily isoflavone intake in Okinawa prefecture was 32 mg/day. These isoflavones intakes were lower than the estimation by Cassidy et al. (150-200 mg/day) [26]. Soybean products intake by Japanese have regional variation [11]. So, the isoflavones intake also would vary. The level of isoflavones intake in our study varies from 48.7 to 450.0 μ mol/day/capita. The intake of isoflavones by Japanese was 15 times higher than that of the Caucasians [27]. Appropriate amount of daily intake of soybean products has not been determined yet for the prevention of cancer.

Ingram et al. [15] carried out a case-control study by 144 pairs of breast cancer patients and controls, and found high urinary excretion of equol and enterolactone being associated with a substantial reduction in breast-cancer risk. Odds ratios between the lowest and highest quartiles of urinary excretion were 0.27 for equol and 0.36 for enterolactone. Their median urinary excretion of equol in control group was 0.108 μ mol/day and of daidzein was 0.91 μ mol/day. Median urinary excretion of equol in our study was 15.7 μ mol/day. Based on Ingram's equol values, our results would suggest a greatly reduced breast cancer risk in the population studied. However, slightly higher equol excretion in the Australian women with low breast cancer risk may only be a biomarker of a more healthy vegetarian diet.

There is a large variation between individuals in urinary excretion of the two daidzein metabolites, equol and *O*-DMA. It has been considered that equol and *O*-DMA is formed by intestinal microflora [3,28]. Breinholt and Larsen [29] reported that equol has slightly stronger estrogenic potency than genistein, determined by the recombined yeast with human estrogen receptor combined with a β-galactosidase receptor gene.

Lample et al. [30] compared habitual diets of individuals who excrete equol and do not excrete significant amounts of equol when presented with a defined soy protein beverage challenge. Twenty-one of the 60 participants (35%) excreted equol after 3-days of consuming the soy supplement. Daily excretion of isoflavones (genistein, daidzein and *O*-DMA) excretion was similar between equol excreter and non-excreter. Among the women, excreter consumed a significantly higher percentage of energy of energy as carbohydrate and greater amounts of plant protein and dietary fibre compared with non-excreter. Rowland et al. [28] studied the extent of inter-individual variation in phytoestrogen metabolism by giving 23 healthy human subjects vegetarian burgers made of extruded soy protein flour containing 56 mg of isoflavonoids/burger, which consumed one per day for 17 days in addition to their regular diet. The eight subjects (35 % of all subjects) excreted over 1000 nmol/day (good equol excreters). They consumed significantly less fat (26 % compared with 35 % of total energy; $p<0.01$) and more carbohydrate (55 % compared with 47 %; $p<0.05$). These data suggested that, among women, diets like Japanese may promote the growth and/or the activity of bacterial populations responsible for equol promotion in the colon. However, there was no significant difference in dietary intake between equol metabolizers and non-metabolizers.

Equol metabolizers were significantly lower the plasma daidzein concentration and urinary daidzein and *O*-DMA excretion. Daidzein would be metabolised to equol by intestinal microflora in equol metabolizers. So, equol metabolizers would be lower the plasma daidzein concentration compared with non-metabolizers and result in the lower urinary excretion of daidzein

The dietary intake of isoflavones, daidzein and genistein, had significant correlation with both plasma concentration and urinary excretion. The plasma concentration of genistein showed a better correlation with the dietary intake than the urinary excretion. The plasma concentration of daidzein, however, showed slightly lower correlation coefficient with the dietary intake. Watanabe et al. [31] reported that the half-lives of the plasma daidzein and genistein in men were 5.79 and 8.36 h, respectively. We collected blood in the fasting state, which weakens the relationship with the dietary intake.

The plasma concentration is important for the consideration of the bioavailability of isoflavones in pharmacokinetic studies, and their possible biological effects e.g. on the hormonal system. The measurement of urinary excretion of isoflavone is useful for estimation of total isoflavone intake and recovery of original food isoflavones in urine. We concluded that measurements of both plasma concentration and the urinary excretion of isoflavones are useful tools in future studies on the association of phytoestrogen intake and incidence of cancer. Particularly the plasma TR-FIA assays are useful because of the small amount of plasma needed and the convenient analytical procedures.

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