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.

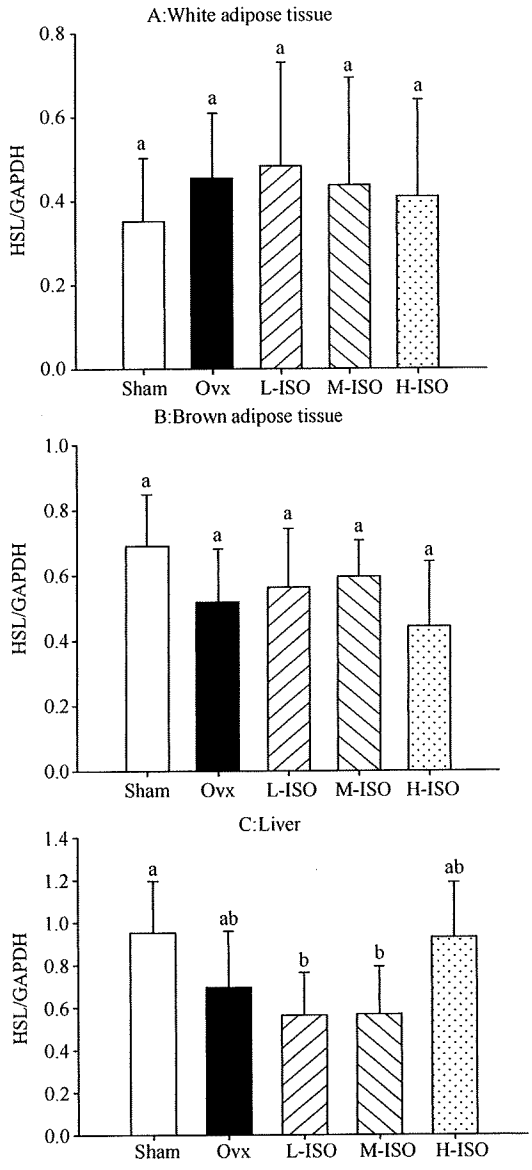

FIG. 3. Effect of isoflavone on HSL mRNA expression in white adipose tissue (A), brown adipose tissue (B), and liver (C). Animal groups comprised the sham-operated controls (Sham), ovariectomized controls (OVX), and OVX rats treated with different doses of isoflavone (L-ISO, M-ISO, and H-ISO). The means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

