Ⅲ．研究成果の刊行物・別冊

Review

Food safety and food labeling from the viewpoint of the consumers

Shaw Watanabe MD,PhD[1,2], Melissa Melby PhD[2], Naomi Aiba PhD,RD[2]

[1]*Life Science Promotion Foundation, Tokyo, Japan*
[2]*National Institute of Health and Nutrition, Tokyo, Japan*

Distrust of food safety has grown among the Japanese people after the occurrence of bovine spongiform encephalitis (BSE) in 2001. The Food Safety Commission was formed under the Cabinet Office and made a network among the ministries. The newly-established Consumer Agency may strengthen the quick response to emergencies. *Shoku-iku* (food and dietary education) Law is being implemented by the Cabinet Office with cooperation from relevant ministries and NGOs. Food Sanitation Law and Health Promotion Law are briefly explained, and the necessity of functional nutriology for non-nutrient biologically active substances is described. With regard to public health nutrition, a new food label showing energy balance and antioxidant unit (AOU) as a surrogate marker of fruit and vegetables has been developed for tailor-made nutrition which makes it easy to for individuals to control energy intake.

Key Words: food safety, food for specified use (FOSHU), functional nutriology, functional food factor (FFF), food labelling

FOOD SAFETY COMMISSION: NETWORK BETWEEN THE MINISTRY OF HEALTH, LABOUR AND WELFARE, MINISTRY OF AGRICULTURE, FORESTRY AND FISHERY, AND CABINET OFFICE

Distrust of food safety has grown among the Japanese people, triggered by various problems beginning with the occurrence of bovine spongiform encephalitis (BSE) in 2001. In response, Japan enacted the Basic Law on Food Safety, a comprehensive law to ensure food safety for the purpose of protecting the health of the nation. Through the development of related laws, Japan has introduced a risk analysis approach as well as a precautious strategy to the food safety network (Figure 1).[1]

Risk assessments are conducted by the Food Safety Commission established under the Basic Law on Food Safety. The approach aims to scientifically assess risks, expressed as the probability and degree of adverse health effects, and develop necessary measures based on the risk assessment. The Food Safety Commission is an organization that undertakes risk assessment, and is independent from risk management organizations such as the Ministry of Agriculture, Forestry and Fisheries, as well as the Ministry of Health, Labour and Welfare. Risk assessment, risk management, and risk communication are a set of solution oriented strategies conducted by exchanging information between the above Food Safety Commission and Ministries. A newly established Consumer Agency should be able to provide early response to an emergency.

THE FOOD SANITATION LAW

In 1947, The Ministry of Health and Welfare (MHLW) enacted the Food Sanitation Law as the first comprehensive law for food safety and hygiene.[2] All food additives have been regulated by this law, and only additives designated as safe by the MHLW are allowed to be used in foods. At first, only chemically synthesized additives were designated, but currently, all types of additives are included under the positive list system. Currently, 345 additives and 46 substances are designated as approved food additives by the MHLW.

The Food Sanitation Law covers various responsibilities such as: the establishment of standards/specifications for food, additives, apparatus, and food containers/ packages; inspection to assess whether these established standards are met; hygiene management of the manufacture process and sale of food; and business licensing. The Abattoir Law and the Poultry Slaughtering Business Control and Poultry Inspection Law cover the regulation of livestock and poultry, including inspection systems for meat. Imported foods are inspected by 31 quarantine stations placed across Japan under the central government.

Local governments and health centres also play an important role. The local governments share responsibilities to conduct inspection of and give advice to food-related businesses.

In recent years the global food trade has been increasing, and imported foods occupy nearly 60 percent of the

Corresponding Author: Dr. Shaw Watanabe, Life Science Foundation, Tokyo, Japan; National Institute of Health and Nutrition, Tokyo, 168-0005, Japan
Tel: +81-3-5379-7785; Fax: +81-3-5379-7786
Email: watashaw@nih.go.jp
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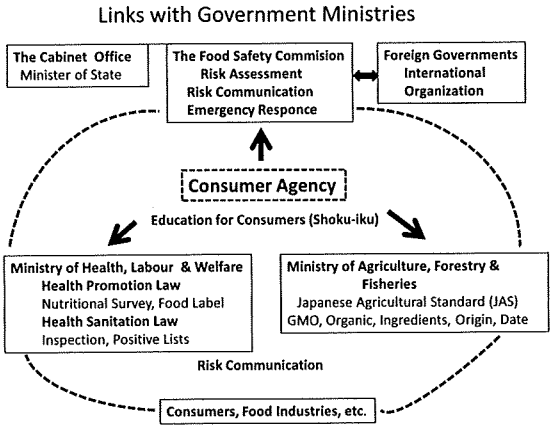

Figure 1. Safety Network of Food Safety Commission. Links between Government Ministries

Japanese market. Also, there is a growing possibility that imported foods contain food additives that are unauthorized in Japan. Safety assessments, conducted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) will facilitate international harmonization of substances that are internationally proven safe and widely used in the world.[3-6]

Ingredients which make up only a small portion of a product may be omitted under JAS Law. Allergenic substances, however, require labelling under the Food Sanitation Law. Mandatory labelling is required at the distribution stage, and is mandatory for eggs, milk, wheat, buckwheat and peanuts, and recommended for abalone, squid, salmon roe, shrimp/prawn, oranges, crab, kiwifruit, beef, tree nuts, salmon, mackerel, soybeans, chicken (poultry), pork, mushrooms, peaches, yams, apples and gelatine.

HEALTH PROMOTION LAW OF MHLW

The "Healthy Japan 21" program was implemented at the beginning of 21st century to prevent life-style related diseases, such as cancer, cardiovascular disease, diabetes mellitus, and hypertension.[2] The Health Promotion Law supports this program. Foods with Health Claims refers to foods that comply with the specifications and standards established by the MHLW and are labelled as having certain nutritional or health functions. These foods are categorized into two groups: Foods with Nutrient Function Claims (FNFC) and Foods for Specified Health Uses (FOSHU).

The former includes foods that contain vitamins and minerals as nutritional ingredients, and the latter are foods officially approved to claim physiological effects on the human body.

FOODS FOR SPECIFIED HEALTH USES (FOSHU)

In 1992, MHLW established "FOSHU" that allows health claims on packaging (Figure.2). Japanese researchers refer to these as "Functional foods".[7-9] FOSHU approval requires scientific evidence of the effectiveness proved by clinical studies, additional safety studies to prove no side effects by oral intake, and exact determination of the specific effective components in foods.

Categories, functional factors and Health Claims for FOSHU are as follows:[2,10]

1. GI (Gastro-intestinal) condition: Carbohydrate, such as oligosaccharides, dietary fiber and chitosan; "Helps maintain a good GI condition."
2. Blood pressure: Lacto-tripeptide from fermented milk, dodecapeptide from casein, a group of peptides from sardine and soy protein; "Suitable for people with mild high blood pressure."
3. Serum cholesterol: Soy protein, chitosan, low molecule sodium alginate and phytosterol "Helps decrease serum cholesterol level."
4. Blood glucose: Indigestible dextrin, wheat albumin, L-arabinose etc.; "Helpful for those who are concerned about their blood glucose level."
5. Absorption of minerals: Fructo-oligosaccharides, caseinphospho peptide; "Improves absorption of calcium." Heme iron from hemoglobin; "Suitable for people with mild iron deficiency anemia."
6. Blood neutral fat: Diacylglycerol and globin degradation product, EPA, DHA; "Helps reduce postprandial

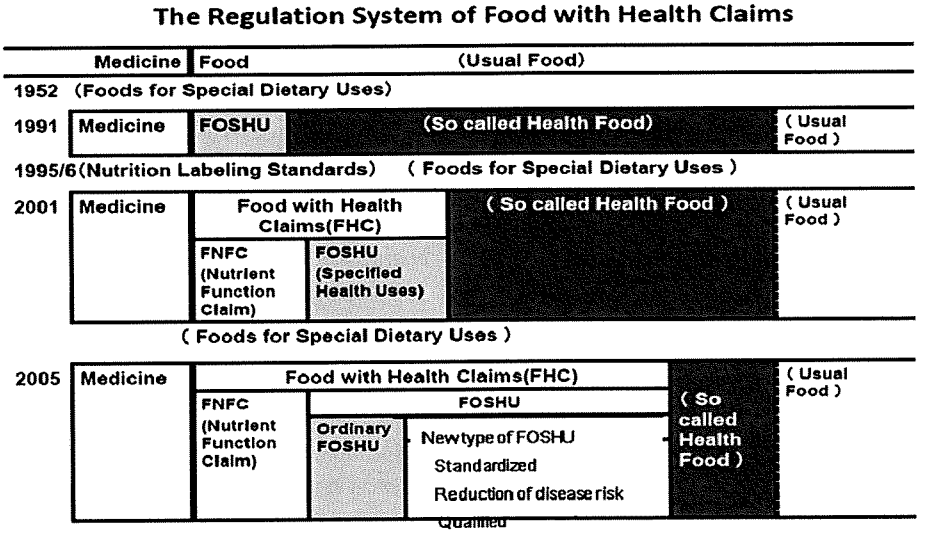

Figure 2. Changes of The Regulation System of Food with Health Claims.

