were performed using Student's *t* test or one-way ANOVA with *post hoc* Dunnett's tests for comparisons between two groups or multiple groups, respectively. Statistical software used was the Statistical Package for the Social Sciences (SPSS; version 13.0 J for Windows). $P<0.05$ was considered statistically significant.

TABLE 1

Sequences for RT-PCR Primers

Gene	Primer Sequence
GAPDH	Forward: 5'-AAA TGG TGA AGG TCG GTG TG-3'
	Reverse: 5'-TGA AGG GGT CGT TGA TGG-3'
FAS	Forward: 5'-CAT TGT GGG CGG GAT CAA C-3'
	Reverse: 5'-GAA CTG CCA CGA CAG CCT CA-3'
HSL	Forward: 5'-GTG GCC GAT TCG CCA TAG AC-3'
	Reverse: 5'-AGT GGC ATC TCA AAG GCC TCA-3'
PPARα	Forward: 5'-CTG ACA TTT GTG ACT GGT CAA GCT C-3'
	Reverse: 5'-TTT CCA GGT CAT CTG CTT CAA GTG-3'
PPARγ	Forward: 5' - GCA CTG CCT ATG AGC ACT TCC ACA-3'
	Reverse: 5' - GGG AGT GGT CAT CCA TCA CAG A-3'

RESULTS

Effects of Isoflavone on Body and Tissue Weight and Food Intake in OVX Rats

The changes in total body weight during experiment are shown in Fig. 1. The body weight gain, liver and fat weight, and food intake are listed in Table 2. The OVX rats had a significantly higher body weight 8 w after experiment than the sham-operated rats ($P<0.05$). The parametrial and perirenal WAT in the OVX rats were higher than those in the sham-operated rats, and statistically significant differences were observed between the isoflavone-treated group and the OVX group and/or the sham-operated group ($P<0.05$). In addition, there were no differences in body weight and fat mass between the sham-operated and isoflavone-treated groups. The weight of brown adipose tissue (BAT) in isoflavone-treated group was significantly lower than that in the OVX and sham-operated groups. OVX and isoflavone had no effect on the ratio of liver to body weight. Food consumption did not differ among the five groups during the 8-week experiment. However, food efficiency was significantly lower in the isoflavone-treated group than in the OVX group ($P<0.05$).

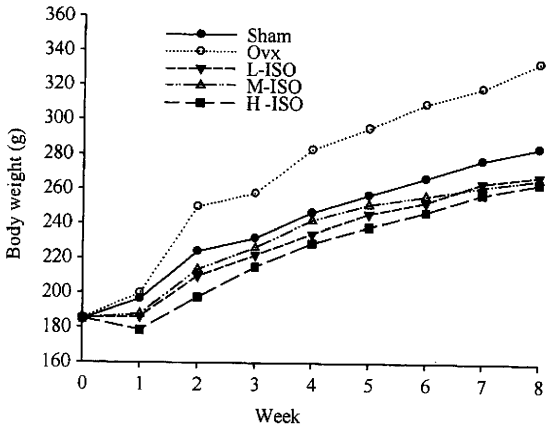

FIG. 1. Effect of isoflavone on body weight of rats 8 weeks after OVX.

TABLE 2

Body Weight, Liver and Fat Weight, and Food Intake

Group	*n*	Final Body Weight (g)	Body Weight Gain (g)	Liver/BW (%)	Parametrial WAT(g)	Perirenal WAT(g)	BAT (mg)	Food Intake (g/kg BW.d)	Food Efficiency (%)
Sham	6	284.3±16.7^{b}	99.6±19.1^{b}	2.61±0.542	10.52±3.65^{a}	4.32±1.33^{b}	463.2±104.4^{a}	50.0±2.0	13.1±2.0^{a,b}
OVX	6	333.3±8.4^{a}	148.0±5.3^{a}	2.03±0.064	13.37±3.10^{a}	6.17±1.87^{a}	408.8±89.8^{a}	48.9±1.4	17.8±0.9^{a}
L-ISO	8	268.0±32.5^{b}	82.7±32.7^{b}	2.20±0.277	6.63±3.11^{b}	3.10±1.79^{b,c}	288.1±54.0^{b}	48.5±1.8	11.4±3.6^{b}
M-ISO	8	266.2±22.6^{b}	81.0±26.6^{b}	2.28±0.304	5.58±2.54^{b}	2.40±0.69^{c}	287.2±32.5^{b}	48.2±2.3	11.4±3.3^{b}
H-ISO	7	263.6±28.1^{b}	78.9±24.0^{b}	2.44±0.461	4.31±2.46^{b}	2.37±1.60^{c}	263.4±61.6^{b}	46.6±2.3	11.8±2.6^{b}

Note. Values are $\bar{x} \pm s$, mean in a row with superscripts without a common letter differ, $P<0.05$. ^{a,b,c} There were significantly differents among a, b, and c ($P<0.05$).

Animal groups were sham-operated control (sham), ovariectomized control (OVX), ovariectomized and treated with different isoflavone (L-ISO, M-ISO, and H-ISO).

Effects of Isoflavone on Serum Biomarkers for Lipid Metabolism in OVX Rats

The serum concentration of total cholesterol in the OVX group was significantly higher than that in the sham-operated group. However, treatment with isoflavones maintained it at the same level as that in the sham-operated group. The serum concentrations of triglycerides and HDL cholesterol were not significantly different in the five groups (Table 3). The serum concentrations of leptin and adiponectin were also significantly higher in the OVX group than in the sham-operated and isoflavone-treated groups.

TABLE 3

Serum Lipids and Adipocytokine Concentrations in the Rats

Group	n	Total Cholesterol (mg/dL)	HDL Cholesterol (mg/dL)	Triacylglycerides (mg/dL)	Leptin (ng/mL)	Adiponectin (μg/mL)
Sham	6	$85.0 \pm 7.5^{a,b}$	39.5 ± 3.9	64.3 ± 16.6	2.20 ± 0.31^{b}	3.03 ± 0.33^{c}
OVX	6	103.0 ± 10.4^{a}	38.0 ± 2.7	48.9 ± 11.3	3.74 ± 0.58^{a}	5.40 ± 0.10^{a}
L-ISO	8	59.2 ± 24.3^{b}	37.3 ± 12.6	43.6 ± 17.7	$1.73 \pm 0.36^{b,c}$	4.19 ± 0.29^{b}
M-ISO	8	66.7 ± 23.4^{b}	33.5 ± 12.4	49.5 ± 18.0	0.96 ± 0.15^{c}	$3.66 \pm 0.41^{b,c}$
H-ISO	7	62.9 ± 22.0^{b}	31.6 ± 11.2	46.5 ± 24.3	$1.43 \pm 0.37^{b,c}$	$3.89 \pm 0.44^{b,c}$

Note. Values are $\bar{x} \pm s$. Mean in a row with superscripts without a common letter differ, $P<0.05$. [a,b,c]There were significantly differents among a, b, and c ($P<0.05$).

Animal groups were Sham-operated control (Sham), ovariectomized control (OVX), ovariectomized and treated with different isoflavone (L-ISO, M-ISO, and H-ISO).

Effects of Isoflavone on mRNA Expression in OVX Rats

The expressions of FAS, HSL, PPAR-alpha (PPARα), and PPARγ genes in WAT, BAT, and the liver were analyzed.

Fig. 2 shows that the absence of ovarian hormone in rats after OVX and treatment with isoflavone reduced, the FAS mRNA expression in WAT and BAT tissue, compared with the rats in the sham-operated group ($P<0.05$). In addition, the FAS mRNA expression in the OVX group was significantly higher than that in the sham-operated group on a high-fat diet ($P<0.05$). Treatment of the OVX rats with isoflavone significantly decreased the mRNA expression of the FAS gene in the liver.

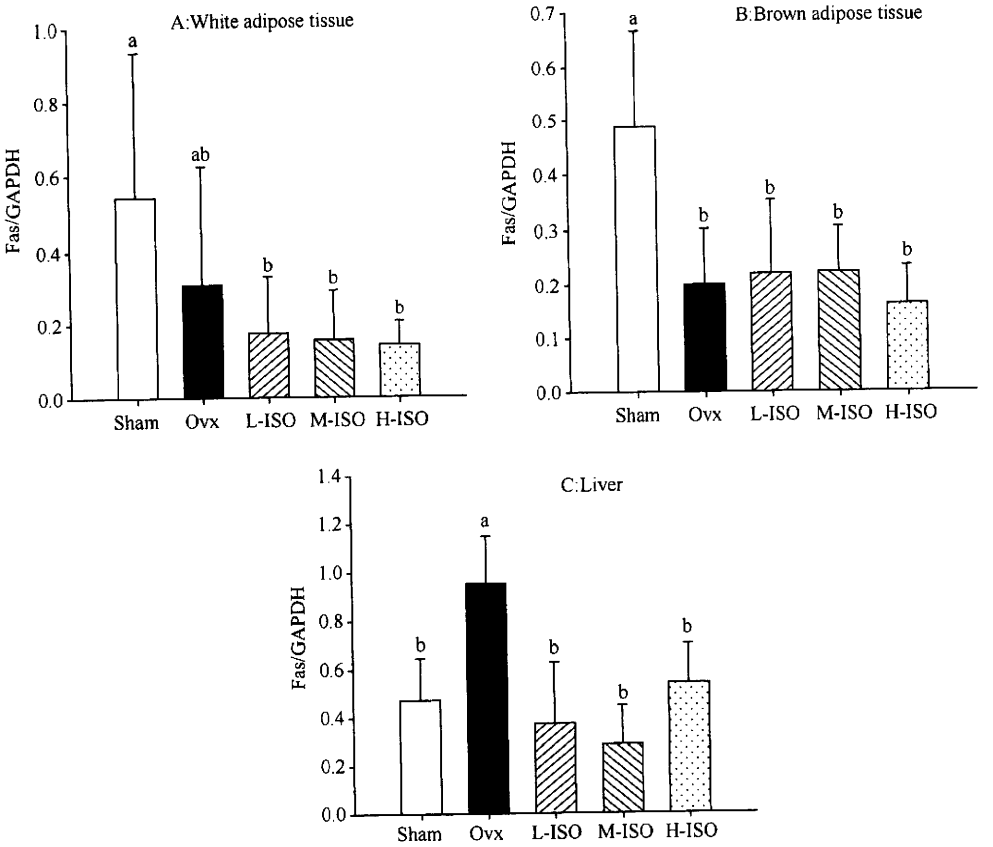

FIG. 2. Effect of isoflavone on FAS mRNA expression in white adipose tissue (A), brown adipose tissue (B), and liver (C). Animal groups comprised sham-operated controls (Sham), ovariectomized controls (OVX), and ovariectomized rats treated with different doses of isoflavone (L-ISO, M-ISO, and H-ISO). The means with the different letters differ ($P<0.05$).

