Intake of dietary phytoestrogen and indices of antioxidant and bone metabolism of pre- and post-menopausal Korean women

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Abstract

A group of 101 women, aged 40-65 years consisted of 48 premenopausal subjects and 53 postmenopausal ones living in Daegu and Gyeongbuk area in Korea were evaluated with their general characteristics, lifestyle factors, nutrient and phytoestrogen intakes, blood and urinary indices concerning antioxidant status and bone metabolism. Body mass index (BMI), waist hip ratio (WHR) and systolic blood pressure (SBP) of the postmenopausal women were significantly higher (23.8, 0.86, and 126.9 mmHg, respectively) than those of the premenopausal women (22.6, 0.82, and 115.9 mmHg; respectively). Nutrient intakes of the postmenopausal and premenopausal groups were not different except lower fat intake and higher dietary fiber and iron intakes in the postmenopausal group. Daily total phytoestrogen intake was significantly higher in the postmenopausal group (48.54 mg) than the premenopausal (31.41 mg) and was resulted mostly from higher intakes of daidzein and genistein from soy and soy products (45.42 mg vs 28.91 mg). Serum genistein level and excretion of enterolactone, major lignan metabolite, were not very different between the two groups. Serum retinal and α- tocopherol levels were higher in the postmenopausal group but TBARS levels were not different between the two groups. Serum osteocalcin (7.18 ng/mL) and urinary deoxypyridinoline (7.15 nmol/mmol creatinine), in the postmenopausal group were significantly higher than those in the premenopausal group (4.80 ng/mL, 5.95 nmol/mmol creatinine). Urinary excretion of enterolactone was positively correlated with serum osetocalcin in premenopausal women and serum genistein negatively correlated with the urinary DPD in postmenopausal women. Dietary phytoestrogen intake was negatively correlated with serum level of TBARS in all subjects. It is concluded that the effect of total phytoestrogen intake is beneficial on body antioxidant status in all middle-aged women regardless of menopause but the effect on bone metabolism appears different by the type of the phytoestrogen and the menopausal state.

Key Words: dietary phytoestrogen, menopausal women, DPD, osteocalcin, TBARS

Introduction

As our country is entering into the aging society, the aging of women is more distinct and the average life span was 75.1 years in males and 81.9 years in females according to the 2005 data of the Korea National Statistical Office (Korea National Statistics, 2005). The population of menopausal women has been increased as the life after menopause becomes about 30 years if the average age of menopause is around 50. Menopause has become a serious health problem for women over 50 because it can increase the incidence of osteoporosis, cardiovascular diseases and senile dementia due to the loss of ovarian follicle and the lack of estrogen. Hormone replacement therapy (HRT) including synthetic estrogens has been used to reduce these postmenopausal problems. However, recent studies (Writing group for the WHI investigators, 2002) have reported that HRT on the contrary increased not only breast cancer and venous thromboembolism but also coronary artery disease and stroke. Thus, alternative methods for HRT have been searched in several ways and studies on phytoestrogens, which are found in foods and plants and have similar structures and functions as estrogen, have been actively performed. Phytoestrogens include isoflavone, lignan and coumestan, and recently added stillbene (Cornwell et al., 2004). Among these, soy isoflavone has been well known for its reported effects on suppressing breast cancer, preventing arteriosclerosis and osteoporosis, alleviating menopausal symptoms and improving cognitive function (Cornwell et al., 2004). Lignans, which are plenty in grains, fruits, vegetables, and seeds, have been reported to be effective in the prevention of breast cancer (Boccardo et al., 2004), uterine cancer, prostate cancer (Lin et al., 2001) and osteoporosis (Ward et al., 2001). Although the intake of coumestans is low in the country, it should be noted that coumestrol contained in soybean sprouts, which is commonly eaten in Korea, is one of representing coumestans. Also, coumestrol has weak estrogen functions (Whitten et al., 2002), with reported effects of preventing bone resorption in ovariectomized rats (Saga et al., 2003) and preventing cancers and cardiovascular diseases (Adlercruts & Mazur, 1997). Besides the estrogenic and antiestrogenic activities (Zava and Duwe 1997, the phytoestrogens have antioxidant activity due to polyphenolic

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structure (Murkies *et al.*, 1998). The antioxidant activities were demonstrated *in vitro* and *in vivo* (Tikkanen *et al.*, 1998) and are regarded as the additional function for the prevention of various chronic diseases (Cornwell *et al.*, 2004).