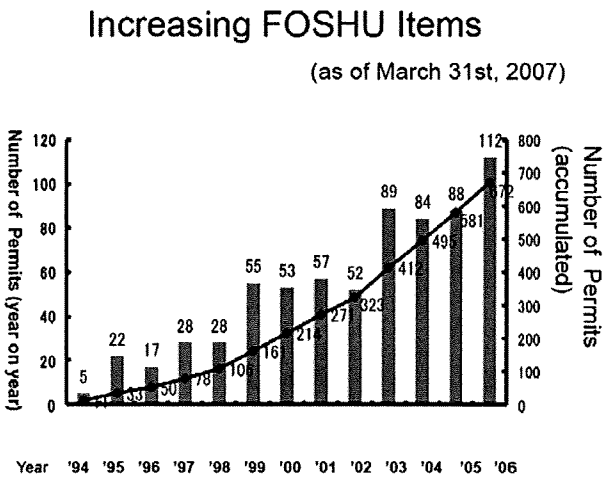

Figure 3. Increasing FOSHU Items as of March 31st, 2007

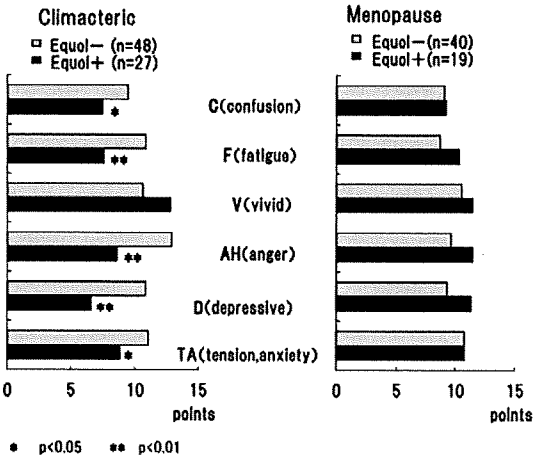

Figure 4. Profile of Mood States (POMS) Feeling Test Scores in Relation to Equol Producibility. Climacteric women, age 40-49.[14]

blood triglyceride levels." "Makes it difficult for fat to cling to the body."

7. Dental health: Some sugar alcohols such as xylitol, maltitol, erythritol, and palatinose (low cariogenic). Green tea polyphenol (non-cariogenic). "This is a low- or non-cariogenic product." "Makes teeth strong and healthy."
8. Bone health: Microorganisms producing high quantities of Vitamin K2, and soy isoflavone. "Promote bone calcification."

Food for Special Dietary Uses (FOSDU) refer to foods that are approved and permitted to display that the food is appropriate for specified dietary use. There are five categories of FOSDU: Formulas for pregnant or lactating women, Infant formulas, Foods for the elderly who have difficulty in masticating or swallowing, Medical foods for the ill.

NECESSITY OF FUNCTIONAL FOOD FACTOR (FFF) DATA-BASE AND FUNCTIONAL NUTRIOLOGY

The market for supplements as well as FOSHU is expanding, and more than 700 supplements are designated as FOSHU at the end of 2007 (Figure 3). Accordingly, reports of adverse effects are increasing. We made a database in NIHN Safety Net containing 1956 cases of adverse effects associated with taking so-called healthy foods, in which 728 were considered to be due to allergic constitution, 456 were due to long-term or excess intake, and 334 were due to interactions with other medicine.[10]

Problems with supplements are differences between in vivo and in vitro effects, differences between product information and those of raw materials, variable quality of natural products due to lack of standards, insufficient data about long term use, and insufficient data about safety for diseased people.

Ingredients in FOSHU and other supplements vary, and functional substances in foods include phytochemicals, certain lipids, amino acids and peptides. Most of these are not ordinary nutrients. It is expected that insufficient intake of macro- and micro-nutrients will result in various physiological manifestations of disease, but nutraceuticals such as FOSHU are expected to have more subtle pharmacological effects. A good example are phytoestrogens, which are believed to be beneficial for maintainingo keep bone density and reducing climacteric symptoms.[11,12]

Antioxidants, however, may prevent cancer and cardiovascular disease,[9] but the necessary doses remain unknown. As with the undesirable interaction between grapefruit and warfarin, unknown interactions between nutraceuticals, drugs and macromolecules inside the body suggest a cautious approach.

Thus, we constructed a database to estimate phytochemical intake from the whole diet; current data allows more than 80 percent this intake to classified and accounted for.[13] Isoflavone intake by the Japanese is very high (Median=15-20 mg) compared to other nations. Recently attention has been called to the isoflavone metabolite equol, because of its stronger estrogenic action. The ability to metabolize daidzein to equol depends on the presence of a certain type of intestinal bacteria. More than half of the older Japanese populationcan convert daidzein to epuol, but this percentage drops to 20-30 percent among the younger generation. Equol producers appear to have differential health profiles (Figure 4). Equol producers showed less severe psychological climacteric symptoms.[14] Caucasians exhibit lower equol producer prevalence rates, so the expected estrogenic effect of isoflavones may differ across populations as well between individuals.

Effective doses of phytochemicals or nutraceuticals can be summarized in a standard table. Large doses of a particular vitamins may cause pharmacological effects, like vitamin C. Such evidence is conceptualized as "Functional Nutriology" in which nutritional or dietary therapy, and use of supplements, effectively makes a bridge to medical treatment (Figure 5). Food industries would benefit by developing supplements, and excluding false or dangerous so-called healthy foods from the market.

TAILOR MADE NUTITION FOR PUBLIC HEALTH

The epidemic increase of obesity in the world mostly results from over-eating of high energy density foods, although several single-nucleotide polymorphisms (SNPs) are considered to influence energy metabolism. Proper

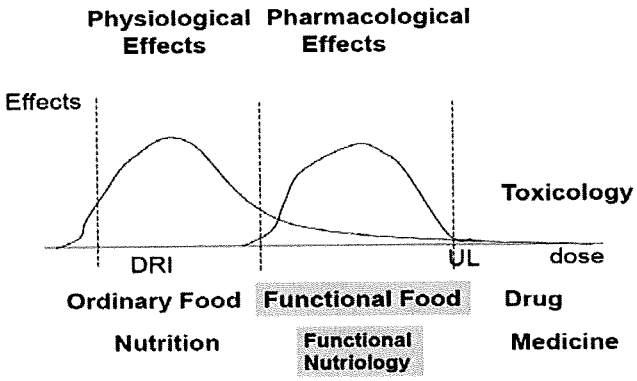

Fig. 5. Concept of Functional Nutrioloty

Fig. 6. Food icon on the menu, showing Energy and com-position of C, P, F and antioxidant unit (AOU) as a surrogate marker of fruit and vegetables. C; carbohydrate, P; protein, L; lipid, AOU; antioxidant unit.

energy intake and physical activity are the most important factors controlling obesity. If energy intake is successfully controlled, other nutrient recommendations can be easily followed. In Japan, a portion size of 80 kcal is a unit widely used for diabetic patients. We have defined a new energy unit (E-unit), as the energy required to melt 1 Kg of ice. Coincidentally it corresponds with a portion size of 80 kcal.