There was no difference in mRNA expression of the HSL gene in WAT and BAT between the groups (Fig. 3). On the other hand, mRNA expression of the HSL gene in the liver was low in the OVX group and in groups after treatment with a low dose of isoflavone, compared with the sham-operated group. A high dose of isoflavone tended to maintain the mRNA expression of the HSL gene in the OVX rats at the same level as that in the sham-operated rats.

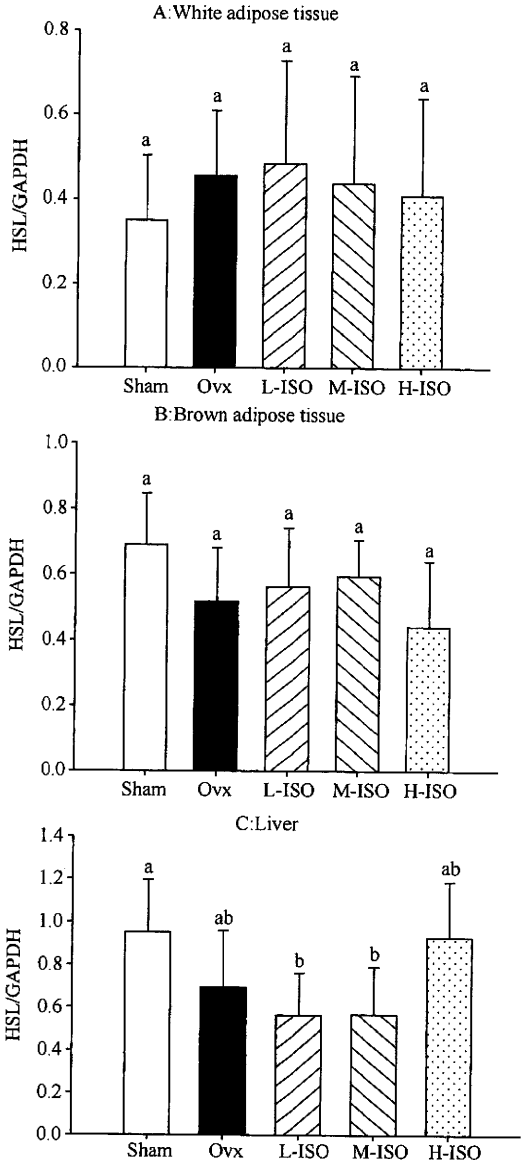

FIG. 3. Effect of isoflavone on HSL mRNA expression in white adipose tissue (A), brown adipose tissue (B), and liver (C). Animal groups comprised the sham-operated controls (Sham), ovariectomized controls (OVX), and OVX rats treated with different doses of isoflavone (L-ISO, M-ISO, and H-ISO). The means with the different letters differ ($P<0.05$).

There were no differences in mRNA expression of PPARα in WAT and liver (Fig. 4). In contrast, the expression of PPARα in BAT of rats treated with a high dose of isoflavones was significantly higher than those in the other groups.

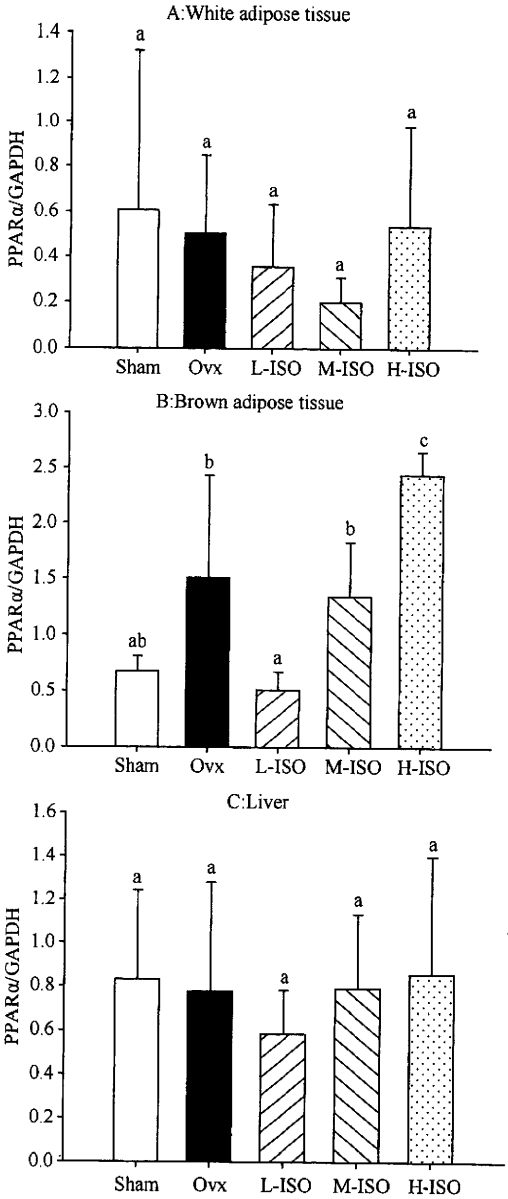

FIG. 4. Effect of isoflavone on PPARα mRNA expression in white adipose tissue (A), brown adipose tissue (B), and liver (C). Animal groups comprised sham-operated controls (Sham), ovariectomized controls (OVX), ovariectomized rats treated with different doses of isoflavone (L-ISO, M-ISO, and H-ISO). The means with the different letters differ ($P<0.05$).

The expression of PPARγ in WAT, BAT, and liver in the OVX group tended to be higher than that in the

sham-operated group (Fig. 5). Isoflavone slightly suppressed the up-regulation of PPARγ mRNA expression. However, this effect was not statistically significant.

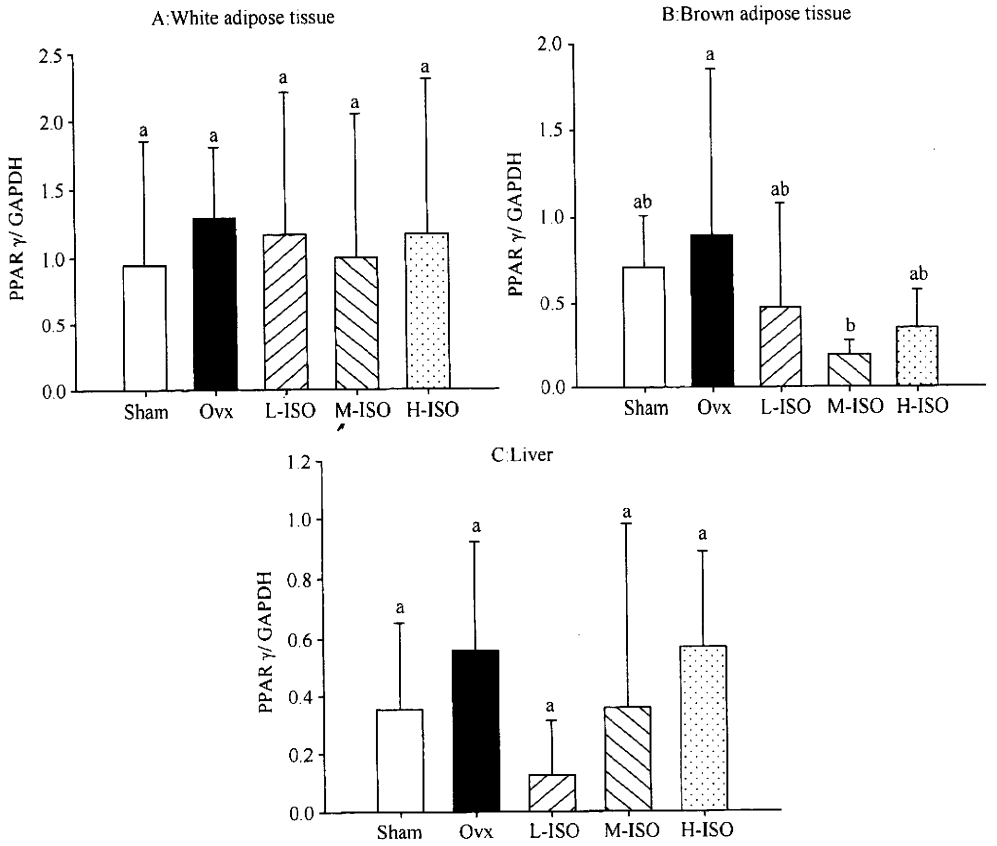

FIG. 5. Effect of isoflavone on PPARγ mRNA expression in white adipose tissue (A), brown adipose tissue (B), and liver (C). Animal groups comprised the sham-operated controls (Sham), ovariectomized controls (OVX), OVX rats treated with different doses of isoflavone (L-ISO, M-ISO, and H-ISO).

DISCUSSION

The relationship between dietary isoflavones, body weight, and fat mass was explored in this study. Isoflavones affected body and fat weights in OVX rats fed on a high-fat diet. Adding different concentrations of isoflavone in the diet (0.68-2.00 g/kg diet) significantly decreased the body weight. Furthermore, the weight of adipose tissue was also significantly lower in the isoflavone group than in the OVX group ($P<0.05$). Although no significant effect of isoflavone on food intake was observed, food efficiency was significantly lower in OVX rats treated with isoflavone than in OVX rats not treated with isoflavone ($P<0.05$), indicating that food consumption does not reflect the change in the amount of adipose tissue. However, isoflavone inhibited food utilization and adipose deposition. Naaz *et al.*[10] and Kim *et al.*[11] reported that the soy isoflavone genistein decreases body weight and adipose deposition in OVX mice. Ali *et al.*[12] reported that isoflavones significantly reduces weight gain and fat deposition in obese and lean male rats. Another research also demonstrated that soy isoflavone prevents body fat accretion and bone loss in OVX mice[13]. Epidemiological surveys and nutrition intervention studies indicated that genistein has beneficial effects on obesity in human beings[14-15], suggesting that an isoflavone-rich diet improves lipid metabolism and has anti-obesity properties.

Serum total cholesterol and adipocytokines, such as leptin and adiponectin, were significantly higher in the OVX rats than those in the sham-operated rats (Table 3). Leptin affects energy homeostasis by inhibiting food intake and stimulating energy expenditure. However, obesity is generally accompanied by a high serum leptin concentration, which is termed as leptin resistance. Isoflavone treatment reduced serum total cholesterol and leptin, suggesting that isoflavones prevents body fat gain, in part, by inhibiting leptin resistance in OVX rats. D'Eon *et al.*[16] reported that 17β-estradiol reduces the serum levels of leptin and adiponectin in OVX mice,

suggesting that the effects of isoflavones on lipid metabolism in animals after OVX animals might, in part, be exhibited *via* an estrogenic pathway.