Such functions of phytoestrogen are widely known but the current status of phytoestrogen intake has been studied mainly for soy isoflavone both in domestic and foreign studies (Cui *et al.*, 2006; Kim & Khil, 2007; Kirk *et al.*, 1999; Lee *et al.*, 2000; Maskarine *et al.*, 1998). Recently in foreign countries, the database for phytoestrogen contents in many foodstuff has been established (De Klein *et al.*, 2001; Reinli & Block, 1996; USDA-IOWA database; Valsta *et al.*, 2003). However, the content may be extremely small depending on the kind of foods and thus its precision can become problematic. To make up for such aspect, Boker *et al.* (2002) has recently introduced the concept of phytoestrogen score. By using this, the reports for total phytoestrogen intake have been increased in other countries and also retrospective study results using the existing data have been reported (De Klein *et al.*, 2001a; De Klein *et al.*, 2001b). However, we have not tried such effort for the investigation of total phyetoestrogen in the country.

Therefore, this study was performed to investigate the phytoestrogen intakes of Korean women before and after menopause by using our phytoestrogen database for 120 kinds of domestic foods developed with reference to the database established in other countries. In addition, the serum and urine levels of some phytroestrogen and its metabolite as well as status of vitamins A and E and lipid peroxidation and bone markers were also measured to evaluate their interrelations with regard to menopause.

Subjects and Methods

Subjects

Subjects were selected by 48 pre-menopausal women and 53 post-menopausal women aged between 40 and 65, who visited the public health center located in Chilgok-gun, Gyeongsangbuk-do during the months of June and July in 2004. Age, lifestyle factors, gynecological and obstetric history of subjects were investigated through the general survey questionnaire. Anthropometry and blood pressure were directly measured by a trained researcher. Fasting blood samples were taken early in the morning for serum preparation and urine samples were taken at the same time and added with a little toluene, and both samples were stored at -70℃ until analyzed.

Dietary intake by 24-hour recall

Nutrients intake was investigated by 24-hour recall method and a total of 3 days including 2 weekdays and 1 weekend day were chosen for recording the type and amount of foods eaten. These were then used for calculating nutrient intake using CAN-pro (version 2.0). Food consumption was recorded by a trained researcher through direct interview, and real-sized food models and pictures were used to help subjects recall the amounts of foods (The Korean Dietetic Association, 1999).

Phytoestrogen intake by food frequency

The phytoestrogen database for 120 types of foods was established on the basis of the preliminary study on types of foods preferred by subjects and with reference to the existing data (Boker *et al.*, 2002; De Klein *et al.*, 2001; Lee *et al.*, 2000; Reinli & Block, 1996; Valsta *et al.*, 2003; USDA-IOWA database). The composition of 120 kinds of foods were 18 grains & grain products, 46 beans & bean products, 16 fruits, 23 vegetables, 10 Kimchis, 3 teas and coffees, 3 alcoholic beverages, and 1 other. Phytoestrogens in the database include isoflvones such as daidzein, genistein, formononetin, and biochain A, and coumestans such as coumesterol, and lignans such as matairesinol, secoisolariciresino, eterolactone and enterodiol. The intake of phytoestrogen was obtained from food frequency survey questionnaire. The questionnaire presented 1 serving size and actual consumption per a food item and the frequency was divided into a total of 11 frequencies including seldom eaten (①), a few times in a year (② record the number), once or 2~3 times a month (③, ④) 1, 2, 3~4, 5~6 times a week (⑤, ⑥, ⑦, ⑧), 1, 2, 3 times a day (⑨, ⑩, ⑪) (Jang, 2007). Phytoestrogen intake was calculated according to the method of Boker *et al.* (2002). The phytoestrogen concentration per 100 g of each food was categorized into 7 levels of scores as shown in Table 1. Thus, if phytoestrogen score (mg/100 g) is known from the range of phytoestrogen contained in 100 g of a certain food, then the phytoestrogen content was calculated by multiplying the amount per serving and daily frequency to that score. Phytoestrogen contents calculated after the frequency survey were added to estimate the daily consumption for 120 food items in the survey questionnaire.