A healthy adult with average activity level requires [body weight (kg) x 0.4] E-units and an active person needs [body weight x 0.5] E-units. For example, a 60 kg man needs 24 E-units, so 8 E-units should be consumed at breakfast, lunch and dinner. In children and adolescents, the body weight multiplier is 1.0 for 10-19 kg body weight, 0.9 for 20-29 kg, 0.8 for 30-39 kg, 0.7 for 40-49 kg, 0.6 for 50-59 kg, and 0.5 for 60-69 kg. The calculated values fit well with those of the dietary reference intake 2010.[15] Desired body weight can be used for the calculation if an individual is overweight or underweight.

If E-units are shown on food labels and restaurant menus, and become popular, this would facilitate control

Table 1. Recommended Energy Intake by DRI2010 in Japan and Calculated Energy Intake by E-Unit System

	Age range	Recommended Energy Intake by PA			kg*1	Energy Intake by E-unit System		
		PAI	PAII	PAIII		Factor	b.w.*0.4	b.w.*0.5
Male	0-5M		550					
	6-8M		650					
	9-11M		700					
	1-2Y		1000		11.7	1.0	936	
	3-5Y		1330		16.2	1.0	1296	
	6-7Y	1350	1550	1700	22.0	0.9	1584	
	8-9Y	1600	1800	2050	27.5	0.9	1980	
	10-11Y	1950	2250	2500	35.5	0.8	2272	
	12-14Y	2200	2500	2750	48.0	0.7	2688	
	15-17Y	2450	2750	3100	58.4	0.6	2803	
	18-28Y	2250	2650	3000	63.0	0.5	2520	2520
	30-49Y	2300	2650	3050	68.5	0.4	2192	2740
	50-69Y	2100	2450	2800	65.0	0.4	2080	2600
	70<	1850	2200	2500	59.7	0.4	1910	2388
Female	0-5M		500					
	6-8M		600					
	9-11M		650					
	1-2Y		900		11.0	1.0	880	
	3-5Y		1250		16.2	1.0	1296	
	6-7Y	1250	1450	1650	22.0	0.9	1584	
	8-9Y	1500	1700	1900	27.2	0.9	1958	
	10-11Y	1750	2000	2250	34.5	0.8	2208	
	12-14Y	2000	2250	2550	46.0	0.7	2576	
	15-17Y	2000	2250	2500	50.6	0.6	2429	
	18-28Y	1700	1950	2250	50.6	0.5	2024	2024
	30-49Y	1750	2000	2300	53.0	0.4	1696	2120
	50-69Y	1650	1950	2200	53.6	0.4	1715	2144
	70<	1450	1700	2000	49.0	0.4	1568	1960

Recommended energy intake by physical activity (PA) is referred from DRI2010
*1Standard body weight in DRI2010 in Japan[2]
Energy is expressed by calorie in the table.

of energy intake for all people (Fig.6). It may be necessary to include E-unit in the standardization and proper quality of agricultural and forestry products (JAS Law), because all consumables (food and beverages) for general consumers are subject to quality standards. This E-unit would be very useful for consumers when choosing between foods. This new energy unit and system was created in response to requests for unified and simplified of foods. This was viewed as necessary due to the diversification of food products resulting from increased imports and new foods on the one hand and rising consumer concerns about diet on the other.

CONCLUDING REMARK

The Food Safety Commission has developed a linkage between the Cabinet Office, Ministry of Health, Labour and Welfare, Ministry of Agriculture, Forestry and Fishery, and the International Organization for analysing information and scientifically assess risks. Shoku-iku (Food and dietary education throughout life) is effective to educate people. The newly established consumer agency should enable faster response to emergencies. A new food labeling system is necessary for producers, providers and consumers so that a healthier society can be formed where we would employ a new energy-unit (80 kcal) for individual energy and nutrient intake as tailor-made nutrition.

AUTHOR DISCLOSURES

Any of the authors does not have conflict with any company.

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Review

Food safety and food labeling from the viewpoint of the consumers

Shaw Watanabe PhD[1,2], Melissa Melby PhD[2], Naomi Aiba RD[2]

[1]*Life Science Foundation, Tokyo, Japan*
[2]*National Institute of Health and Nutrition, Tokyo, Japan*

由消費者的觀點來看食品安全與食品標示

在 2001 年發生狂牛症事件之後，日本民衆漸漸開始質疑食品的安全性。於是日本在內閣府下成立食品安全委員會並且在各部會之間組成一個連繫網路。新成立的消費者服務處加強對緊急事件的快速回應。內閣府與相關部會及非政府組織合作執行食品教育(食品及膳食教育)法。本文對日本食品衛生法及健康促進法做簡短的說明，並敘述功能性營養學的必要性，以研究非營養但具生物活性的物質。關於公共衛生營養，依個體需要而設計的營養已發展出一種新的食品標示，可以顯示熱量平衡及用抗氧化單位(AOU)作為水果及蔬菜的替代指標，這可使每個人更容易控制熱量攝取。

關鍵字：食品安全、特定保健食品(FOSHU)、功能性營養學、功能性食品因子(FFF)、食品標示

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New equol supplement for relieving menopausal symptoms: Randomized, placebo-controlled trial of Japanese women

Naoko Ishiwata, PhD,[1] Melissa K. Melby, PhD,[2] Shoichi Mizuno, PhD,[2] and Shaw Watanabe, MD, DSc[2]

Abstract

Objectives: Equol, a metabolite of the isoflavone daidzein, is hypothesized to play a major role in the health benefits of soy. We examined the effect of a new S-equol supplement on menopausal symptoms and mood states.

Design: We conducted a randomized, double-blind, placebo-controlled trial with our new equol supplement for 12 weeks with 134 Japanese women (aged 40-59 years). They were randomly assigned to three groups: placebo (n = 44), 10 mg of equol per day (EQ-1; n = 44), and 10 mg of equol three times per day (EQ-3; n = 46). Habitual isoflavone intake was limited to 20 mg/d. Participants completed menopausal symptom and Profile of Mood States questionnaires at baseline and postintervention. Physical examination and blood and 24-hour urine collection were performed at baseline and postintervention.

Results: At baseline, total menopausal symptom score varied by menopausal and equol producer status (34.3% producers). A total of 127 participants (94.8%) completed the trial. No adverse effects were reported, except for a systemic rash in one EQ-3 woman. The anxiety scores of equol producers were lower than those of nonproducers ($P < 0.05$). Significant differences between premenopausal and perimenopausal/postmenopausal symptom scores were observed for anxiety, somatic, and total scores. After the EQ-3 intervention, perimenopausal/postmenopausal equol nonproducers showed significant decreases from baseline in all menopausal symptom scores except depression ($P < 0.01$). Compared with placebo, the EQ-3 group showed significant decreases in depression scores ($P < 0.05$), as well as significant decreases in Tension-Anxiety ($P < 0.05$), Depression-Dejection ($P < 0.05$) and Fatigue ($P < 0.01$) and increases in Vigor ($P < 0.05$) of the Profile of Mood States.

Conclusion: S-equol supplement improved mood-related symptoms in perimenopausal/postmenopausal equol nonproducers.

Key Words: Equol – Isoflavones – Supplement – RCT – Menopausal symptoms – Mood.

Epidemiological studies suggest that high consumption of soy isoflavones may account for the lower frequency of menopausal symptoms in Asian populations.[1] Recent studies found a 2-week hot flash prevalence of 22.1% among Japanese women compared with 36.6% and 46.5% for white American and African American women, respectively.[2-4] Adlercreutz et al[5] suggested that the estrogen-like properties of isoflavones might account for the low prevalence of hot flashes experienced by Japanese women.