Despite the beneficial effects of dietary isoflavone on metabolic abnormalities and obesity, studies on the effect of isoflavone on the expression of lipid metabolism-related genes in OVX rats are limited. Therefore, we performed RT-PCR to reveal the possible mechanism underlying the effects of isoflavone on lipid metabolism in OVX rats with diet-induced obesity.

FAS is one of the primary lipogenic enzymes and plays a central role in lipogenesis of mammals and converts dietary calories into a stored form of energy. The activity of FAS has a positive correlation with body fat mass. It was reported that inhibition of the FAS gene expression can sharply decrease the body fat in mice[17]. In this study, the change in FAS gene expression in liver and adipose tissues varied. FAS mRNA expression in the liver was higher in OVX rats than in sham-operated rats, and was significantly inhibited after treatment with isoflavone ($P<0.05$), demonstrating that estrogen deficiency induces body weight gain with fatty acid synthesis in the liver and these changes were improved after treatment with isoflavone. On the other hand, mRNA expression of the FAS gene in WAT and BAT of the OVX group was significantly lower than that in the sham-operated group ($P<0.05$), which is consistent with the previously reported data in OVX mice[1]. It appears that down-regulation of FAS may occur in adipose tissue of rodents after OVX. Further study is needed to clarify this mechanism.

HSL is an intracellular, neutral lipase, and the rate-limiting enzyme in lipolysis[18]. HSL is expressed in various tissues and plays an important role in lipid metabolism, including neutral cholesteryl ester hydrolase[19-20]. Palin *et al.*[21] showed that 17β-estradiol at high doses up-regulates HSL expression. However, a low dose of 17β-estradiol does not significantly alter the HSL expression in subcutaneous abdominal adipocytes isolated from a woman. Although Banz *et al.*[5] reported that soy protein enhances the expression of HSL mRNA in the liver of obese male Zucker diabetic fatty (ZDF) rats. There is no report concerning the direct influence of isoflavone on HSL-gene expression in OVX rats. In this study, the expression of HSL mRNA was lower in the liver of OVX rats treated with a low dose of isoflavones than in sham-operated rats. However, the gene expression tended to restore to the level in the sham-operated group following treatment with a high dose of isoflavones, which is consistent with the previously reported data[21], suggesting that the decreased lipolysis in liver is, in part, responsible for the body weight gain in OVX rats, and isoflavone intake inhibits this change.

PPARs belong to the nuclear steroid receptor super-family. PPARα is mainly expressed in the liver, heart, skeletal muscles, kidney, and intestine. PPARγ is mainly expressed whereas PPAR-delta (PPARδ is widely distributed in adipose tissue and the immune system[22]. Of the three PPAR isoforms known to date, PPARα and PPARγ play an important role in the regulation of whole-body and cellular lipid metabolism[23]. The activation of PPARα increases fatty acid β-oxidation and insulin sensitization, whereas blood and liver lipid concentrations are typically reduced. Ovariectomy decreases expression of the PPARβ gene, and estrogen treatment can stimulate the expression of this gene[24]. On the other hand, PPARβ controls the expression of genes involved in adipocyte differentiation and lipogenesis. The increased adipocyte differentiation attributing to PPARγ activation often results in weight gain because of increased body fat; resulting in the paradoxical effect of PPARγ agonist treatment i.e., increased fat mass along with improvement in other metabolic lipid variables.

It has been demonstrated that isoflavone-containing soy extracts or soy isoflavone can activate PPAR-mediated gene expression and induce metabolic changes suggestive of a PPAR agonist-like effect of soy isoflavone[25]. Similarly, Sujong *et al.*[4] reported that genistein up-regulates PPARα and PPARγ gene expression in the liver of C57BL/6J mice fed on a high-fat diet. It has been shown that soflavone enhances PPARα and PPARγ mRNA expression in WAT of rats[26].

Expressions of the PPARα and PPARγ gene in WAT, BAT, and liver of OVX mice were examined in this study. The expression of PPARα in liver and WAT tended to be lower in the OVX rats treated with a low dose of isoflavone than in sham-operated rats, whereas treatment of OVX rats with a high dose of isoflavone tended to up-regulate the gene expression (Fig. 4). Meanwhile the expression of PPARγ in liver and BAT in OVX rats tended to be higher than that in sham-operated rats, and treatment with isoflavone improved these changes. However, dose response was not observed (Fig. 5), suggesting that OVX increases lipogenesis while decreases lipolysis, and isoflavone improves lipid metabolism in the liver of OVX rats.

In summary, isoflavone is a type of phytoestrogen that can inhibit body weight gain and obesity induced by OVX with a high-fat diet by decreasing food utilization and modulating the gene expression related to lipid metabolism. These effects of isoflavones may involve not only the reduction of the expression of lipogenic genes related to fatty acid synthesis and adipocyte differentiation but also increase the expression of lipolysis-related genes in the liver of OVX rats.

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報　文

「健康食品」中の大豆イソフラボンの定量と表示に関する調査研究

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Study on Food Labeling and the Content of Soybean Isoflavones in Health Foods

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Recent growing interest in health and the diet has led to an increased focus on soy foods and their functional components, *e.g.*, isoflavones. The labeling and isoflavone content of 20 commercially available health foods were assessed. The isoflavone content (aglycone equivalents) in one serving of soymilk was in the range of 20–29 mg, these values being almost the same as those reported on the labels of these foods. However, one soft drink had an isoflavone content 1/3 of the value reported on its label. Some powdered foods, *e.g.*, kinako, contained 6–18 mg of isoflavones in one serving; the isoflavone content reported on the labels of these foods was almost equivalent to the actual isoflavone content of the foods. The isoflavone content of such solid foods as tablets, dried soybean, and soy snacks was in the range of 3–46 mg. One tablet contained only 2/3 of the isoflavone content reported on its label. Based on the presence of 12 isoflavone components, a wide variety of isoflavone components and contents was noted, and the foods were classified into three types: soymilk, soy extract preparation, and soybean. Differences in the isoflavone components of the health foods would vary the absorption rate and effect of the foods. Furthermore, differences in the age, sex, and individual characteristics of consumers also need to be considered when assessing the effectiveness and safety of isoflavone-containing health foods.

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Key words: soy, isoflavone, quantitative analysis, health food, food labeling

緒　　言

近年，人々の健康志向が高まる中，大豆あるいは大豆の機能性成分を含む「健康食品」が数多く市販されるようになった。大豆には，主要な栄養素としてたんぱく質，炭水化物，脂質，ミネラル，ビタミン，食物繊維，などが含まれるほか，微量成分として，大豆イソフラボン，サポニン，レシチン等が含まれている。

1999年，米国食品医薬品局（FDA）は，25 g/日の大豆たんぱく質の摂取は虚血性心疾患を予防するとして，大豆たんぱく質を含む食品に対して心臓病のリスク低減表示を認めた。一方，2006年，米国心臓病協会は大豆たんぱく質及び大豆イソフラボンに関する最近の無作為割付比較試験のメタアナリシスを行ない，大豆たんぱく質及びイソフラボンの血清脂質に対する改善効果は期待されている程ではないとした[1)]。

本邦では，大豆たんぱく質と大豆イソフラボンが特定

キーワード：大豆，イソフラボン，定量分析，「健康食品」，食品表示
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保健用食品の関与成分として厚生労働省から許可されているが，他方，大豆イソフラボンに関しては，その安全性が内閣府食品安全委員会で再評価され，2006年5月に「大豆イソフラボンを含む特定保健用食品の安全性評価の基本的な考え方」が報告された[2]。

これらの背景から，昨今，人びとの大豆及び大豆イソフラボンに対する関心が特に高まっている。そこで本研究では，大豆イソフラボンの適切な表示と摂取方法を提案する目的で，現在市販されている大豆または大豆イソフラボンを原材料に用いた「健康食品」の実態を調査し，表示に関する調査を実施するとともに，食品中に含まれる大豆イソフラボンの定量及びその成分分析を行った。なお，本論文でいう「健康食品」は，保健機能食品（特定保健用食品及び栄養機能食品）といわゆる健康食品をさす。

方　　法

1．「健康食品」の入手

豆乳など液状食品4品目は平成19年4月，新宿区内の食料品店で購入した。その他の液状食品2品目，粉末状食品5品目及び固体状食品9品目は平成18年12月，都内薬局の健康食品コーナーで販売されているイソフラボン含有食品を購入した。

2．試料の調製

食品中の大豆イソフラボンの定量は，厚生労働省が通知した「大豆イソフラボンを含む特定保健用食品等の取扱いに関する指針（食安発第0823001号）」別紙の方法に従って行った[3]。

大豆イソフラボンは15種類の成分が存在するが，標準品が入手可能な12種類について分析を行った（表1）。各標準品（長良サイエンス，岐阜）を70%エタノールに溶解し，濃度が1 mg / 5 m*l* になるように調製した。

液状食品は，イソフラボンとして1～10 mg が含まれる量の食品を 50 m*l* 容メスフラスコに正確に分取し，70%エタノールで 50 m*l* に定容して試験溶液とした。

粉末状食品は，イソフラボンとして1～10 mg が含まれる量の食品を 50 m*l* ビーカーに精密に秤量し，70%エタノール 25 m*l* を加えて溶解させた。沈殿物が析出した場合には，必要に応じて振とうあるいは超音波処理を行った後に遠心分離し，上清を試験溶液とした。試料を完全に溶解させた後，50 m*l* 容メスフラスコに移し変え，70%エタノールで 50 m*l* に定容して試験溶液とした。

固体状食品は，試料を均一に粉砕もしくは混合した後，イソフラボンとして1～10 mg が含まれる量の食品を 50 m*l* ビーカーに精密に秤量し，70%エタノール 25 m*l* を加えて溶解させた。溶解し難い試料の場合には，振とうあるいは超音波処理を行って溶解させた。試料が完全に溶解した後，50 m*l* 容メスフラスコに移し変え，70%エタノールで 50 m*l* に定容して試験溶液とした。軟カプセルは40度に加熱して溶解し，同様の操作を行った。

粉末状及び固体状食品において振とうあるいは超音波処理をしても完全に溶解せず不溶物が認められる場合には，30分間室温で攪拌抽出した後，遠心分離して上清を 50 m*l* 容メスフラスコに移し，残渣についても同様の抽出操作を更に2回行い，計3回の分の上清を集め，70%エタノールで 50 m*l* に定容した。試料は全て 0.45 μm のメンブランフィルターでろ過した後，分析に供した。

3．分　　析

厚生労働省の「大豆イソフラボンを含む特定保健用食品等の取扱いに関する指針」[3] の方法に従い，高速液体クロマトグラフィー法（HPLC 法）により定量した。HPLC による分析条件を以下に示す。