Biochemical analysis

Serum genistein was quantified by using TR-FIA (time-resolved fluoroimmunoassay) with Labmaster (Turku, Finland) genistein Kit (1212-2003) and urinary enterolactone was quantified in the same manner with Labmaster enterolactone Kit

Table 1. Grouping of phytoestrogen concentrations into score categories*

Phytoestrogen, mg/100 g	Phytoestrogen score, mg/100g
0	0
0.00001-0.00099, trace	0.0005
0.001-0.0099	0.005
0.01-0.099	0.05
0.1-0.99	0.5
1-9.99	5
10 and over	50

* Boker LK *et al.*, 2002

(1212-2001).

Serum retinol and α-tocopherol were quantified at the same time according to the method of Bieri (Bieri *et al.*, 1979) by using HPLC (column, Bondapak C_{18}, mobile phase, methanol/H_2O (97/3)). At this time, the internal standards used were retinyl acetate and tocopheryl acetate, respectively. Serum lipid peroxides were measured by using Yagi method (Yagi, 1976) for the measurement of TBARS, the substances that reacted with thiobarbituric acid (TBA), and 1,1,3,3-tetramethoxypropane was used as a standard.

Serum osteocalcin and urinary deoxypyridinoline (DPD) were measured in Samkwang Reference Laboratories (Seoul, Korea) by IRMA method with the Osteocalcin-IRMA kit from Bio-sourse[TM], and the urinary deoxypyridinoline (DPD) excretion was measured by using Gamma-BCT DPD flow Diagram[R] kit from Immuno Diagnostic System[R] and with a 1470-Wizard ɣ-Counter.

Statistical analysis

All data obtained from this study were analyzed by SAS program to produce the mean and standard deviation. General characteristics of subjects were analyzed using χ^2-test and the significance of difference between premenopausal and postmenopausal women was verified using *t*-test at $p < 0.05$.

Results

General characteristics of the subjects

The significance between anthropometry and maternal factors of pre- and post-menopausal women is shown in Table 2. Total number of subjects was 101, including 48 pre-menopausal and 53 post-menopausal women. The average age of pre-menopausal women was 45.6 years and that of post-menopausal women was 55.0 years, and the average age of menopause was 50 years. Two of the post-menopausal women were at the age of 41 and 45 because they had undergone ovariectomy. The age of menarche was one year higher in post-menopausal women group. There was no significant differences in average height and weight between two groups but the body mass index (BMI) was 22.6 in pre-menopausal group and 23.8 in post-menopausal group, which was significantly higher ($p < 0.05$). This is because the number of subjects with the BMI of 18.5~22.9 was 29 in pre-menopausal women, which was more than half of the group, while it was only 38% in post-menopausal group, which had more in the overweight range of 23.0~24.9 and the obese range of over 25.0. Waist-hip ratio (WHR) was 0.82 in pre-menopausal group and 0.86 in post-menopausal group, which was significantly higher. Blood pressure was in the normal range in both groups but the systolic blood pressure of post-menopausal group was higher compared to the pre-menopausal group.