Although estrogen therapy effectively alleviates vasomotor symptoms,[6,7] the results of the Women's Health Initiative showed overall harmful effects of estrogen plus progestin on risks of myocardial infarctions, strokes, and venous thrombosis in the lungs and legs.[8-10] Isoflavone supplements may provide an alternative to estrogen for relief of menopausal symptoms because Japanese women with high soy intake experience fewer hot flashes.[11] However, intervention studies with soy protein or isoflavones showed small or inconclusive results.[12-15]

The clinical effectiveness of soy isoflavones for menopausal symptoms may depend on the ability to metabolize the isoflavone daidzein to the isoflavan equol, mediated by equol's greater estrogenic activity and affinity for both estrogen receptors.[16,17] The ability to produce equol depends on the presence of certain intestinal microflora.[18,19] The prevalence of equol producers varies from 30% to 59% in human populations and seems to be higher in Asian and vegetarian populations.[20-24] Uchiyama et al[25] successfully used isolated *Lactococcus garvieae* spp to yield S-equol from daidzein-rich soy germ. Preliminary research indicated that equol producers have fewer hot flashes.[26] Thus, efficacy of isoflavones for alleviation of menopausal symptoms may depend on an individual's ability to produce equol.

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From the [1]Department of Life Environments Management, Atomi University, Nakano, Niza-shi, Saitama, Japan; and [2]National Institute of Health and Nutrition, Toyama, Shinjyuku-ku, Tokyo, Japan.

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Address correspondence to: Naoko Ishiwata, PhD, Department of Life Environments Management, Atomi University, 1-9-6 Nakano, Niza-shi, Saitama 352-8501, Japan. E-mail: ishiwata@atomi.ac.jp

The objectives of this double-blind, randomized, placebo controlled trial were to examine differences in symptoms, mood states, and biomarkers (1) between Japanese midlife female equol producers and nonproducers with habitual soy diet and (2) after an intervention with a recently developed S-equol supplement administered once or three times daily. We hypothesized that equol producers would have fewer symptoms and better mood states at baseline compared with equol nonproducers and that equol nonproducers would show more significant improvements after intervention, approaching scores similar to those of equol producers at baseline.

MATERIALS AND METHODS

Participants

Women aged 40 to 59 years were recruited by direct mail from the mothers of students in a Japanese women's university. The response rate was 21% (n = 141), and 134 women participated in the study. A total of 127 women completed the 12-week intervention, and their data were used for all analyses. Women were excluded if they reported previous or current use of hormone therapy, history of ovariectomy or hysterectomy, or no menopausal symptoms. They were enrolled between February and May 2006. Using baseline menopausal symptom scores, the women were randomly assigned to one of four treatment groups: EQ-1, EQ-3, P-1, or P-3 (described in the "Equol Supplements" section). Because no significant differences were observed between the two placebo groups, results for only one combined placebo group are reported. Menopausal status was assigned based on self-reported menstrual patterns in the previous 12 months according to the following definitions: no changes, premenopausal; irregular menstrual periods, perimenopausal; no menstrual periods, postmenopausal. The study protocol was approved by the institutional review board of the National Institute of Health and Nutrition, and all women gave written informed consent to participate before beginning the study and were free to withdraw from the study at any time without obligation.

Equol supplements

The equol supplement was produced from soy germ by fermentation with *L garvieae* spp by Otsuka Pharmaceutical Co Ltd.[25] One capsule contained 10.9 mg (41.7 μmol) equol; 66.7% of the powder was fermented soy material, and 33.3% was erythritol and other additives to improve taste. One equol supplement package contained 10.0 mg S-equol, 0.8 mg daidzein, 2.0 mg genistein, and 4.5 mg glycitein in granulated form. The EQ-1 group consumed one pack containing 10 mg equol per day at breakfast; the EQ-3 group consumed one pack containing 10 mg equol at each meal (three per day). The placebo groups took one (P-1) or three (P-3) placebo packs that were identical in appearance, size, color, taste, and smell to equol supplements. The placebo supplements contained lactose instead of equol and isoflavones. Participants were permitted to ingest up to 20 mg isoflavones daily from meals during the 12-week protocol. Participants were given an illustrated isoflavone content table of soy products, and they recorded their soy product intake amount in a daily diary during the intervention period.

Intervention

The study design was a randomized, double-blind, placebo-controlled, 12-week clinical trial. After completing the baseline tests, women were randomly assigned to the EQ-1, EQ-3, P-1 or P-3 group. Group assignments were generated using a computer algorithm that allocated participants by baseline menopausal symptom score into four groups with approximately equal numbers. Participants, investigators, study staff, and laboratory technicians were blinded to group assignment until the final analysis of the trial. To determine compliance, the participants were asked to report how many supplement packs they had missed or forgotten to take in their daily diaries. Compliance was also assessed by counting returned empty supplement packs at the end of intervention.

Measurements

Participants were asked to collect 24-hour urine samples using Urinmate (Sumitomo Bakelite Co Ltd) at baseline and after 12 weeks. To determine equol producer status, participants consumed soy products containing approximately 50 mg isoflavones at dinner and began 24-hour urine collection the following morning starting with the second-void urine. Participants who excreted more than 10 ng/mL (41.28 nM) equol (HPLC detection limit) in 24-hour urine samples were defined as equol producers.

Urinary concentrations of equol were measured by HPLC using a modified method of Lundh et al.[27] Urine samples were extracted twice in ethyl acetate and evaporated. Mobile phase consisted of 17% methanol and 3% ethyl acetate in 0.05% phosphate (A) and 2% ethyl acetate in methanol (B). Isoflavones and metabolites were separated at 40°C by reversed-phase HPLC on a 4.5 × 250 mm Capcell pak C18 column (Shiseido Co, Tokyo, Japan) using a linear gradient of 0% to 40% B. Equol detection was at 280 nM using a model SPD-10A VU-VIS detector (Shiseido Co, Tokyo, Japan). The equol sensitivity and interassay CV were 0.04 μmol/L and 1.5%, respectively. Given the sensitivity of the assay, women with urinary equol concentrations less than 10 ng/mL (0.041 μmol/L) were considered to be nonproducers.

Fasting blood samples were collected at baseline and after 12 weeks of intervention. Samples were centrifuged at 3,000 rpm for 10 min at 4°C within 2 hours of blood collection. Serum estradiol, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were measured by fluoroimmunoassay at SRL Inc (Tokyo, Japan). Height, body weight, body fat, and blood pressure were measured at baseline and the end of intervention.

Questionnaires

General questionnaire

Questionnaires (self-report) were filled out at baseline and at the end of intervention. It included information

TABLE 1. *Component symptoms grouped according to the Greene Climacteric Scale factors in this study (symptom score out of 69 total)*

	Psychological (24)			
Vasomotor (9)	Anxiety (18)	Depression (6)	Somatic (21)	Other (15)
Hot flash	Difficulty falling asleep	Depression	Dizziness	Ringing in the ear
Sweating		Fatigue	Chest tightness	Eye fatigue
Chilliness	Sleep lightly		Headache	Forgetfulness
	Excitement		Shoulder stiffness	Vaginal dryness
	Anxiety		Backache	Decreased libido
	Nervousness		Arthritis	
	Palpitations		Numbness	

regarding general health, physical activity, smoking status, menstruation states, years since last menstrual cycle, medication, and soy product consumption. A 1-month food frequency questionnaire was used to assess dietary intake at baseline.

Menopausal symptom scale

At baseline and the end of intervention, participants reported the severity of 23 menopausal symptoms on a four-point scale (0, not at all; 1, a little, but daily life is not affected; 2, quite a lot; 3, very much) in the past 1 month. Twenty-one symptoms were derived from the Menopausal Symptom Scale for Japanese women[28] and two additional symptoms were added to assess sexual and urogenital dysfunction. In addition to the total symptom score, the Greene Climacteric Scale[29] was used to calculate component symptom scores for vasomotor, psychological (anxiety and depression), and somatic symptom factors (Table 1).

Profile of Mood States

To evaluate the effects of the equol supplement on mood, participants answered the validated Japanese version of the Profile of Mood States (POMS) at baseline and the end of intervention.[30] The POMS consists of 65 questions concerning 1-week recall of six mood states: Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor, Fatigue, and Confusion.