カラム：YMC-Pack ODS-AM-303（size ϕ 4.6 × 250 mm）（ワイエムシィ社，京都）

移動相：A　アセトニトリル／水／酢酸混液（15:85:0.1 v/v/v）（和光，東京）

B アセトニトリル／水／酢酸混液（35:65:0.1 v/v/v）

濃度勾配：A から B までの直線濃度勾配を50分間行う

流速：1 m*l*/min

カラム温度：35℃

測定波長：254 nm

試料注入量：10 μl

4．食品中のイソフラボン量の算出

試料中の大豆イソフラボン濃度は，分子量の比を用いて全てアグリコン当量として次の式により求めた。

例：試料中のダイゼイン型イソフラボンの濃度（アグリコン当量）の求め方。

$$TDe = TADe \times CD/AD \times 0.611$$

TDe ：ダイゼイン型イソフラボンの濃度（アグリコン当量）[mg/*l*]

表1　大豆イソフラボン標準品

分　　類	ダイゼイン型イソフラボン	グリシテイン型イソフラボン	ゲニステイン型イソフラボン
配糖体	ダイジン（D）	グリシチン（Gl）	ゲニスチン（G）
マロニル配糖体	マロニルダイジン（MD）	マロニルグリシチン（MGl）	マロニルゲニスチン（MG）
アセチル配糖体	アセチルダイジン（AD）	アセチルグリシチン（AGl）	アセチルゲニスチン（AG）
アグリコン	ダイゼイン（De）	グリシテイン（Gle）	ゲニステイン（Ge）

TADe：ダイゼイン型イソフラボンのピーク面積
CD　：イソフラボン標準溶液中のダイジンの濃度 [mg/l]
AD　：イソフラボン標準溶液クロマトグラム上のダイジンのピーク面積

グリシテイン型及びゲニステイン型イソフラボンアグリコンの濃度も同様に求め，その総和を試料中の総イソフラボン濃度（アグリコン当量）とし，試料中のイソフラボン含有量（アグリコン当量）を下記の式により求めた。グリシテイン型は0.637，ゲニステイン型は0.625を乗じてアグリコン当量を求めた。

試料中のイソフラボン含量（アグリコン当量）[mg/100 g または mg/100 ml]
＝総イソフラボン濃度（mg/l）×50/1,000×1/試料採取量（g/ml）×100

試料の測定は2連で行い，平均値で示した。CV値（%）は，測定内0.7%，測定間2.8%であった。なお，対象食品に表示されている総イソフラボン量のアグリコン換算値は，分析で求めたイソフラボン成分の構成比をもとに分子量の比から求めた。

結　　果

1．表　　示

表2に対象とした食品中の，名称，大豆イソフラボンに関する原材料名，一日または一回あたりの摂取目安量に関する表示，大豆イソフラボン含有量に関する表示，そのアグリコン換算値を示した。

厚生労働省の通知では，特定保健用食品のほか，錠剤，カプセル剤，粉末剤，液状剤の形状のいわゆる健康食品のうち，大豆イソフラボンを含む食品には大豆イソフラボンアグリコンとしての含有量を表示すること，また一日あたりの摂取目安量を大豆イソフラボンアグリコンとして30 mgを超えないようにとされた。大豆イソフラボンを含む特定保健用食品には大豆イソフラボンアグリコンとしての含有量の表示があったが，その他の食品では錠剤型の食品一品目と煎り黒大豆にアグリコン表示があったのみで，多くはアグリコン表示がなかった。また，一日あたりの摂取目安量のアグリコン換算値は，錠剤の形状をした食品4品目中3品目で若干ではあるが30 mgを超えていた。固体状食品のうち，錠剤の形状をした食品には，美容サポート食品，健康補助食品の表示が，また，菓子には栄養食品などの表示があった。なお，20品目中11品目に「遺伝子組換えでない」の表示があった。

2．「健康食品」中の大豆イソフラボン量

表2に各食品の一日または一回摂取目安量当たりの大豆イソフラボンの表示量とそのアグリコン換算値及び分析値（アグリコン当量）を示した。

液状食品のうち豆乳類及びお茶では，摂取目安量あたりに含まれる大豆イソフラボンアグリコンの分析値は20～29 mgであり，表示量のアグリコン換算値ともほぼ一致した。一方，清涼飲料水（液状剤型）には表示量に対して大豆イソフラボン分析値が1/3のものがあった。

粉末状食品はコーヒーミックス，コラーゲン食品，きなこ，青汁豆乳であり，一回摂取目安量あたりに含まれる大豆イソフラボンアグリコンの分析値は，6～18 mgであった。表示量に対して分析値が著しく逸脱している食品はなかった。

固体状食品のうち錠剤型の食品では，一日の摂取目安量あたりに含まれる大豆イソフラボンアグリコンの分析値は3～46 mgの範囲であり，このうち大豆胚芽抽出物（錠剤型）では，分析値が表示量の2/3のものがあった。黒豆茶，煎り黒大豆，菓子の一回摂取目安量に含まれる大豆イソフラボンアグリコンの分析値は4～19 mgであり，表示量に対して分析値が著しく逸脱している食品はなかった。

3．「健康食品」中の大豆イソフラボン成分の解析

表3に各食品100 gあたりに含まれる12種類の大豆イソフラボンの成分分析の結果を示す。

大豆イソフラボン含量が最も多い食品は，大豆イソフラボン加工食品の表示がある錠剤型の食品で，100 gあたり1.6～4.5 gの大豆イソフラボンが含まれていた。次いで大豆発酵抽出物の表示がある錠剤型等が100 gあたり0.5 g程度のイソフラボンを含有していた。その他の通常の食品は100 gあたり数十ミリグラムのイソフラボンが含まれていた。

イソフラボンの成分分析では，全ての食品に12種類のイソフラボン成分が含まれていたが，食品によって成分に特徴があり，大きく分けて豆乳型，大豆抽出物添加型，大豆型に分類された。

豆乳型食品は配糖体のダイジン，ゲニスチン及びマロニル配糖体のマロニルダイジン，マロニルゲニスチンを主に含有する食品で，豆乳及び大豆菓子がこれに該当する。これらの配糖体とマロニル配糖体は全体の80%以上を占めていた。豆乳に大豆胚芽抽出物または大豆イソフラボンが添加されている食品には，これらに加えてグリシチンが含まれていた。

大豆抽出物添加型食品は，原材料に大豆胚芽抽出物あるいは大豆イソフラボンの表示がある食品で，形状によらず，全体の約90%が配糖体のダイジン及びグリシチンで占められる食品が多かった。一方，原材料に大豆胚芽抽出物の表示がある錠剤型の食品のうち，アグリコン型のゲニステインのみが主成分である食品が1品目あった。

大豆型食品は，比較的多種類のイソフラボン成分を含

表2　食品の表示とイソフラボン含有量（アグリコン換算値）

形態	名　　称	原材料名	1日または1回摂取目安量に関する表示	表　示　量	表示量のアグリコン換算値[1)] (mg)	分析値（アグリコン当量） (mg)
液状食品	調整豆乳（特定保健用食品 大豆たんぱく質）	大豆（遺伝子組換えでない）	1日1本 (200 ml)	大豆イソフラボンアグリコンとして16 mg	16	20
	豆乳飲料（特定保健用食品 大豆イソフラボン）	黒大豆（遺伝子組換えでない）大豆イソフラボン	1日1本 (125 ml)	大豆イソフラボンアグリコンとして25 mg	25	29
	まるごと大豆飲料	大豆（遺伝子組換えでない）	1本 (125 ml)	大豆イソフラボン50 mg	32	27
	豆乳飲料	大豆　大豆イソフラボン	1本 (125 ml)	イソフラボン30 mg	19	20
	黒豆茶	黒大豆（遺伝子組換えでない）　大豆イソフラボン	1本 (500 ml)	大豆イソフラボン25 mg（アグリコン換算）	25	24
	清涼飲料水：液状剤型	焙煎大豆粉末（イソフラボン含有）	1本 (50 ml)	大豆イソフラボン40 mg	26	8
粉末状食品	コーヒーミックス（栄養機能食品　ビタミンD）	黒豆（大豆）大豆イソフラボン	1杯分 (11 g)	イソフラボン10 mg	6.3	9.4
	コラーゲン食品	大豆胚芽抽出物	1日5 g	大豆イソフラボン配合	記載なし	6
	きな粉調製品	黒大豆（遺伝子組換えでない）	1袋 (12 g)	イソフラボン13.2 mg	10	14
	大豆加工食品（青汁豆乳）	青大豆粉末 大豆胚芽抽出物	1袋 (18 g)	大豆イソフラボン 15.14 mg	10	11
	黒ごまきなこ	大豆（国内産）	1杯分 (24 g)	大豆イソフラボン 24.24 mg	20	18
固体状食品	大豆イソフラボン加工食品：錠剤型（美容サポート食品）	大豆抽出物（遺伝子組換えでない）	1日6粒 (1.26 g)	大豆イソフラボン50 mg	31	46
	大豆イソフラボン加工食品：錠剤型（健康補助食品）	大豆抽出物（遺伝子組換えでない）	1日4粒 (1 g)	大豆イソフラボン60 mg	38	45
	大豆イソフラボン加工食品：錠剤型	大豆胚芽抽出物（遺伝子組換えでない）	1日8粒 (2.0 g)	大豆胚芽抽出物125 mg（イソフラボンとして50 mg）	32	33
	コエンザイムQ10含有食品：軟カプセル型（栄養機能食品 ビタミンE）	大豆イソフラボン	1日2粒 (900 mg)	イソフラボン3.7 mg	2.3	3
	大豆発酵抽出物：錠剤型	大豆胚芽抽出物（遺伝子組換えでない）	1日5粒 (1.25 g)	イソフラボンアグリコン 9 mg	9	5.9
	菓子A（栄養食品）	大豆粉	1本 (30 g)	イソフラボン22 mg	14	14
	菓子B（栄養食品）	大豆粉	1本 (30 g)	イソフラボン20 mg	13	16
	黒豆茶	黒大豆（遺伝子組換えでない）	1包 (15 g)	イソフラボン7.4 mg	4.6	4.2
	煎り黒大豆	黒大豆（遺伝子組換えでない）	10 g	大豆イソフラボン15 mg（アグリコン換算）	15	15