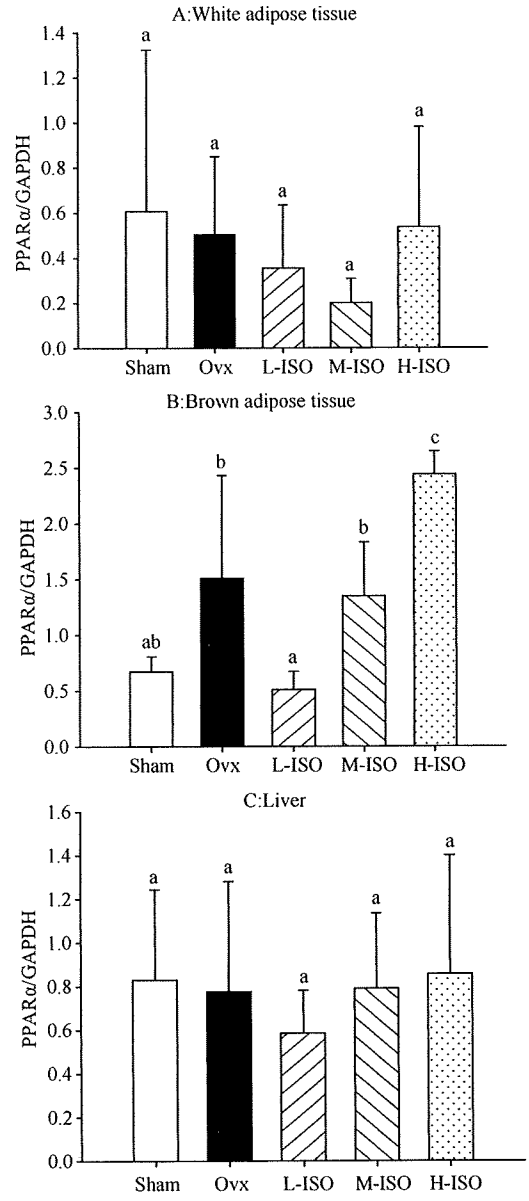

FIG. 4. Effect of isoflavone on PPARα mRNA expression in white adipose tissue (A), brown adipose tissue (B), and liver (C). Animal groups comprised sham-operated controls (Sham), ovariectomized controls (OVX), ovariectomized rats treated with different doses of isoflavone (L-ISO, M-ISO, and H-ISO). The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

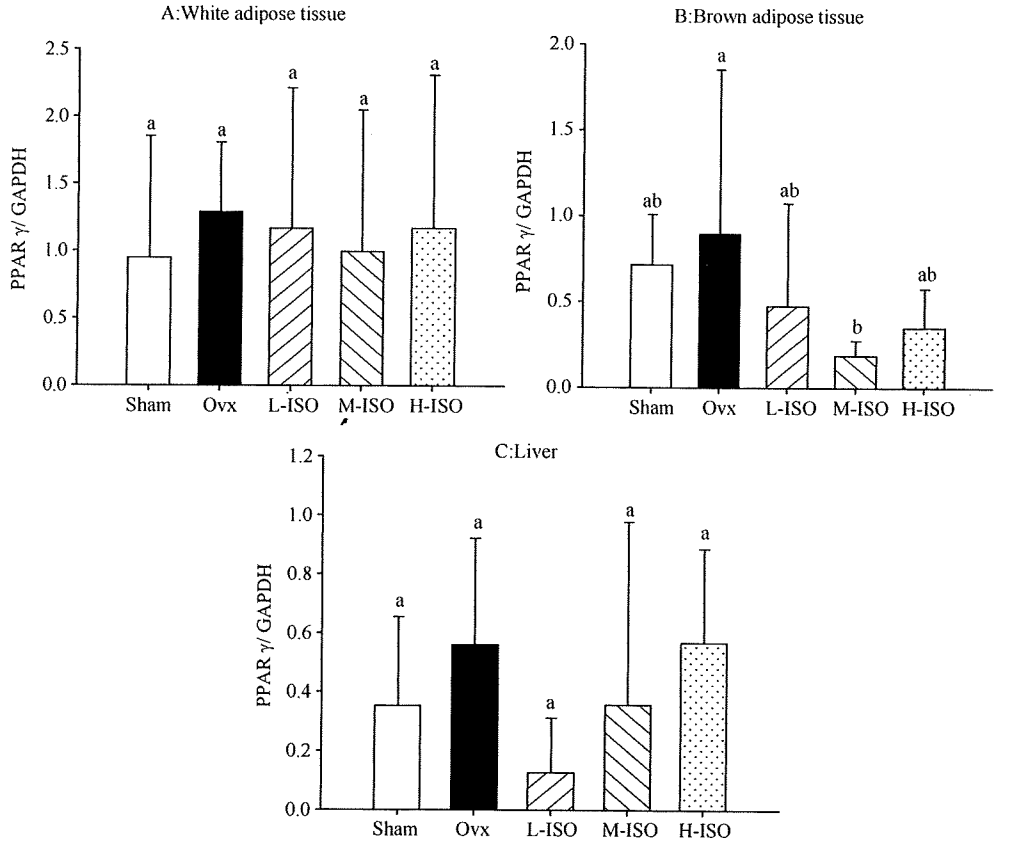

FIG. 5. Effect of isoflavone on PPARγ mRNA expression in white adipose tissue (A), brown adipose tissue (B), and liver (C). Animal groups comprised the sham-operated controls (Sham), ovariectomized controls (OVX), OVX rats treated with different doses of isoflavone (L-ISO, M-ISO, and H-ISO).

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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