Sample size calculation

To detect a difference of 4 points in menopausal symptom score, using $\alpha = 0.05$ and $\beta = 0.2$ (power, 80%), and assuming a mean menopausal score of 20 points and SD of 8 points, the necessary sample size was estimated to be 47 per group. With two placebo and two treatment groups, a total sample size of 141 was needed. We were able to recruit 141 women, although the sample size of women who began the intervention was 134 and the number of women who completed the intervention was 127. Because perimenopausal and postmenopausal women were expected to have the most frequent and severe symptoms, we also calculated sample size for this group and determined that 90 individuals would give sufficient power. Thus, we recruited 90 perimenopausal and postmenopausal women for the study.

Statistical analysis

We compared baseline symptom scores and changes after intervention among the three study groups and two menopausal groups (premenopausal vs perimenopausal and postmenopausal groups combined) using non-parametric Mann-Whitney and Wilcoxon tests. Based on previous literature, perimenopausal and postmenopausal women were expected to have the highest prevalence of symptoms, compared with premenopausal women of similar age. Thus, to increase sample size and power of analyses, perimenopausal and postmenopausal groups were combined into a perimenopausal/postmenopausal group. Statistical analyses were performed using SPSS statistical software version 14.0 (SPSS, Tokyo, Japan). A P value of <0.05 was considered significant.

RESULTS

General

Of 134 women who began the study, 39 were premenopausal (age, 46.6 ± 3.8 years), 25 were perimenopausal (age, 48.4 ± 4.2 years), and 70 were postmenopausal (age, 53.3 ± 3.1 years). Seven individuals did not complete the post-intervention questionnaire and sample collection. Therefore, statistical analysis was conducted using the complete data of 127 participants (94.8%).

Baseline characteristics at randomization indicated that there were no significant differences among the three treatment groups (Table 2). As expected, significant differences were observed in hormones between menopausal groups (Table 3). The frequency of equol producers among all participants was 34.3%. Except for one woman from the EQ-3 group who experienced a generalized rash at the second week of intervention, no adverse effects were reported during the study. There were no significant changes in FSH, LH, estradiol, and progesterone between baseline and after the 12-week intervention (Tables 4 and 5) in perimenopausal/postmenopausal women.

TABLE 2. *Baseline characteristics of participants by treatment group*

	Placebo (n = 44)	EQ-1 (n = 44)	EQ-3 (n = 46)
Age, mean ± SD, y	50.6 ± 4.9	50.5 ± 4.7	50.5 ± 4.7
Height, mean ± SD, cm	155.5 ± 3.7	157.6 ± 4.4	154.7 ± 5.0
Body weight, mean ± SD, kg	55 ± 8.7	57.9 ± 9.1	54.9 ± 6.9
Body mass index, mean ± SD, kg/m^2	22.3 ± 3.0	22.3 ± 3.0	22.0 ± 3.0
Body fat, mean ± SD, %	30.3 ± 4.0	31.2 ± 4.1	30.9 ± 3.8
Age of menarche, mean ± SD, y	12.9 ± 1.1	12.9 ± 1.4	13.0 ± 1.5
Menopausal symptom score, mean ± SD	19.7 ± 10.7	17.4 ± 9.9	18.4 ± 8.1
Parity, mean ± SD	2.47 ± 1.5	2.61 ± 1.5	2.3 ± 1.1
Premenopausal women, % (n)	22.7 (10)	29.5 (13)	34.8 (16)
Perimenopausal women, % (n)	25.0 (11)	18.2 (8)	13.0 (6)
Postmenopausal women, % (n)	52.3 (23)	52.3 (23)	52.2 (24)
Smoking, %	18.2	22.7	15.2
Equol producer, %	41.9	29.3	37.2

The EQ-1 group took 10 mg equol once a day, the EQ-3 group took 10 mg equol three times a day. There were no significant differences in groups by analysis of variance.

TABLE 3. *Baseline menopausal scores, POMS scores, and hormones by menopausal and equol producer status*

	Equol producer						
	Premenopausal (n = 12)	Perimenopausal (n = 12)	Sig[a]	Postmenopausal (n = 22)	Sig[b]	Sig[c]	Perimenopausal/ postmenopausal (n = 34)	Sig[d]
Menopausal score								
Psychological	2.9 (2.2)	2.8 (3.6)		5.5 (4.8)			4.5 (4.5)	
Anxiety	2.3 (1.9)	2.0 (2.4)		4.1 (4.1)			3.4 (3.7)	
Depression	0.6 (0.7)	0.8 (1.2)		1.4 (1.2)			1.2 (1.2)	
Somatic	3.2 (1.8)	4.8 (2.3)		7.6 (4.4)	[i]		6.6 (4.0)	[i]
Vasomotor	1.6 (1.4)	1.1 (1.2)		3.0 (1.9)	[h]	[i]	2.3 (1.9)	
Total	10.8 (4.9)	14.0 (5.9)		21.3 (11.1)	[i]	[h]	18.7 (10.1)	[i]
POMS								
Tension-Anxiety	46.4 (4.6)	44.3 (4.8)		47.8 (7.5)			46.6 (6.8)	
Depression-Dejection	48.3 (4.1)	44.6 (3.3)	[h]	49.2 (8.4)			47.6 (7.3)	
Anger-Hostility	49.3 (5.6)	46.0 (5.2)		50.0 (9.2)			48.6 (8.2)	
Vigor	49.5 (7.1)	47.2 (7.5)		48.0 (7.9)			47.8 (7.6)	
Fatigue	46.6 (5.5)	44.5 (6.8)		50.1 (9.2)			48.1 (8.7)	
Confusion	47.3 (6.9)	47.1 (5.3)		50.6 (10.1)			49.3 (8.8)	
Hormones								
LH, nmol/L	3.1 (2.3)	12.7 (9.4)	[i]	20.5 (11.6)	[i]		17.8 (11.4)	[i]
FSH, U/L	5.5 (3.3)	27.0 (25.2)	[i]	55.5 (26.3)	[i]	[i]	45.5 (29.0)	[i]
Estradiol, pg/mL	169.0 (139.9)	130.1 (126.7)		30.9 (24.6)	[i]	[h]	78.3 (100.4)	[i]
Progesterone, ng/mL	5.59 (6.76)	0.70 (1.14)		0.60 (1.17)	[i]		0.64 (1.14)	[i]

(Continued on next page)

Values are presented as mean (SD). POMS, Profile of Mood States; Sig, significance; LH, luteinizng hormone; FHS, follicle-stimulating hormone; ANOVA, analysis of variance.
[a]Significant differences between premenopausal and perimenopausal women by Mann-Whitney test.
[b]Significant differences between premenopausal and postmenopausal women by Mann-Whitney test.
[c]Significant differences between perimenopausal and postmenopausal women by Mann-Whitney test.
[d]Significant differences between premenopausal and perimenopausal/postmenopausal women by Mann-Whitney test.
[e]Significant differences between equol non-producer and producer of peri/postmenopausal women by Mann-Whitney test.
[f]Significant differences between premenopausal and perimenopausal/postmenopausal women by Mann-Whitney test.
[g]Significant differences between equol producer status assessed by Mann-Whitney test.
[h]$P < 0.05$.
[i]$P < 0.01$.

Urine S-equol concentrations demonstrated good compliance with the protocol (Fig. 1). After the intervention, mean (median) equol concentrations of equol producers and nonproducers were the following: 6.28 (0.041) μmol/L and 0.33 (0.041) μmol/L for the placebo group, 38.50 (38.25) μmol/L and 25.24 (26.40) μmol/L for the EQ-1 group, and 125.11 (108.15) μmol/L and 88.43 (87.00) μmol/L for the EQ-3 group, respectively. Urine equol concentrations showed good correlation with the equol supplement intake. Compliance with the study protocol was also confirmed by written dietary records. Based on the remnants of supplement packs returned at the end of the intervention, compliance was estimated to be 94.5%.