Table 2. General characteristics of the study subjects

Variables	Premenopausal women (n=48)	Premenopausal women (n=53)	Significance
Age (yrs)	45.6 ± 4.9 (40~51)	55.0 ± 5.2 (41~57)	$P < 0.1$[6]
Menopause (yrs)	–	50.0 ± 4.9	–
Menarche (yrs)	15.3 ± 1.5	16.3 ± 1.8	$p < 0.001$[6]
Height (cm)	156.9 ± 4.5	155.9 ± 4.6	NS[7]
Weight (kg)	55.6 ± 6.0	58.0 ± 7.3	NS
BMI (kg/m²)[1]	22.6 ± 2.7	23.8 ± 2.5	$p < 0.05$[6]
< 18.5	–	1(1.9)	NS
18.5~22.9	29(60.4)[5]	20(37.7)	
23.0~24.9	11(22.9)	14(26.4)	
> 25.0	8(16.7)	18(34.0)	
WHR[2]	0.82 ± 0.05	0.86 ± 0.06	$p < 0.001$[6]
< 0.85	35(72.9)	23(43.4)	$p < 0.01$[8]
≥ 0.85	13(27.1)	30(56.6)	
SBP (mmHg)[3]	115.9 ± 15.0	126.9 ± 20.3	$p < 0.01$[6]
DBP (mmHg)[4]	72.7 ± 9.3	76.2 ± 15.4	NS

[1] BMI : Body mass index
[2] WHR : Waist hip circumference ratio
[3] SBP : Systolic blood pressure
[4] DBP : Diastolic blood pressure
[5] N (%)
[6] NS : Not significant
[7] Significantly different by *t*-test
[8] Significantly different by χ^2-test

Table 3. Daily nutrient intakes of the study subjects

Variables	Premenopausal women (n=48)	Postmenopausal women (n=53)	Significance[2]
Energy (kcal)	1,537.2 ± 331.9[1]	1,509.7 ± 321.7	NS[3]
Carbohydrate (g)	241.9 ± 58.8	253.0 ± 55.2	NS
Fat (g)	37.4 ± 14.1	31.4 ± 10.2	$p < 0.05$
Protein (g)	54.5 ± 15.0	53.9 ± 14.4	NS
Dietary Fiber (g)	12.0 ± 4.1	14.0 ± 5.2	$P < 0.05$
Vitamin A (μg RE)	598.3 ± 300.1	682.3 ± 367.2	NS
Carotene (μg)	2438 ± 1556	2594 ± 1314	NS
Vitamin E (mg α-TE)	9.4 ± 4.6	9.9 ± 3.9	NS
Vitamin C (mg)	67.3 ± 24.0	73.1 ± 33.3	NS
Vitamin B_1 (mg)	1.3 ± 0.6	1.4 ± 0.7	NS
Vitamin B_2 (mg)	1.1 ± 0.4	1.1 ± 0.5	NS
Niacin (mg)	2.0 ± 3.8	12.4 ± 3.1	NS
Vitamin B_6 (mg)	1.5 ± 0.5	1.7 ± 0.5	NS
Folate (μg)	186.4 ± 55.9	195.2 ± 62.3	NS
Calcium (mg)	454.3 ± 166.5	466.8 ± 158.8	NS
Phosphorus (mg)	807.4 ± 218.8	828.8 ± 222.9	NS
Na (mg)	3,381.1 ± 1,057.1	3,217.8 ± 1,081.4	NS
Iron (mg)	10.3 ± 3.1	11.6 ± 3.0	$p < 0.05$
Zinc (mg)	6.6 ± 1.6	6.9 ± 1.7	NS
Cholesterol (mg)	193.9 ± 112.4	160.4 ± 75.7	NS

[1] Mean ± SD
[2] Significantly different by *t*-test
[3] NS : Not significantly different

Daily nutrient intakes

The results for the average daily nutrient intakes from 3 days of food consumption survey using 24-hour recall method are shown in Table 3. There was no significant differences in nutrient intakes between two groups, except fat and iron. Energy intakes were similar in pre-menopausal group and post-menopausal group as 1,537 kcal and 1,510 kcal, respectively, but pre-menopausal women consumed more fat as 37 g compared to 31 g in post-menopausal women. Dietary fiber and iron intakes were 12.0 g and 10.3 mg in pre-menopausal women, respectively, and 14.0 g and 11.6 mg in post-menopausal women, which were significantly higher. The average intakes of vitamins and minerals examined in the present study were not significantly different between groups but tended to be higher in post-menopausal women compared to pre-menopausal women.