[1)] 表示量のアグリコン換算値は、分析により求めたイソフラボン成分の割合を基に分子量の比から求めた。

有している食品で，特に配糖体のダイジン，グリシチン，ゲニスチンに加え，アセチルグリシチンを多く含んでいた。これには，きな粉やお茶が該当した。

なお，今回は分析の対象とはしていないが，粉末状及び固体状食品には，サクシニルゲニスチンと溶出時間が一致する小さなピークが検出されるものが多かった。

表3　食品100 g 当りのイソフラボン成分量（アグリコン換算値）[1)]　mg/100 g（100 ml）

形態	名称	原材料名	D*	MD*	AD*	De*	Gl*	MGl*	AGl*	Gle*	G*	MG*	AG*	Ge*	総イソフラボン
液状食品	調整豆乳（特定保健用食品　大豆たんぱく質）	大豆（遺伝子組換えでない）	2.4	1.7	0.3	0.0	0.0	0.0	0.0	0.0	3.5	2.9	0.0	0.1	11.0
	豆乳飲料（特定保健用食品　大豆イソフラボン）	黒大豆（遺伝子組換えでない） 大豆イソフラボン	9.1	1.1	0.1	0.6	1.6	0.1	0.6	0.0	7.2	1.4	0.4	0.8	23.0
	まるごと大豆飲料	大豆（遺伝子組換えでない）	4.1	3.4	0.4	0.1	0.5	0.0	1.4	0.0	4.7	5.4	0.0	0.7	20.8
	豆乳飲料	大豆　大豆イソフラボン	6.0	0.3	0.0	0.2	3.6	0.0	0.4	0.2	3.7	0.7	0.0	0.5	15.8
	黒豆茶	黒大豆（遺伝子組換えでない） 大豆イソフラボン	2.3	0.7	0.0	0.0	1.2	0.0	0.2	0.1	0.0	0.0	0.1	0.0	4.8
	清涼飲料水：液状剤型	焙煎大豆粉末（イソフラボン含有）	5.9	0.2	0.0	0.3	2.9	0.2	0.5	0.3	4.4	0.5	0.0	0.5	15.8
粉末状食品	コーヒーミックス（栄養機能食品　ビタミンD）	黒豆（大豆）　大豆イソフラボン	39.1	0.3	0.0	3.4	34.0	0.1	4.2	0.2	12.3	0.4	0.0	0.5	94.4
	コラーゲン食品	大豆胚芽抽出物	47.0	0.1	0.0	2.9	45.5	0.0	6.7	3.1	11.4	1.1	0.0	0.8	118.6
	きな粉調製品	黒大豆（遺伝子組換えでない）	18.9	2.2	0.2	32.2	2.0	0.4	32.8	3.3	18.6	0.9	0.0	6.2	117.6
	大豆加工食品（青汁豆乳）	青大豆粉末　大豆胚芽抽出物	13.2	0.2	1.9	0.7	8.0	0.2	2.7	3.4	9.9	19.6	0.2	0.7	60.8
	黒ごまきなこ	大豆（国内産）	7.2	0.0	0.1	1.5	3.1	1.0	7.3	33.5	14.4	0.7	0.1	5.5	74.5
固体状食品	大豆イソフラボン加工食品：錠剤型 （美容サポート食品）	大豆抽出物（遺伝子組換えでない）	1511.0	0.0	2.6	6.3	1821.1	6.8	33.3	0.5	248.1	5.8	0.0	2.2	3637.8
	大豆イソフラボン加工食品：錠剤型 （健康補助食品）	大豆抽出物（遺伝子組換えでない）	1954.3	13.0	2.9	24.8	1953.4	0.8	84.0	1.3	427.9	8.0	0.0	25.2	4495.6
	大豆イソフラボン加工食品：錠剤型	大豆胚芽抽出物（遺伝子組換えでない）	656.6	8.4	1.4	26.1	647.9	1.8	105.7	2.3	155.3	16.1	0.1	6.5	1628.0
	コエンザイムQ10含有食品：軟カプセル型 （栄養機能食品　ビタミンE）	大豆イソフラボン	113.8	0.0	0.1	5.2	139.8	3.3	18.7	11.6	38.5	1.9	0.0	1.1	334.0
	大豆発酵抽出物：錠剤型	大豆胚芽抽出物（遺伝子組換えでない）	0.6	2.6	1.0	0.9	0.6	2.4	10.0	2.6	0.4	0.0	0.0	448.9	470.1
	菓子A（栄養食品）	大豆粉	6.9	5.1	0.7	0.5	0.1	0.0	0.0	1.6	13.9	13.7	2.6	0.8	45.7
	菓子B（栄養食品）	大豆粉	9.6	6.0	1.1	1.6	0.0	0.0	0.0	0.1	18.6	12.8	2.8	2.2	55.0
	黒豆茶	黒大豆（遺伝子組換えでない）	3.5	0.0	0.3	0.4	1.3	1.6	13.3	1.3	4.6	0.7	0.0	1.2	28.1
	煎り黒大豆	黒大豆（遺伝子組換えでない）	25.4	0.3	0.4	0.7	3.3	8.5	75.0	5.4	25.7	0.2	0.0	6.3	151.3

1) アグリコン換算値は分子量の比から求めた。
■ 豆乳型食品に多く含まれる成分、■ 大豆抽出物添加型食品に多く含まれる成分を示す
*表1を参照

4.「健康食品」中の型別イソフラボン含有量（重量%）

大豆イソフラボンは大きく分けてダイゼイン型，グリシテイン型，ゲニステイン型に分類される（表1）。食品中の12種類のイソフラボン成分の合計量のうち，各型のイソフラボンが占める割合（重量%）を表4に示した。豆乳型食品は，ダイゼイン型とゲニステイン型が約90%を占めた。大豆抽出物添加型は錠剤型の食品に多く，ダイゼイン型とグリシテイン型が約90%を占めていたが，中にはゲニステイン型のみが含まれている錠剤型食品があった。大豆抽出物が添加された豆乳飲料は3種の型をほぼ等しく含んでいた。一方，大豆型食品は，ダイゼイン型，グリシテイン型，ゲニステイン型をほぼ等しく含

表4　食品中の型別イソフラボンの割合（重量比）

形態	名　　称	原　材　料　名	ダイゼイン型（%）	グリシテイン型（%）	ゲニステイン型（%）	総量（%）
液状食品	調整豆乳 （特定保健用食品　大豆たんぱく質）	大豆 （遺伝子組換えでない）	40.5	0.5	59.0	100
	豆乳飲料 （特定保健用食品　大豆イソフラボン）	黒大豆（遺伝子組換えでない） 大豆イソフラボン	47.2	10.1	42.7	100
	まるごと大豆飲料	大豆（遺伝子組換えでない）	38.4	9.5	52.1	100
	豆乳飲料	大豆　大豆イソフラボン	42.2	26.6	31.2	100
	黒豆茶	黒大豆（遺伝子組換えでない） 大豆イソフラボン	64.0	32.7	3.3	100
	清涼飲料水：液状剤型	焙煎大豆粉末 （イソフラボン含有）	41.1	24.5	34.5	100
粉末状食品	コーヒーミックス （栄養機能食品　ビタミン D）	黒豆（大豆） 大豆イソフラボン	45.3	40.7	13.9	100
	コラーゲン食品	大豆胚芽抽出物	42.1	46.6	11.2	100
	きな粉調製品	黒大豆 （遺伝子組換えでない）	45.4	32.7	21.9	100
	大豆加工食品（青汁豆乳）	青大豆粉末　大豆胚芽抽出物	26.2	23.7	50.1	100
	黒ごまきなこ	大豆（国内産）	11.8	60.4	27.9	100
固体状食品	大豆イソフラボン加工食品：錠剤型 （美容サポート食品）	大豆抽出物 （遺伝子組換えでない）	41.8	51.2	7.0	100
	大豆イソフラボン加工食品：錠剤型 （健康補助食品）	大豆抽出物 （遺伝子組換えでない）	44.4	45.4	10.3	100
	大豆イソフラボン加工食品：錠剤型	大豆胚芽抽出物 （遺伝子組換えでない）	42.5	46.5	10.9	100
	コエンザイム Q10 含有食品：軟カプセル型（栄養機能食品　ビタミン E）	大豆イソフラボン	35.6	51.9	12.4	100
	大豆発酵抽出物：錠剤型	大豆胚芽抽出物 （遺伝子組換えでない）	1.1	3.3	95.6	100
	菓子 A （栄養食品）	大豆粉	28.8	2.7	67.5	100
	菓子 B （栄養食品）	大豆粉	33.3	0.5	66.2	100
	黒豆茶	黒大豆 （遺伝子組換えでない）	14.9	62.0	23.1	100
	煎り黒大豆	黒大豆 （遺伝子組換えでない）	17.7	61.0	21.3	100

□ 豆乳型食品に多く含まれるイソフラボン型、■ 大豆抽出物添加型食品に多く含まれるイソフラボン型を示す

有していた。

考　　察

大豆イソフラボンを含む「健康食品」に着目し，一日または一回の摂取目安量に含まれる大豆イソフラボン量を求めるとともに，12種類のイソフラボン成分量を比較した。

これらの食品には，大豆イソフラボンまたは大豆たんぱく質を関与成分とする特定保健用食品といわゆる健康食品が含まれる。これらを，形状により，液状食品，粉末状食品，固体状食品に分類し，各々に適した方法に従って大豆イソフラボンの抽出を行い，HPLC 法にて定量及び成分分析を行った。

1．大豆イソフラボンの含有量と表示

分析した食品のなかで重量あたり総イソフラボン含量が最も多かったのは，主原料が大豆胚芽抽出物である錠剤型の大豆加工食品であり，次いで多いのがきな粉，煎り黒大豆，大豆菓子などの大豆を丸ごと使用した食品で，最も少ないのは豆乳などの液状食品であった。豆乳は，イソフラボンが浸水などの加工中に流出することやおからに移行することから含有量が低下すると考えられる。

表2より，一日または一回の摂取目安量に含まれる大豆イソフラボン（アグリコン換算）は液状食品，粉末状食品とも表示量とほぼ一致した。液状食品のなかで，液状剤型食品では表示量の 1/3 しか大豆イソフラボンが含まれていないものがあった。一方，固体状食品のうち錠剤型の食品では 3～46 mg と幅があり，錠剤型の食品 5 品目中 3 品目で 30 mg を超えるものがあること，また含有量が表示量の 2/3 の食品があった。これらのことから，「健康食品」のうち，液状剤型及び錠剤型のいわゆる健康食品には，表示量と含有量が一致しない食品があることが明らかになった。これらの食品は形状及び重量あたりのイソフラボン含有量が高いことから，過剰摂取にも注意を要すると考えられた。

厚生労働省は，平成18年 8 月に「大豆イソフラボンを含む特定保健用食品などの取扱いに関する指針」において，平成19年 4 月以降に製造される大豆イソフラボンを含む特定保健用食品及びイソフラボンが強化，濃縮されたいわゆる健康食品については，大豆イソフラボンアグリコンとして一日の摂取量が 30 mg を超えないように設定すること，またアグリコンとしての含有量を表示することとした。本調査において，これらの食品にアグリコン表示のないものや，分析値が 30 mg を超える食品が散見されたが，この理由として，食品の多くが平成18年12月～平成19年 4 月に購入されたものであることから，時期的に指針に十分に対応していない可能性が考えられた。なお，平成18年 3 月に国民生活センターが実施した，「大豆イソフラボンを多く含むとうたった「健康食品」」に関する調査では，24銘柄中14銘柄が，最大摂取目安量当たりの大豆イソフラボン量（アグリコンとして）が 30 mg を超えていた[4]。