Intervention

Table 3 shows the baseline menopausal scores, POMS scores, and hormones by menopausal and equol producer status. The anxiety scores of equol producers were lower than those of nonproducers ($P < 0.05$). Significant differences between the premenopausal and perimenopausal/postmenopausal groups were observed for anxiety, somatic, and total symptom scores.

Table 4 shows the menopausal scores, POMS scores, and hormone results for the subgroup of perimenopausal/postmenopausal equol producers. Significant differences between baseline and 12 weeks were observed only in the placebo group.

Table 5 shows the results for the subgroup of perimenopausal/postmenopausal equol nonproducers, the group with the highest symptom scores and the group most likely to benefit from the equol supplement. After the EQ-3 intervention, perimenopausal/postmenopausal equol nonproducers showed significant decreases from baseline in all menopausal symptom scores, except depression ($P < 0.05$), and significant decreases in somatic and total menopausal scores ($P < 0.05$) compared with the placebo group (Table 5). The EQ-3 group also showed significant decreases in Depression-Dejection ($P < 0.05$) and increases in Vigor ($P < 0.01$). Compared with the placebo group, the EQ-3 group showed significant decreases in Tension-Anxiety ($P < 0.05$), Depression-Dejection ($P < 0.05$) and Fatigue ($P < 0.01$) and increases in Vigor ($P < 0.05$).

DISCUSSION

Japanese menopausal women with high soy intake have fewer vasomotor symptoms than their North American counterparts do,[11,31] although Japanese menopausal women commonly report chilliness, shoulder stiffness, and psychological symptoms.[2-4,32-34] This observation led to the hypothesis that isoflavones may act as natural selective estrogen receptor modulators at menopause.[35] Although circumstantial evidence for the beneficial effects of isoflavones is increasing, the results of clinical trials are inconsistent.[12-15] Recent evidence suggests that it is important to stratify study

TABLE 3. *Continued*

	Equol nonproducer								Total population	
	Premenopausal (n = 25)	Perimenopausal (n = 10)	Sig[a]	Postmenopausal (n = 46)	Sig[b]	Sig[c]	Perimenopausal/ postmenopausal (n = 56)	Sig[d]	Sig[e]	Perimenopausal/ postmenopausal vs premenopausal significance[e]	Equol nonproducer vs producer significance[f]
Menopausal score											
Psychological	4.6 (4.2)	9.0 (3.9)	i	6.1 (4.1)		h	6.6 (4.2)	h	h	h	i
Anxiety	3.2 (2.2)	6.6 (2.9)	i	4.7 (3.2)	h		5.0 (3.2)	h	i	h	i
Depression	1.4 (1.6)	2.4 (1.6)		1.4 (1.3)		h	1.6 (1.6)				
Somatic	5.5 (3.2)	7.6 (3.2)		6.3 (3.2)			6.5 (3.2)			h	
Vasomotor	2.2 (1.7)	3.1 (1.7)		2.7 (1.9)			2.8 (1.9)				
Total	16.6 (8.6)	25.6 (9.2)	i	19.9 (9.5)			21.0 (9.6)	h		i	h
POMS											
Tension-Anxiety	47.5 (7.2)	51.8 (5.8)	h	48.7 (7.3)			49.3 (7.1)		h		
Depression-Dejection	48.3 (8.6)	53.0 (7.7)		49.1 (6.8)			49.8 (7.0)				h
Anger-Hostility	50.9 (9.3)	53.2 (8.3)		50.0 (8.7)			50.5 (8.6)				
Vigor	48.7 (11.3)	42.2 (5.3)		44.3 (7.4)			43.8 (7.5)		h		h
Fatigue	50.4 (7.8)	51.5 (7.9)		49.5 (7.6)			49.7 (7.6)				
Confusion	49.6 (10.0)	57.1 (7.8)	h	51.9 (9.1)			52.7 (9.0)		h		
Hormones											
LH, nmol/L	4.7 (4.9)	12.4 (9.7)	h	26.0 (14.1)	i	i	23.5 (14.3)	i		i	
FSH, U/L	9.2 (4.4)	21.0 (19.9)	h	65.2 (25.0)	i	i	56.9 (29.3)	i		i	
Estradiol, pg/mL	192.3 (161.6)	206.0 (172.6)		20.3 (23.7)	i	i	87.5 (136.6)	i		i	
Progesterone, ng/mL	4.65 (7.14)	1.73 (2.56)		0.27 (0.12)	i	h	0.53 (1.20)	i		i	

Values are presented as mean (SD). POMS, Profile of Mood States; Sig, significance; LH, luteinizng hormone; FHS, follicle-stimulating hormone; ANOVA, analysis of variance.
[a]Significant differences between premenopausal and perimenopausal women by Mann-Whitney test.
[b]Significant differences between premenopausal and postmenopausal women by Mann-Whitney test.
[c]Significant differences between perimenopausal and postmenopausal women by Mann-Whitney test.
[d]Significant differences between premenopausal and perimenopausal/postmenopausal women by Mann-Whitney test.
[e]Significant differences between equol non-producer and producer of peri/postmenopausal women by Mann-Whitney test.
[f]Significant differences between premenopausal and perimenopausal/postmenopausal women by Mann-Whitney test.
[g]Significant differences between equol producer status assessed by Mann-Whitney test.
[h]$P < 0.05$.
[i]$P < 0.01$.

TABLE 4. *Menopausal scores, POMS scores, and hormones at baseline and after 12 weeks of equol supplementation in perimenopausal/postmenopausal equol producers*

	Placebo (n = 14)			EQ-1 (n = 10)		EQ-3 (n = 10)	
	Baseline	12 weeks	Significance[a]	Baseline	12 weeks	Baseline	12 weeks
Menopausal scores							
Psychological	5.3 (5.2)	3.4 (3.7)		2.4 (2.1)	1.9 (1.9)	5.6 (4.9)	4.4 (4.5)
Anxiety	4.2 (4.2)	2.3 (2.8)	b	1.7 (1.6)	1.3 (1.5)	3.8 (4.3)	3.2 (3.6)
Depression	1.1 (1.3)	1.1 (1.2)		0.7 (1.1)	0.6 (0.7)	1.8 (1.0)	1.2 (1.4)
Somatic	7.0 (5.1)	5.8 (4.4)		6.1 (3.4)	5.8 (2.6)	6.5 (3.0)	5.7 (3.1)
Vasomotor	3.3 (2.2)	1.8 (2.2)	c	1.2 (1.0)	1.1 (0.9)	2.1 (1.8)	1.6 (1.6)
Total	20.8 (12.5)	14.6 (9.3)	c	13.7 (6.3)	11.7 (4.1)	20.8 (8.3)	15.9 (9.6)
POMS							
Tension-Anxiety	45.6 (7.4)	45.0 (6.1)		45.9 (7.0)	44.7 (5.3)	48.6 (6.0)	47.9 (7.9)
Depression-Dejection	48.0 (9.2)	48.1 (6.3)		46.2 (6.2)	44.5 (3.5)	48.3 (5.8)	50.1 (8.5)
Anger-Hostility	46.8 (9.0)	47.1 (6.4)		47.7 (9.0)	45.8 (4.9)	52 (5.2)	50.6 (7.5)
Vigor	47.4 (9.5)	42.5 (8.2)	b	49.5 (5.9)	46.4 (7.1)	46.6 (6.4)	48.8 (6.8)
Fatigue	46.5 (9.6)	47.6 (10.6)		47.8 (9.9)	47.6 (8.5)	50.6 (6.1)	51.8 (8.1)
Confusion	50.1 (9.3)	48.3 (9.2)		47.1 (8.0)	46.1 (2.2)	50.4 (9.4)	48.5 (7.4)
Hormones							
LH, nmol/L	19.4 (9.0)	19.3 (11.9)		18.0 (16.2)	18.3 (14.9)	15.2 (9.2)	13.7 (6.8)
FSH, U/L	54.7 (32.7)	55.0 (35.8)		37.1 (28.9)	40.1 (27.3)	40.8 (21.7)	40.2 (26.9)
Estradiol, pg/mL	35.1 (25.4)	43.0 (32.8)		103.8 (97.1)	85.2 (55.6)	134.2 (159.5)	143.6 (199.5)
Progesterone, ng/mL	0.27 (0.14)	0.24 (0.12)		1.17 (1.79)	1.72 (2.59)	0.63 (1.01)	0.34 (0.28)

Values are presented as mean (SD). The EQ-1 group took 10 mg equal once a day, and the EQ-3 group took 10 mg equal three times a day. POMS, Profile of Mood States; LH, luteinizing hormone; FSH, follicle-stimulating hormone.
[a]Significant differences between baseline and 12 wk in placebo group by paired Wilcoxon test.
[b]$P < 0.05$.
[c]$P < 0.01$.