Daily phytoestrogen intakes and body status

Daily phytoestrogen intakes of subjects were calculated using the food frequency survey for 120 foods as shown in Table 4. Isoflavone was 90~93% of ingested phytoestrogen and was 28.91 mg in pre-menopausal women and 45.42 mg in post-menopausal women. Almost all of these isoflavones were made of diadzein and genistein and each of which took half of the total isoflavone intake, suggesting that these are most important sources of soybean-derived phytoestrogen. Coumesterol is a single component but was about 1 mg/day, which was 3% of the total phytoestrogen intakes, and lignan intake was 1.5~1.8 g, which was 4% of the total phytoestrogen. Phytoestrogen intake between pre- and post-menopausal women showed that the intake of post-menopausal women was greater by 50% than that of pre-menopausal women. This increment was due to the increased intake of isoflavone. However, coumesterol intake tended to be higher in post-menopausal women and most lignan types were higher in post-menopausal women. Total phytoestrogen intake

Table 4. Daily intakes of various dietary phytoestrogens of the subjects

Phytoestrogen	Mean intake score, mg/day		
	Premenopausal women (n=46)	Postmenopausal women (n=53)	Significance
Daidzein	14.43±10.06[1]	22.61±10.96	$p<0.0001$[2]
Genistein	14.47±10.09	22.80±10.90	$p<0.0001$
Formononetin	0.008±0.007	0.006±0.006	NS[3]
Biochanin A	0.0006±0.0010	0.0011±0.0020	NS
Total isoflavones	28.91±20.14	45.42±21.85	$p<0.0001$
Coumesterol	1.04±0.91	1.32±1.39	NS
Total coumestans	1.04±0.91	1.32±1.39	NS
Matairesinol	0.07±0.82	0.13±0.19	$p<0.05$
Secoisolariciresinol	0.41±0.20	0.45±0.31	NS
Enterolactone	0.48±0.26	0.58±0.42	$p<0.05$
Enterodiol	0.50±0.21	0.64±0.53	$p<0.01$
Total lignans	1.46±0.29	1.80±0.53	$p<0.01$
Total phytoestrogen	31.41±21.63	48.54±23.77	$p<0.0001$

[1] Values are mean±SD
[2] Significantly different by t-test
[3] NS : Not significantly different

Table 5. Serum genistein levels and urinary enterolactone excretion of the subjects

Variables	Premenopausal women (n=48)	Postmenopausal women (n=53)	Significance[2]
Serum genistein (nmol/L)	n=42	n=45	NS[3]
	120.5±117.4[1]	169.3±142.1	
Urine enterolactone (nmol/mmol creatinine)	n=38	n=38	NS
	228.0±265.5	173.1±116.6	

[1] Values are mean±SD
[2] Significantly different by t-test
[3] NS : Not significantly different

in diet was significantly higher in the postmenopausal group (48.54 mg) than in the premenopausal group (31.41 mg).

The kinds of phytoestrogen ingested by subjects were several, but in this study, only serum genistein level and urinary excretion of enterolactone, which is one of major metabolites of lignans, were measured for the content of these substances in the body. As presented in Table 5, the average concentration of serum genistein in pre-menopausal women was 120.5 nmol/L while that in post-menopausal women was 169.3 nmol/L, which was slightly higher. Urinary enterolactone excretion was 173.1 nmol/L in post-menopausal women, while 228.0 nmol/L in pre-menopausal women.

Body antioxidant status and bone marker levels

Serum retinol, α-tocopherol and lipid peroxide measured as thiobarbituric acid reactive substances (TBARS) were measured from the study subjects and the results are shown in Table 6. Serum retinol was 0.41 μg/ml in pre-menopausal women while 0.46 μg/ml in post-menopausal women, which was significantly higher, and also the concentration of α-tocopherol was higher in post-menopausal women. However, there was no difference between two groups when serum α-tocopherol was compared to serum cholesterol (α-tocopherol μg/mg chlesterol). The average serum TBARS was 1.48 nmol/ml in pre-menopausal women and 1.65 nmol/ml in post-menopausal women, which was slightly higher but not significant.