2．大豆イソフラボンの成分分析

大豆イソフラボンの成分分析では，12種類の組成により豆乳型，大豆抽出物添加型，大豆型に分類された。豆乳型にはダイゼイン型とゲニステイン型のイソフラボンが多く，そのなかでも水溶性の配糖体とマロニル配糖体が多くを占めた。この結果は先行研究とよく一致している[5]。大豆抽出物添加型は食品の形状によらず，大豆胚芽抽出物，大豆抽出物あるいは大豆イソフラボンが添加された食品で，ダイジンとグリシチンが全体の80％以上を占めた。大豆胚芽（胚軸）には配糖体のダイジン及びグリシチンが豊富であることから，これらの食品には同様の成分が多く含まれていることとよく一致する[6]。大豆型の食品はきな粉，焙煎黒大豆で，ダイゼイン型，グリシテイン型，ゲニステイン型のイソフラボンをおおよそ等しく含んでおり，なかでも配糖体のダイジン，グリシチン，ゲニスチンに加え，アセチルグリシチンを多く含んでいた。きな粉や焙煎大豆は大豆を高温で焙煎するため，熱によって容易に分解されるマロニル配糖体は殆んど検出されなかった。また，水分が少ない状態で高温加熱処理を行うため，アセチル配糖体が多い。これに比べ，大豆菓子は豆乳と類似したイソフラボン組成を持っていたことから，焙煎ほど高温で加工されたものではないと考えられた。

3．「健康食品」中の大豆イソフラボンの生体利用性

大豆イソフラボンは成分によって吸収性や生物活性が異なることが報告されている。アグリコン型のイソフラボンは腸管で直接吸収されるが，配糖体は腸内細菌によってアグリコンに代謝されてから吸収される。ヒトにおける単回投与試験では，アグリコンの吸収は早く，吸収量も多いことが報告されている[7]。これらのことから，アグリコンのみを含む錠剤を単独で摂取した場合は，大豆食品を食事とともに摂取する場合とは異なる体内動態を示すとともに，生物活性も異なる可能性があると考えられる。

一方，配糖体のダイジンはアグリコンのダイゼインに代謝され吸収されるが，一部はさらにエクオールまたは *O*-デスメチルアンゴレンシン（*O*-DMA）に代謝されてから吸収される[8]。腸内細菌叢はヒトによって個人差があり，エクオール産生菌を保有しているのは日本人閉経後女性では約50％であると報告されている[9]。エクオールはイソフラボンのなかでも最もエストロゲン様作用が強いことから，大豆イソフラボンの臨床的な作用はエクオール産生能に依存するとの報告も多い[10]。Duncan ら

は，エクオール産生者は非産生者に比べて，乳がんの罹患率が低いことを報告している[11]。我々も，閉経後女性を対象にした大豆イソフラボンの介入試験において，エクオール産生者では非産生者に比べて骨量の減少率が低いことを確認している[12]。したがって，ダイゼイン型のイソフラボンを多く含む食品は，摂取側の腸内細菌叢の違いにより異なる効果を発揮する可能性がある。

本調査では，ゲニステインのみを含む錠剤型の食品が1品目あった。ゲニステイン型のイソフラボンは，他の成分とは異なり，チロシンキナーゼ活性やトポイソメラーゼ活性を阻害する作用を持っているため[13]，細胞増殖に影響を与えることや[14]，乳がんリスクを低下させる可能性が報告されている[15]。一方，大豆イソフラボンは17β-エストラジオールに構造が類似しているため，そのレセプターに対して親和性を示すが，ゲニステインは，ダイゼインやグリシテインに比べてエストロゲンレセプターへの親和性が高く，エストロゲン様作用も強い[16]。また，特にβレセプターへの親和性が高いことから，βレセプターに特異的な作用を示す可能性もある。これらのことから，ゲニステイン型のイソフラボンを多く含む食品は他のイソフラボン型を含む食品と異なる健康効果を示す可能性がある。

以上より，今回の調査は今後のイソフラボンの適正表示の提案に役立つと考えられた。また，大豆イソフラボンを含む「健康食品」の有効性と安全性は，含まれるイソフラボン成分の違いによる吸収性や生物活性を把握し，摂取する側の年齢，性別，個体特性等も考慮して評価する必要があると考えられた。

ま と め

大豆イソフラボンを含む「健康食品」に着目し，一日または一回の摂取目安量に含まれる大豆イソフラボン量を求めるとともに，12種類のイソフラボン成分量を比較した。これらを，形状により，液状食品，粉末状食品，固体状食品に分類し，厚生労働省の「大豆イソフラボンを含む特定保健用食品等の取扱いに関する指針」の方法に従い，高速液体クロマトグラフィー法（HPLC法）により定量した。豆乳などの液状食品の摂取目安量あたりに含まれる大豆イソフラボン（アグリコン換算）は20～29 mgであり，表示量のアグリコン換算値ともほぼ一致した。液状剤型食品には表示に対して大豆イソフラボン分析値が1/3のものがあった。きな粉などの粉末状食品の1回摂取目安量あたりに含まれる大豆イソフラボン（アグリコン換算）は，6～18 mgであり，表示量に対して分析値が著しく逸脱している食品はなかった。固体状食品のうち錠剤型食品の1回の摂取目安量あたりに含まれる大豆イソフラボン（アグリコン換算）は3～46 mgの範囲であり，このうち一品目では，分析値が表示量の2/3のものがあった。

一方，12種類の大豆イソフラボンの成分分析により，これらの食品は豆乳型，大豆抽出物添加型，大豆型に分類された。このように大豆イソフラボンを含む食品は，含まれるイソフラボンの量や組成に違いがあることから，その吸収性や生物活性が食品によって異なる可能性があること，またその評価の際には，摂取する側の年齢，性別，個体特性等も考慮する必要があると考えられた。

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Original Article

Effect of soy isoflavone extract supplements on bone mineral density in menopausal women: meta-analysis of randomized controlled trials

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This study was conducted to clarify the effect of ingesting soy isoflavone extracts (not soy protein or foods containing isoflavones) on bone mineral density (BMD) in menopausal women. PubMed, CENTRAL, ICHUSHI, CNKI, Wanfang Data, CQVIP, and NSTL were searched for randomized controlled trials published in English, Japanese, or Chinese reporting the effects of soy isoflavone extracts on lumbar spine or hip BMD in menopausal women. Trials were identified and reviewed for inclusion and exclusion eligibility. Data on study design, participants, interventions, and outcomes were extracted. Eleven, seven, five, and five trials were finally selected for estimation of the effects on spine, femoral neck, hip total, and trochanter BMD, respectively. Meta-analysis including data from1240 menopausal women revealed that daily ingestion of an average of 82 (47–150) mg soy isoflavones (aglycone equivalent) for 6–12 months significantly increased spine BMD by 22.25 mg/cm^2 (95% CI: 7.62, 32.89; p=0.002), or by 2.38% (95% CI: 0.93, 3.83; p=0.001) compared with controls (random-effects model). Subgroup analyses indicated that the varying effects of isoflavones on spine BMD across trials might be associated with study characteristics of intervention duration (6 vs. 12 months), region of participant (Asian vs. Western), and basal BMD (normal bone mass vs. osteopenia or osteoporosis). No significant effects on femoral neck, hip total, and trochanter BMD were found. Soy isoflavone extract supplements increased lumbar spine BMD in menopausal women. Further studies are needed to address factors affecting the magnitudes of effect on spine and to verify the effect on hip.

Key Words: meta-analysis, isoflavones, dietary supplements, menopause, bone density

INTRODUCTION

Osteopenia and osteoporosis are major health problems in postmenopausal women, who experience a sharp decrease in estrogen concentration that leads to an increased rate of bone remodeling.[1,2] The yearly decline in bone mineral density (BMD) of the lumbar spine and hip in postmenopausal women is reported to be at least 1% and up to 2.4%.[1,3] Although hormone replacement therapy (HRT) has positive effects in increasing BMD in postmenopausal women with low bone mass,[1,4] it is associated with a higher risk of hormone-related cancer[5-7] and other unfavorable adverse events.[8,9]

Epidemiological studies indicate that women who have high soy intake have a lower risk of osteoporosis than women who consume a typical Western diet.[10-12] Consequently, many menopausal women use phytoestrogens to maintain their BMD because they are unlikely to cause the undesirable effects associated with steroid hormones.[8,13] The primary dietary phytoestrogens ingested are soy isoflavones, which have structures similar to that of estrogen.[14]

A meta-analysis of randomized controlled trials (RCTs) has estimated the effect of ingesting soy isoflavones on lumbar spine BMD.[15] This included 10 RCTs of both soy isoflavone tablets and isolated soy protein containing isoflavones, and revealed a significant increase of BMD by 20.6 mg/cm^2 (magnitude in term of percentage and effect on hip not presented) resulting from soy isoflavones. Given the result in units of mg/cm^2, whether the magnitude of increase can prevent the naturally occurring postmenopausal bone loss remains unclear. Subgroup analysis of three trials testing isoflavone tablet revealed no significant effect, however one trial testing soy isoflavone extract was mistakenly included in the isolated soy

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protein subgroup.[16] In addition, two[17] and three[18] comparisons from the same trial respectively with two and three soy isoflavone groups compared to the same control group were included simultaneously as separate studies in the meta-analysis. This is not recommended because it is considered to induce a serious unit-of-analysis problem.[19] Another recently published meta-analysis included 10 RCTs of soy isoflavones supplementation of at least one year duration (four RCTs testing isoflavones extracts), and did not find significantly beneficial effects of soy isoflavones on spine and hip BMD.[20]

Supplements of soy isoflavone extracts were easily ingested by the people who want to benefit from soy isoflavones, but are unable to usually consume and/or do not like to intake products of soy protein or soy foods containing isoflavones. In addition, the beneficial effects of soy protein might require synergistic reactions between isoflavones and other soy components.[15] Thus, clarifying the effects of extracted soy isoflavones (not as a constituent part in soy protein) is of more clinically important. However, both the two meta-analyses failed to reveal significant effects of soy isoflavone extracts in subgroup analysis, which might be due to the fact that only data from four RCTs were included.[15,20] We have identified 12 RCTs of soy isoflavone extracts (not of soy protein or foods containing isoflavones) that reported effects on spine BMD in menopausal women,[8,16-18,21-29] and performed the present meta-analysis to clarify the effects of soy isoflavone extract both in terms of change (mg/cm^2) and percentage change (%) from baseline for lumbar spine and hip BMD, without influence on the same parameters by soy protein per se or other components in soy protein.