TABLE 5. *Menopausal scores, POMS scores, and hormones at baseline and after 12 weeks of equol supplementation in perimenopausal/postmenopausal equol nonproducers*

	Placebo (n = 19)			EQ-1 (n = 18)		EQ-3 (n = 19)			
	Baseline	12 weeks	Significance[a]	Baseline	12 weeks	Baseline	12 weeks	Significance[b]	Significance[c]
Menopausal scores									
Psychological	6.4 (3.7)	6.4 (4.0)		6.9 (5.4)	5.1 (3.4)	6.5 (3.4)	4.4 (3.1)	[e]	
Anxiety	4.7 (2.8)	4.5 (3.2)		5.3 (4.0)	3.9 (2.7)	5.1 (2.7)	3.4 (2.3)	[e]	
Depression	1.7 (1.5)	1.9 (1.1)		1.7 (1.7)	1.2 (1.0)	1.4 (1.1)	1.0 (1.2)		
Somatic	6.8 (3.2)	6.3 (3.2)		6.7 (3.8)	6.1 (2.0)	6.1 (2.7)	4.7 (2.5)	[d]	[d]
Vasomotor	3.2 (1.9)	2.2 (1.5)	[d]	2.7 (1.8)	2.2 (1.7)	2.4 (1.9)	1.4 (1.3)	[d]	
Total	21.4 (9.0)	18.4 (7.6)		21.3 (12.3)	17.3 (6.9)	20.2 (7.5)	13.6 (6.2)	[e]	
POMS									[d]
Tension-Anxiety	48.9 (6.4)	50.7 (9.1)		49.0 (8.8)	47.9 (7.5)	49.8 (6.2)	46.6 (4.6)		
Depression-Dejection	51.3 (7.6)	53.3 (9.6)		49.4 (7.9)	48.6 (6.7)	48.5 (5.6)	46.5 (4.4)	[d]	[d]
Anger-Hostility	50.4 (8.4)	52.4 (7.1)		50.7 (10.1)	50.1 (7.7)	50.4 (7.7)	48.7 (4.7)		
Vigor	44.0 (7.9)	42.6 (6.5)		44.2 (8.1)	45.2 (7.6)	43.4 (6.7)	45.6 (7.1)	[d]	[d]
Fatigue	50.3 (9.3)	54.3 (9.7)		49.8 (8.4)	49.7 (7.5)	49.2 (4.7)	46.5 (5.6)		[e]
Confusion	54.5 (6.2)	54.3 (8.8)		50.4 (10.1)	50.4 (7.0)	53.1 (10.2)	51.6 (6.6)		
Hormones									
LH, nmol/L	21.3 (9.2)	21.0 (9.1)		25.3 (13.3)	23.9 (12.2)	23.9 (19.0)	23.0 (14.3)		
FSH, U/L	50.8 (24.5)	53.4 (27.9)		63.9 (29.2)	65.4 (31.0)	56.5 (33.5)	59.6 (31.4)		
Estradiol, pg/mL	116.0 (154.6)	178.1 (127.8)		43.0 (56.0)	17.5 (7.9)	129.5 (182.9)	96.8 (166.3)		
Progesterone, ng/mL	0.76 (1.61)	1.38 (3.26)		0.28 (0.12)	0.29 (0.14)	0.55 (1.31)	0.46 (0.92)		

Values are presented as mean (SD). The EQ-1 group took 10 mg equal once a day, and the EQ-3 took 10 mg equal three times a day. POMS, Profile of Mood States; LH, luteinizing hormone; FSH, follicle-stimulating hormone.
[a]Significant differences between baseline and 12 wk in placebo group by Wilcoxon test.
[b]Significant differences between baseline and 12 wk in EQ-3 by Wilcoxon test.
[c]Significant differences in 12-wk's changes between placebo and EQ-3 by Mann-Whitney test.
[d]$P < 0.05$.
[e]$P < 0.01$.

populations by the ability to produce equol,[36] because of equol's greater estrogenic activity and affinity for both estrogen receptors.[16,17] In the present study, mood-related symptom scores showed significant improvements after equol supplementation (10 mg, three times per day) compared with placebo in perimenopausal/postmenopausal equol nonproducers.

The first trial assessing the efficacy of soy for the alleviation of menopausal symptoms was published in 1995,[37] and since then, more than 40 others have examined the impact of soy foods or soy-derived isoflavone supplements on hot flash frequency and/or severity. However, numerous published reviews have found inconsistent results.[13,14,35,38] Nagata et al[11] found that the prevalence of hot flashes was inversely related to both the amount of soy foods consumed and the calculated intake of isoflavones. A recent study of 108 Japanese women that examined equol producer status found that hot flashes occurred in only 5% of menopausal women.[26] On the basis of urinary concentrations, equol producers (53.5%) reported less severe symptoms as assessed by a simplified menopausal index score ($P < 0.05$).[26] These data suggest that equol producers comprise a distinct subpopulation that may gain the most benefit from soy isoflavones for relief of menopausal symptoms and that equol nonproducers may benefit from an equol supplement.

Only 34% of participants in this study were equol producers, compared with previous studies that reported close to half for Japanese menopausal women.[26] We recruited women with symptoms, as opposed to conducting a general population study. To the extent that equol-producing ability may lead to decreased symptoms, selecting a population with higher symptom prevalence is likely to result in a lower prevalence of equol-producer ability, such as that observed in this study.

It seems that equol does not have any serious adverse effects. Only one adverse event (generalized rash) occurred in this study, in the second week of the EQ-3 intervention. The participant had previously experienced a similar allergic

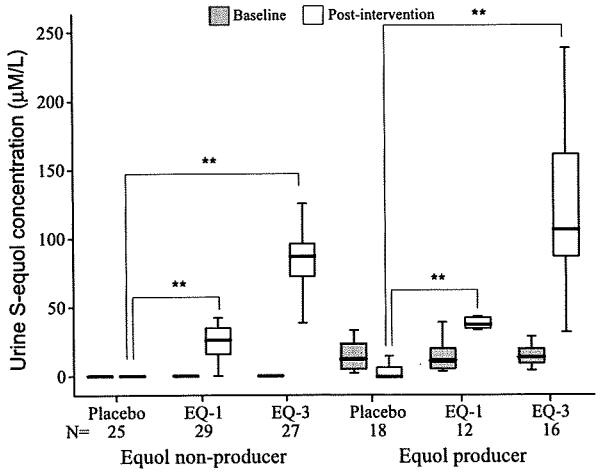

FIG. 1. Equol concentrations (μmol/L) in 24-hour urine samples at baseline and after 12-week intervention. The EQ-1 group took 10 mg equal once a day, and the EQ-3 group took 10 mg equal three times a day. Participants who excreted more than 10 ng/mL (0.041 μmol/L) equol in their 24-hour urine samples were defined as equol producers. Significant differences between baseline and after the intervention by Mann-Whitney rank sum test are shown as $*P < 0.05$ and $**P < 0.01$.

reaction after using propolis. Equol supplementation may affect steroid hormones, but in this study, plasma levels of FSH, LH, and progesterone in premenopausal, perimenopausal, and postmenopausal women did not change after 12 weeks of intervention with 10 mg equol administered once or three times daily. Epidemiological data on populations with habitual soy consumption suggest that long-term therapeutic soy use poses minimal risks.