Serum osteocalcin level, which is a bone formation index, and urinary deoxypyridinoline (DPD), which is a bone resorption index, are shown in Table 7. Serum osteocalcin in most subjects

Table 6. Serum levels of retinol, α-tocopherol and TBARS of the subjects

Variables	Premenopausal women (n=48)	Postmenopausal women (n=53)	Significance[2]
Retinol (μg/ml)	0.41±0.08[1]	0.46±0.10	$p<0.05$
α-Tocopherol (μg/ml)	9.34±2.07	10.76±2.65	$p<0.01$
α-Tocopherol (μg/mg cholesterol)	5.35±0.94	5.64±1.06	NS[3]
TBARS (MDA nmol/ml)	1.48±0.49	1.65±0.62	NS[3]

[1] Values are mean±SD
[2] Significantly different by t-test
[3] NS : Not significantly different

Table 7. Serum Osteocalcin and urinary deoxypyridinoline (DPD) excretion of the subjects

Variables	Premenopausal women (n=48)	Postmenopausal women (n=53)	Significance
Osteocalcin (ng/ml)	4.80±2.16[1]	7.18±3.05	p<0.0001[3]
1.02-15.1 (Normal range)	48(100.0)[2]	52(98.1)	NS[5]
>15.1	-	1(1.9)	
Deoxypyridinoline (nmol/mmol creatinine)	5.95±2.44	7.15±2.39	p<0.05[3]
2.5-6.5 (Normal range)	35(72.9)	21(39.6)	p<0.001[4]
>6.5	13(27.1)	32(60.4)	

[1] Values are mean±SD
[2] N(%)
[3] Significantly different by t-test
[4] Significantly different by X^2-test
[5] NS: Not significantly different

Table 8. Correlation coefficients between dietary phytoestrogen intake, serum osteocalcin, urine deoxypyridinoline (DPD) and serum osteocalcin, urine DPD and serum TBARS levels of the pre- and post-menopausal women

Subjects		Serum Osteocalcin	Urine DPD	Serum TBARS
Premenopausal	Dietary phytoestrogen intake	-0.2143	0.1734	-0.5912***
women	Serum genistein	0.0904	-0.0092	-0.2515
	Urine enterolactone	0.4797**	0.1547	0.0075
Postmenopausal	Dietary phytoestrogen intake	-0.1057	0.3341	-0.4649**
women	Serum genistein	-0.1383	-0.3957*	-0.0657
	Urine enterolactone	-0.2149	-0.0461	-0.1358

* p<0.05
** p<0.01
*** p<0.005

was within the normal range, 1.02~15.1 ng/ml, but the average level was 4.8 ng/ml in pre-menopausal women and high as 7.18 ng/ml in post-menopausal women. The average urinary DPD concentration was 5.95 nmol/mmol creatinine in pre-menopausal women, while 7.15 nmol/mmol creatinine in post-menopausal women, which was significantly higher. Also, the number of subjects outside the normal range of 2.5~6.5 nmol/mmol creatinine was 13 (27.1%) in pre-menopausal women, but 31 (60.4%) in post-menopausal women, showing a significant difference between two groups. The correlations among bone marker, serum TBARS value, total phytoestrogen intake, serum genistein and urinary DPD levels in pre-menopausal and post-menopausal women are presented in Table 8. While there was a positive correlation between urine enterolactone and serum osteocalcin level in pre-menopausal women, no correlation was found in post-menopausal women. Serum genistein level showed a negative correlation with urine DPD level in post-menopausal women. Serum TBARS values showed higher negative correlations with dietary phytoestrogen intakes in both groups.