MATERIALS AND METHODS

PubMed (1966–2008), CENTRAL (1966–2008), ICHUSHI (1983–2008), and CNKI (1979–2008) were searched for relevant studies that had been published by September 2008. We also searched Wanfang Data, CQVIP and NSTL, which are other major search engines in China. Reference lists of relevant studies were manually searched. Studies were eligible for inclusion if they met all of the following criteria: (1) randomized parallel-group controlled trials published in English, Japanese, or Chinese; (2) trials with a crossover design that contained data for the first period;[19,30] (3) tested the effects of ingesting supplements of soy isoflavone extracts (not of soy protein or foods containing isoflavones) on lumbar spine or hip (femoral neck, total hip, or trochanter) BMD in menopausal women; and (4) BMD data were measured by dual X-ray absorptiometry. When duplicate data were reported for the same study subjects, only the article with the largest sample was included.[19] Two reviewers independently reviewed and evaluated the studies, and consensus was reached by discussion when there were disagreements.

Data on study design, number of participants, interventions, and outcomes for BMD were also independently extracted by two reviewers and confirmed by each other. When necessary, data on outcomes for BMD were obtained from graphs. If possible, we obtained necessary data not reported in the articles by contacting to the authors. We calculated mean change (follow-up − baseline) and percentage change [(follow-up − baseline) ÷ baseline × 100%] from baseline in BMD, when the data were not directly available. We primarily determined missing SD of the changes if statistical analyses comparing the changes themselves were presented (e.g., confidence intervals, standard errors, t values, p values, F values). Alternatively, we imputed them by computing mean correlations between the baseline and final values from included trials in which SD for change, as well as for baseline and final measurements were available.[19] Standard deviation for percentage change was calculated by dividing SD for change with mean baseline value.

We used the Jadad scale to assess the quality of included RCTs, a score of < 3 indicating low quality.[31] We also used a 3-category grading system (A, B, C) to denote the methodological quality of each study.[32] Category A studies have the least bias and results are considered valid; B studies are susceptible to some bias, but not sufficient to invalidate the results; and C studies have significant bias that may invalidate the results. We arbitrarily defined category C as of low quality. Concealment of treatment allocation in RCTs was assessed as adequate, inadequate or unclear.[33] Two reviewers independently assessed the studies, and consensus was reached by discussion when there were disagreements.

We performed meta-analysis to determine the overall treatment effect of soy isoflavones on BMD, using the weighted mean difference method in Review Manager (version 5.0.20; Nordic Cochrane Center, Oxford, England). Treatment effect of each trial was estimated as the mean difference between changes (or percentage changes) from baseline in BMD for each comparison group (i.e., the change from the baseline for participants ingesting soy isoflavones minus that for controls). When data of more than one time points for the same trial were reported in one article or reported separately in two articles, we primarily used the data set for the short duration in order not to induce unit-of-analysis error. The data set for other time points were used for sensitivity analysis to prevent reporting bias. For trials had more than one isoflavone group compared with one control group, we combined the multiple isoflavones groups into a single group for each of these trials without inducing unit-of-analysis error.[34]

We used both a fixed effect model or a random effects model to calculate weighted mean differences (WMD), 95% CIs for each comparison, a combined overall effect with p-value, and the p-value for testing heterogeneity ($p < 0.1$ was considered significant); when there was significant heterogeneity across included trials, the results based on the random effects model were shown.[19,30,35]

We conducted sensitivity analyses to evaluate the effects of degree of correlation between baseline and final values, time point of measurement (using data for long duration instead of data for short duration in trials with multiple time points of evaluation), study design (selecting only placebo-controlled trials), and study quality (eliminating low-quality trials). If at least 10 trials were available, subgroup analyses and meta-regressions were performed to investigate possible factors that might related to varying effects of soy isoflavones on BMD across trials, on the basis of pre-specified factors of intervention

duration, isoflavone dosage, region of participants, and basal BMD.[15,20] We used a cut-off point of 75 mg/day in subgroup analysis for isoflavone dosage, because daily isoflavone intake of up to 75 mg (aglycone form) is considered safe by the Japan Food Safety Commission. Significant tests based on test for heterogeneity, chi-squared statistics, were performed to investigate differences between two subgroups.[19,34] We examined potential publication bias by using funnel plots and by performing Egger's test to assess the asymmetry of funnel plots. Meta-regressions and Egger's test were respectively performed with the use of user-written "metareg" and "metabias" commands for Stata 10.1 for Windows (StataCorp LP, College Station, Tex).

RESULTS

The search strategy (Figure 1) yielded 16 potentially appropriate reports of RCTs to be included in the meta-analysis. After excluding one article[36] reporting only duplicate femoral data that had appeared in another article,[25] and two articles[37,38] describing a smaller sample than that analyzed in another article,[23,17] 13 articles on 12 trials were included for meta-analysis.[8, 16-18, 21-29] Two articles reported outcomes for durations of six months[27] and one year[28] for the same trial participants.

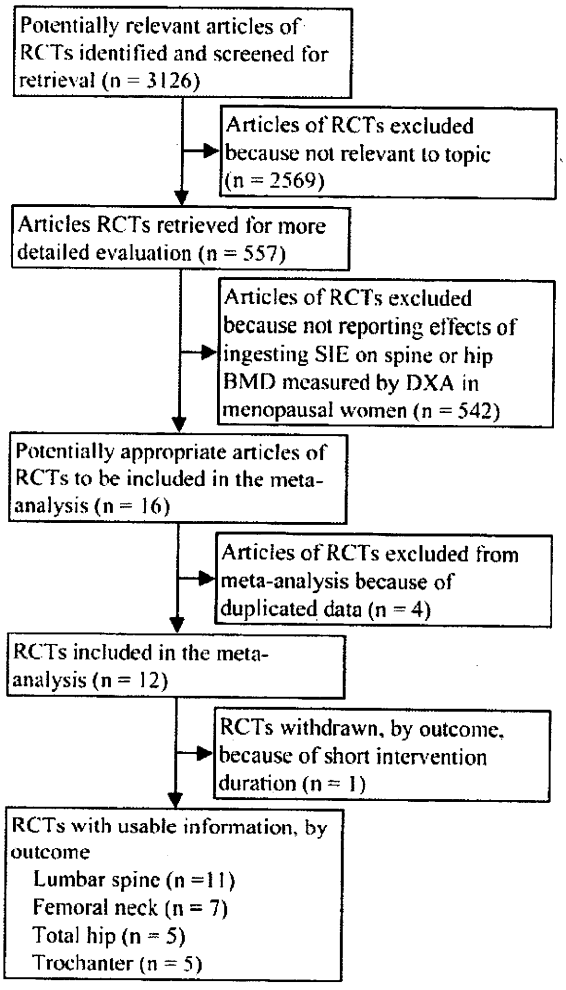

Figure 1. Search and selection of trials. Abbreviations: RCTs, randomized controlled trials; BMD, bone mineral density; SIE, soy isoflavone extracts, DXA, dual X-ray absorptiometry.

The characteristics of 12 trials are summarized in Table 1. Two articles for each trial contained data for two time points.[21,25] Three trials tested two isoflavone groups[17,22,24] and one tested three isoflavone groups[18] compared with one identical control group. One trial did not address the form and composition of soy isoflavones tested,[18] we assumed the dose as aglycone equivalent to calculate the mean dosage. Four, six, and two trials included participants of normal bone mass (T-score > –1 SD, corresponds to BMD > 0.937g/cm^2), low bone mass or osteopenia (–1 SD ≥ –2.5, corresponds to 0.937 g/cm^2 ≥ BMD ≥ 0.772 g/cm^2), and osteoporosis (T-score < –2.5 SD, corresponds to BMD < 0.772 g/cm^2) on the basis of averaged basal spine BMD, respectively.[39] Only one trial was assessed as "adequate" for concealment of treatment allocation,[22] and the remaining trials were assessed as "unclear" due to insufficient information. Participants in the comparison groups had similar dietary intakes of soy isoflavones, calcium, and vitamin D and physical activities. Most of the studies were designed to maintain the participants' usual diets, lifestyle and body weight. Adverse events were generally similar for both the isoflavone and control groups and no serious adverse events were noted in the included trials, although they were not well addressed in several trials.

Because bone is a slowly responding organ, a complete bone remodeling cycle takes up to 6 months, and therefore a study duration of less than 6 months is not sufficient to evaluate the effect of any intervention on bone BMD.[28] Thus, one 3-month trial of low-quality that reported negative effect of soy isoflavones on spine BMD was then withdrawn.[26] From 3126 relevant articles identified, 11,[8,16-18,21-25,27-29] 7,[8,17,22-25,27,28] 5,[16,17,22,27-29] and 5[17,22-24,27,28] trials were finally selected for estimating the effects on lumbar spine, femoral neck, total hip, and trochanter BMD, respectively (Figure 1, Table 1). Fourteen correlation coefficients between baseline and follow-up values were calculated from 5 reports of 4 trials,[17,23,24,27,28] which were consistent and resulted in a mean value of 0.98 (0.96–1).

Meta-analysis of the 11 trials with 1240 participants using the fixed effect model resulted in significant heterogeneity ($p < 0.001$), and revealed that daily ingestion of an average of 82 (47–150) mg (aglycone equivalent) soy isoflavones for 6 months to one year significantly increased lumbar spine BMD by 12.08 mg/cm^2 (95% CI: 9.83, 14.33 mg/cm^2; $p < 0.001$), or by 1.47% (95% CI: 1.21, 1.74%; $p < 0.001$) compared with controls. Meta-analysis using the random effects model, revealed an significant overall effect of soy isoflavones in increasing spine BMD by 20.25 mg/cm^2 (95% CI: 7.62, 32.89 mg/cm^2, $p = 0.002$), or by 2.38% (95% CI: 0.93, 3.83%, $p = 0.001$; Figure 2). Of the11 selected trials, 7 trials revealed significant positive mean difference between changes or percentage change from baseline in spine BMD for isoflavone and control groups (favors isoflavone). The mean difference was negative at 27-week time point and was positive at 53-week time point in one trial,[21] the mean difference at 2-year duration was about two times of that at 1-year time point;[25] whereas, the