Most study participants had few symptoms, and thus, sample size limitations may have led to decreased statistical power to identify some true effects. Significant effects of equol supplementation were observed only in perimenopausal/postmenopausal nonproducers, the subpopulation most likely to have symptoms and benefit from equol supplementation. In addition, assessment of long-term effects may be limited by the relatively short 12-week treatment period. Although the short half-life of the S-equol supplement (85 minutes) minimizes cumulative adverse effects,[39] repeat administration is necessary to achieve effective plasma concentrations. In this study, three-times-a-day administration resulted in significant improvement in mood-related symptoms. No significant differences between the EQ-1 and placebo groups (in perimenopausal/postmenopausal equol producers and nonproducers) suggest that a one-time dose of 10 mg equol supplement is insufficient. Given the rapid pharmacokinetic elimination of the equol supplement, it is likely that administration several times daily is required for effectiveness. Lower doses, such as 5 mg, might be sufficient to observe significant improvement in menopausal symptoms and requires further research.

CONCLUSIONS

In conclusion, this is the first study to show the effectiveness of an S-equol supplement made by *L garvieae* spp. For relief of mood-related symptoms in equol nonproducers, three times a day of 10 mg equol supplementation for 12 weeks produced significant improvement in symptoms. This supplement, in combination with 20 mg isoflavones from the habitual diet, did not cause any serious adverse effects, except for one allergic rash. Thus, this equol supplement is a promising alternative to estrogen therapy for menopausal equol nonproducers. Further research is needed on mood-related symptoms and potential underlying hormonal mechanisms.

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A cross-sectional study on the effects of long term very low protein diets in patients with Chronic Kidney Disease: serum and urine DEXA and amino acid profiles

Shaw Watanabe[1], Mikie Noboru[1,2], Misae Yasunari[1,2], Terukuni Ideura[2,3]

[1]Life Science Promotion Foundation, Keio University School of Medicine
[2]Adequate Protein Intake Group, [3]Showa University Fujigaoka Hospital

25-3-1004, Daikyo-cho, Sinju-ku, Tokyo 156-0015, Japan
watashaw@lifescience.or.jp

Abstract

Background: Chronic renal failure has increased among aged population in Japan. Protein-restricted diets have been successfully used to treat chronic renal failure. However, concerns over sarcopenia and other nutritional disorders have made doctors in Japan reluctant to recommend low-protein diets.

Study Design: A cross-sectional study was carried out based on dietary records, urine and blood samples and DEXA measurements to evaluate body composition.

Setting & Participants: The study was carried out at Keio University Hospital and National Institute of Health and Nutrition, Tokyo, over the 3-day period in June, 2009. Subjects were 10 CKD patients (1 male, 9 female); ten members of the patients' families (3 male, 7 female) and 11 dieticians (all female) were used as control subjects.

Factor: The CKD patients maintained a daily protein intake of less than 0.5 g/kg body weight (VLPD) for periods averaging 7 years. Members of the control group all had a daily protein intake of over 1g/kg body weight.

Outcomes: Indicators of nutritional disorders, metabolic abnormalities or changes in body composition were sought.

Measurements: Intake of various nutrients was calculated from dietary records. Blood plasma and urine content was analyzed. Body composition was measured using DEXA.

Results: The CKD subjects were found not to suffer from sarcopenia, osteoporosis, hyperkalemia, hyperphosphatemia, hyperuremia or high levels of uric acid, although slight anemia was observed. Vitamin and mineral intakes were lower than controls, but no recognizable symptoms from nutrient deficiency occurred. Urinary excretion of amino acids was different from controls.

Limitations: Results are limited by the relatively small number of test subjects, variation in time on the VLPD, and gender imbalance.

Conclusions: Results suggest that VLPD did not show any abnormality in body composition when energy requirement was fulfilled. Different amino acid metabolism would lead to cautious prescription of amino acid supplement.

Indexed Words:
CKD, Low protein diet, nutrition, amino acid, DEXA, cross sectional study, epidemiology.

Introduction

Chronic kidney disease (CKD) has increased among aging population in Japan. It is important to save glomerular filtration rate (GFR) for anti-aging medicine. Over the past 50 years, protein-restricted diets have been successfully used to treat chronic renal failure. Long-term therapy with protein- and phosphorus-restricted diets has been shown to reduce damage to the kidneys and prevent renal failure complications.[1-3] A meta-analysis of 7 randomized controlled trials with 1494 non-diabetic patients found that protein restriction decreased the incidence of composite outcomes during the trial with an odds ratio of 0.61.[4,5]

However, since the poor prognosis of very low protein diet (VLPD) was reported in the MDRD study,[6-8] Japanese doctors have become reluctant to prescribe protein restriction, and regarded a protein intake of 0.8 g per kg body weight to constitute a low protein diet.[9] Protein restriction is believed to lead to nutritional disorders such as protein-calorie malnutrition, and concerns over sarcopenia and other nutritional deficiencies make doctors unwilling to treat chronic kidney disease (CKD) patients with low protein diets.

We have supported a group of patients with (CKD) aiming to avoid hemodialysis as far as possible[10]. Adhering to diets prescribed for renal failure is frequently a difficult and frustrating task for patients and their families, so we have tried over many years to develop appealing diets for the patients. The patients expressed a wish to learn more about the function and mechanism of kidney diseases; we thus arranged a course of kidney seminars on Saturday afternoons for 3 months from April 2009. The course was attended by a group of 10 CKD patients, 10 family members, and 11 dietitians; following the course, they wanted to carry out a cross sectional study of the 3 groups to ascertain the positive and negative effects of a prolonged VLPD.

The CKD patients in this study followed a VLPD on a prescription of less than 0.5 g/kg body weight for 7 years on average; they were therefore interested to ascertain their actual protein intake, metabolic state of protein and amino acids, and body composition such as bone density and lean body mass.

Subjects and Methods

Subjects were 31 people who took part in the 2009 kidney seminar course planned

by the "Adequate Protein Intake Promotion Group". Ten CKD patients (one man and nine women), 10 family members (3 men and 7 women), and 11 dietitians (all women) registered and completed the course. At the end of course a cross-sectional study of the participants was planned, and group members themselves designed the study under the guidance of authors which was approved by the Ethical Committee of the Life Science Promotion Foundation.

A profile of the CKD patients is given in Table 1. The average age of the 3 family men was 53.4±9.9 and that of the 7 family women was 51.3±10.3; the 11 dietitians had an average age of 36.7±15.2 years. The CKD group was prescribed a VLPD with a daily protein intake of less than 0.5 g/kg body weight, and given care by a single doctor (T.I.)every 1-3 months for periods averaging 7 years.

All participants were asked to provide dietary records and 24-hour urine collection by the urimeter^{R} over a 3 day period; on the 3rd day, 10 ml blood samples were collected and DEXA examinations performed.

Total intake of energy, water, protein, fat, carbohydrate, NaCl, Mg, Mn, Ca, P, K, ash, retinol, carotene, vitamin A, vitamin E, vitamin K, vitamin B1, folic acid, vitamin C, and amino acids was calculated from the dietary records using the FFF database.[11]

Sera were used to measure total protein (TP), albumin, AST, ALT, gammaGTP, ProBNP, TG, total cholesterol, HDL cholesterol, LDL cholesterol, BUN, creatinine, BUN/Cr ratio, UA, CRP, Na, K, Cl, Ca and Mg. Whole blood was used to measure WBC, RBC, Hb, Ht, Pt, MCV, MCH, and MCHC. Blood and biochemical measurements were taken at the Serum Research Laboratory in Tokyo.

Body composition was measured in the National Institute of Health and Nutrition using DEXA (model DPX-IQ, Lunar Radiation) with subjects in the supine position. Whole body scans provided bone, fat and lean mass composition in the heel, arm, trunk and leg tissues; values were processed using a computer. DEXA measurements of muscle and fat mass in the arm and leg have been well validated against other standards.[12-15]

Analysis of serum biochemistry and urine, in addition to analysis of amino acids in plasma and urine using HPLC chemical detection, was also performed by the Serum Research Laboratory, Tokyo. eGFR was calculated using an equation given by the Japanese Nephrology Group. [16]