Discussion

Physical conditions of subjects in this study showed higher BMI, WHR and blood pressure in post-menopausal women when compared to pre-menopausal women, which were consistent with other studies (Haarbo *et al.*, 1991; Wing *et al.*, 1991) and confirmed higher chances for chronic diseases. Such recognition is considered as a factor for reducing fat intake from the meals in post-menopausal women. However, when the nutrient intakes of subjects in this study were compared to the Dietary Reference Intakes for Koreans (Korean Nutrition Society, 2005), most subjects were in the range under the recommended intake (RI) but close to the estimated average requirement (EAR). But, dietary fiber was only 55% of adequate intake (AI) in pre-menopausal women and 64% in post-menopausal women, suggesting a considerably low intake level. Calcium intake was 454 mg in pre-menopausal women, which was 65% of RI (700 mg), but post-menopausal women maintained the same level. Actually, the RI of women over 50 was increased to 800 mg and thus the lack became larger compared to pre-menopausal state. Therefore, it seems necessary to actively induce dietary changes to increase the calcium intake in relation to osteoporosis, which is the most serious problem after menopause.

As the importance of phytoestrogen for the prevention of chronic diseases such as osteoporosis in women is highlighted, many studies on its intake, metabolism and functions have been performed. Soybean isoflavone is the first to be known among phytoestrogens but the degree of its intakes have shown great differences depending on races and regions. A study on the isoflavone intake among races in Hawaii using a frequency survey for soybean food intake showed that the average intakes of Chinese and Japanese people were 10~20 mg but those of Caucasian and Philippine was only 5 mg (Maskarine *et al.*, 1998). The study for the isoflavone intake of Dutch women using a food frequency questionnaire (FFQ) for 227 foods developed to investigate total phytoestrogen intake including isoflavone and lignan showed the result of 0.88 mg (Boker *et al.*, 2002), and the result calculated by using the Framingham study data was 0.78 mg (De Klein *et al.*, 2001a), and the intake of Americans except Asians in the year of 2000 was calculated as 2.87 mg (Horn-Ross *et al.*, 2000). These results were lower than those from the studies performed before 2000 (Adlercreuts *et al.*, 1991; Kirk *et al.*, 1999; Maskarine *et al.*, 1998) and considered as the differences among subjects but may possibly be the differences of investigation methods. Phytoestrogen intake studies in Korea have been performed mainly on soy isoflavone, and its average intake was in the range of 15 mg to 48 mg with differences (Choi *et al.*, 2005; Cui *et al.*, 2006; Kim & Khil 2007; Kim & Kwon, 2001; Lee *et al.*, 2000), which were considered as differences due to subjects and regions. However, a common aspect found in any survey study is great individual differences ranging from 0 to 200 mg. In this study, the isoflavone intake calculated by using a recently developed phytoestrogen score was

in the range of 29~45 mg, which was rather higher compared to the average values calculated from frequency surveys for soybean food intake in Korea. Diadzein and genistein took almost 100% of total isoflavone and formononectin and biochanin A were negligible, which is different from the results of foreign studies. The most noticeable result in this study was that the isoflavone intake of post-menopausal women was increased by 50% compared to that of pre-menopausal women, which was similar to the result from the study by Cui *et al.* (2006) in which menopausal women with osteoporosis showed higher isoflavone intake compared to women in the control group (48.56 mg vs 29.19 mg). It is considered that this result was caused by increased intakes of soybean foods such as tofu (~20 g/d), bean paste (10~15 g/d) and other bean containing foods (~5~15 g/d) due to increased information on health and nutrition of menopausal women in these days. Foods containing coumestan among phytoestrogens are very limited and one foreign study showed its amount as 0.2~1.7. μg/day (De Klein *et al.*, 2001a) and up to 0.2 mg/day when considering Asian population (Boker *et al.*, 2002). Coumesterol, a representing form of coumestan, is plenty in one of our common food, soy bean sprout (~1 mg/100g ; phytoestroen score 5; Relini & Block, 1996) and thus expected to have higher intake in Korean people, which was confirmed in this study for the first time. Coumesterol in mung bean sprout, which is largely consumed in China and Japan, was not detectable unlike soy bean sprout (Relini & Block, 1996), suggesting that the coumesterol intake in Koreans may be higher among Asians. It is meaningful that the lignan intake in daily meals of Koreans was first reported in this study. Lignan intake has been reported as 0.20~1.11 mg/day in Europe (Boker *et al.*, 2002; Kikkinen *et al.*, 2003) and in the USA (De Klein *et al.*, 2001a). The result of this study showed that the lignan intake of Korean women was higher than that of Western people. This is considered as reasonable in view of the fact that the major sources of lignan are grains and seeds such as sesame seed. However, when considering the fact that all domestic foods were not included in the database used in this study, it is possible that lignan intake was underestimated. Under the same condition, all kinds of lignan intakes were significantly higher in post-menopausal women compared to pre-menopausal women, reflecting the health-oriented multi-grain intake of this group.