Table 1. Characteristics of included randomized controlled trials

Study	Design[†]	Follow-up	Participants[‡]	Intervention[§]	Baseline mean BMD outcomes (g/cm^2)[¶]	Jadad scale	Quality category
Brink 2008[21]	P; R, DB+, WD	27, 53 wk	N: 300/237 (21%) PoW; mean age: 53 y; TSM = 33 (12–60) mo; non-osteoporotic (spine Z-score ≥ 2); Netherlands, Italy, France	110 mg IAE [25–35% De, 60–75% Ge, 1–5% Gle] vs. placebo	L1–4: 0.990, mean (SD) T-score = −0.0 ± 1.1	4	C (dropout > 20%)
Chen 2003[22]	P; R+, DB+, WD	1 y	N: 203/175 (14%) PoW; mean age: 54.2 y; TSM = 4.1 (1–10) y; Hong Kong	40 and 80 mg IAE [46% De, 15% Ge, 39% Gle] vs. placebo	L1–4: 0.860; FN: 0.682; TH: 0.819; Tr: 0.605	5	A
Dong 2008[23]	P; R, WD	12 mo	N: 60/52 (13%) PoW; mean age: 54.7 y, TSM = 6.2 (≥ 1) y; T-score < −1.5 China	100 mg IC [66 mg IAE: 39% De, 61% Ge, 1% Gle] + calcium vs. calcium only (control)	L2–4: 0.756; FN: 0.719; Tr: 0.552	2	B
Gao 2006[18]	P; R	24 wk	N: 50/50 PoW; age: 48–62 y; TSM ≥ 1y; China	60, 90, and 150 mg IF vs. no-treatment (control)	L1–4: 0.974	1	B
Harkness 2004[16]	CO; R+, DB, WD	6 mo × 2	N: 20/19 (5%) PoW; mean age: 70.6 y; TSM = 19.1 (> 8) y; T-score < 2.5; USA	110 mg IAE [40% De, 52% Ge, 9% Gle] vs. placebo	L1–4: 0.881; TH: 0.800	4	B
Huang 2006[24]	P; R, OL, WD	1 y	N: 43/42 (2%) PoW; mean age: 52.4 y; TSM = 4.4 (1–13) y; Taiwan	100 and 200 mg IAE [29% De, 71% Ge] vs. regular diet only (control)	L1–4: 0.881; FN: 0.812; Tr: 0.715	2	B
Marini 2007[25]	P; R+, DB+, WD	12, 24 mo	N: 389/389 (10, 22%) PoW; mean age: 54.5 y; TSM = 63 mo (≥ 1 y); femoral neck BMD < 0.795 g/cm^2 (−1.0 T-score); Italy	54 mg pure Ge vs. placebo	L: 0.840; FN: 0.670	5	A, C (dropout > 20%)
Morabito 2002[8]	P; R, DB+	1 y	N: 90/90 PoW; mean age: 51.5 y; TSM = 6.5 (≥ 1) y; femoral neck BMD < 0.795 g/cm^2 (−1.0 T-score); Italy	54 mg pure Ge vs. placebo	L: 0.925; FN: 0.688	3	A
Uesugi 2003[26]	P; R, WD	3 mo	N: 22/21 (4%) PoW; mean age: 53.7 y; TSM = 6 (5–10) y; non-osteoporosis; Japan	62 mg IC [38 mg IAE: 52% De, 11% Ge, 37% Gle] vs. placebo	L2–4: 1.040	2	C (unclear analyzed N)
Wu 2006a[27], b[28]	P; R, DB+, WD	6, 12 mo	N: 136/128, 108 (6, 21%); mean age: 54.4 y; TSM = 3.2 (1–5) y; Japan	75 mg IC [47 mg IAE: 54% De, 13% Ge, 34% Gle] vs. placebo	L2–4: 0.899; FN: 0.672; TH: 0.782; Tr: 0.595	4	A, C (dropout > 20%)
Xin 2006[29]	P; R, DB	6 mo	N: 76 MW; age: 45–55 y; TSM ≤ 5 y; China	50 mg pure De + calcium vs. calcium only (control)	L2–4: 0.715; TH: 0.643	2	C (unclear analyzed N)
Ye 2006[17]	P; R+, SB, WD	6 mo	N: 90/84 (7%) PoW; mean age: 52.3 (1–5) y; TSM = 2.6 (1–5) y; China	84 and 126 mg IAE [52% D(e), 15% G(e), 33% Gl(e)] vs. placebo	L1–4: 0.864; FN: 0.702; TH: 0.800; Tr: 0.588	3	B

[†]CO, crossover, DB, double-blinded (gives 1 point to Jadad scale); DB+, double-blinded by appropriate method (gives 2 point); OL, open-labeled; P, Parallel; R, randomized (give 1 point); R+, randomized by appropriate method (gives 2 point); SB, single-blinded; WD, withdrawals and dropouts described (gives 1 point).
[‡]BMD, bone mineral density; N, randomize/analyzed number (dropout rate) of participants; MW, menopausal women; PoW, postmenopausal women; TSM, averaged time since menopause.
[§]IAE, isoflavone aglycone equivalents; IC, isoflavone conjugate containing glycoside and aglycone forms; IF, isoflavones (form and composition unknown); D(e), daidz(e)in; De, daidzein; Ge, genistein; G(e), genist(e)in; Gl(e), glycit(e)in; Gle, glycitein.
[¶]FN, femoral neck, L, lumbar spine; TH, total hip; Tr, trochanter.

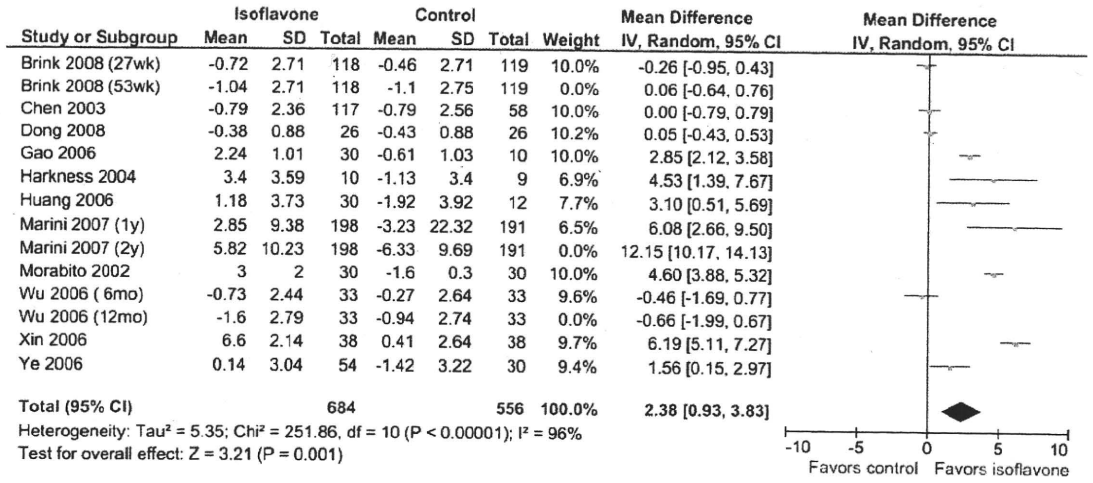

Figure 2. Effects of soy isoflavones on spine BMD (%). Mean Difference, weighted mean difference between percentage changes (%) of spine bone mineral density (BMD) from baseline for isoflavone and control groups; random, random effects model. Horizontal lines denote the 95% CI. Data sets for long duration not included in the meta-analysis were signed 0.0% Weight. ■ Point estimate (size of the square corresponds to its weight); ♦ Combined overall effect.

mean difference for 6 months duration[27] was similarly negative to that for 1 year duration.[28]

Sensitivity analyses assuming the level of correlation coefficient between baseline and follow-up values to be 0.75 and 0.5, using data sets of longer duration instead of short duration for trials with two time points of measurements, selecting only placebo-controlled trials, and eliminating low-quality trials (Jadad scale < 3 or Category C) did not result in significantly different overall effects of soy isoflavones on spine BMD.

Results of subgroup analyses of the effects of soy isoflavones on spine BMD were shown in Table 2. Each subgroup analysis resulted in significant heterogeneity and revealed significant effect of soy isoflavones in increasing spine BMD compared with controls using the fixed effect model. Results based on fixed effect model revealed that effects of soy isoflavones on spine BMD in subgroups of 6 months duration and of Asian region were significantly different with the effects in subgroup of 1 year duration and of Western region, respectively. Two subgroups of each subgroup analysis using the random effects model, show similarly significant effects of soy isoflavones in increasing spine BMD, except for a subgroup of participants with normal bone mass at baseline. Meta-regressions analyzing each of or all of the four pre-specified categorical study characteristics (intervention duration, isoflavone dosage, region of participants, and basal spine BMD), did not reveal that these pre-specified factors were significantly associated with the varying effects of soy isoflavones on spine BMD across trials. The funnel plots (Figure 3) and Egger's test of effects of soy isoflavones on spine BMD among the 11 trials (p = 0.251 and p = 0.267 for effects in terms of change and percentage change, respectively) did not indicate any obvious publication bias.

Meta-analysis of the 7 trials with 868 participants using the fixed effect model resulted in significant heterogeneity ($p < 0.001$). Meta-analysis using the random effects model, revealed that daily ingestion of an average of 76 (47–150) mg (aglycone equivalent) soy isoflavones for 6 months to one year non-significantly increased femoral neck BMD by 10.24 mg/cm^2 (95% CI: −3.73, 24.20 mg/cm^2, p = 0.15), or by 1.48% (95% CI: −0.54, 3.50%, p = 0.15) compared with controls. Sensitivity analysis assuming the level of correlation coefficient between baseline and follow-up values to be 0.75 and 0.5, did not result in significantly different overall effects of soy isoflavones on femoral neck BMD. Whereas, sensitivity analysis using data sets of longer duration for trials with two time points of measurements, found that ingestion of soy isoflavones for 6 months to 2 years tended to increase femoral neck BMD by 16.89 mg/cm^2 (95% CI: −2.34, 36.11 mg/cm^2, p = 0.09), or by 2.45% (95% CI: −0.31, 5.21, p = 0.08; Figure 4) compared with controls (random effects model). Sensitivity analyses selecting only placebo-controlled trials and eliminating low-quality trials were not performed because of the small number of available trials.

Meta-analysis of the 5 trials with 420 participants using the fixed effect model resulted in non-significant heterogeneity ($p \geq 0.1$), revealed that daily ingestion of an average of 74 (47–110) mg (aglycone equivalent) soy isoflavones for 6 months to one year non-significantly change total hip BMD by 2.45 mg/cm^2 (95% CI: −1.41, 6.30 mg/cm^2, p = 0.21), or by 0.05% (95% CI: −0.53, 0.63%, p = 0.86) compared with controls. Sensitivity analyses assuming the level of correlation coefficient between baseline and follow-up values to be 0.75 and 0.5 and using data sets of longer duration for trials with two time points of measurements, did not result in significantly different overall effects of soy isoflavones on total hip BMD.

Meta-analysis of the 5 trials with 419 participants revealed that daily ingestion of an average of 85 (47–150) mg (aglycone equivalent) soy isoflavones for 6 months to one year non-significantly change trochanter BMD by −0.40 mg/cm^2 (95% CI: −6.58, 5.78 mg/cm^2, p = 0.90), or by −0.07% (95% CI: −1.15, 1.02%, p = 0.91) compared with controls (random effects model). Sensitivity analyses assuming the level of correlation coefficient between