Phytoestrogens in foods are mainly in the form of glycosides containing sugars and changed into aglycone and absorbed in the digestive process. Also, considerable amount of these agly-cones can be fermented by colonic bacteria and isoflavone is converted into O-desmethylangolensin and equol, and lignan is converted into enterolactone and enterodiol, and then absorbed and some are excreted in feces (Lampe, 2003). As the accuracy of phytoestrogen intake can be problematic, efforts have been made to measure these metabolites in blood and urine in addition to phytoestrogen itself to understand the degree of its intake. Most studies (Adlercreutz *et al.*, 1991; Hutchins *et al.*, 1995; Lampe, 2003) reported that the intake and urinary excretion of

isoflavone or its metabolites were more strongly correlated with their dietary intakes than those of lignans. But one Finnish study (Kikkinen *et al.*, 2003) supported the strong correlation of lignan intakes and serum enterolactone. In this study, phytoestrogen and metabolites content in the body were investigated in a very limited way and such correlation was not shown although serum genistein appeared to be higher in post-menopausal women whose phytoestrgen intake was higher compared to pre-meno-pausal women. The increase in urinary DPD as well as serum osteocalcin in post-menopausal women was consistent with other study results (Choi *et al.*, 2000; Kawana *et al.*, 1994). The result in this study showed lower urinary DPD as serum genistein became higher in post-menopausal women. It is interpreted as an important role of suppressing bone resorption by increasing soybean isoflavone intake in daily meals. However, the excretion of enterolactone, unlike lignan intake, was not high in post-menopausal women, suggesting that the lignan intake was possibly underestimated as mentioned above. Thus, further research is needed to figure out this result. Urinary enterolactone excretion in Korean menopausal women reported by Kim *et al.* (2002) was 180~360 μmol/day and that from this study was 170 ~228 μmol/L. The 24-hour urine content was not measured in this study but when considering the daily urine volume as 1.0~1.5 L, the urinary enterolactone result of this study and that of Kim *et al.* (2002) were in the same range. It was reported in these results that subjects with osteopenia or osteoporosis showed lower urinary enterolactone than normal subjects, but the cause was not clear because lignan intake of subjects was not reported. In this study, the osteocalcin content, a bone formation marker, was increased in post-menopausal women, which is thought as increased overall bone turnover due to accelerated bone loss (Peichl *et al.*, 1998). This basic physiological change after menopause seems to have much higher impact on urinary DPD and serum osteocalcin levels than the dietary phytoestrogn, resulting in different correlations between these bone markers and the phytoestrogen intake in pre- and post-menopausal women. On the other hand, it is very important that serum lipid peroxide was decreased as dietary phytoestrogen intake became higher in all subjects. Considering that most of the phytoestrogens are polyphenolic compounds and possess the antioxidant capacity (Tikkanen *et al.*, 1998, Hutchins *et al.*, 2005), this result was not unexpected. However, the higher phytoestrogen intake appears to have significance for post-menopausal women whose serum TBARS level were not reduced despite of higher serum α-tocopherol level compared with pre-menopausal women to maintain proper body oxidant/antioxidant status.

It is concluded that the dietary phytoestrogen is beneficial by decreasing oxidative stress in all middle-aged women regardless of menopause and can help to prevent the incidence of several chronic diseases that are markedly increased after menopause. But the effect of phytoestrogen on bone metabolism and health appears different by the type of the phytoestrogen and the menopausal state and is to be further investigated in the future.

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