Letter to the Editor

Effect of Soy Isoflavone Crude Extract Supplementation on High Fat Diet-induced Insulin Resistance in Ovariectomized Rats*

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Female Wister rats aged 8 weeks were randomly divided into sham operation group, ovariectomized (OVX) control group, and 2 OVX groups fed with soy isoflavone crude extract supplementation. The rats had free access to high fat diet and water for 9 weeks. No significant difference was found in body weight (BW), total abdominal fat, food intake and food utilization rate between OVX control group and 2 OVX groups. However, the fasting blood glucose and blood lipid levels were significantly higher in 2 OVX groups than in OVX control group (*P*<0.05). Intraperitoneal glucose tolerance test (IGTT) showed that the area under AUC was smaller in 2 OVX groups than in OVX control group (*P*<0.05). These findings showed that soy isoflavone crude extract supplementation can improve glucose tolerance and prevent high fat diet-induced insulin resistance in ovariectomized rats.

Cardiovascular diseases (CVD), especially coronary heart disease (CHD) and stroke, are the leading causes of death in women after menopause. Insulin resistance (IR) may contribute to the high risk of accelerated CVD[1].

Several factors are involved in the pathogenesis of IR, such as dyslipidemia and altered glucose and insulin homeostasis. Postmenopausal hormone therapy exerts some beneficial effects on glycemic and lipid metabolism, but many postmenopausal women are either unwilling or unable to undergo hormone replacement therapy. It is therefore important to find alternative strategies for the increased risk of cardiovascular events occurred in women after menopause[2].

Isoflavones with a similar molecular structure to estrogen can effectively reduce the CVD risk in postmenopausal women. Choi et al.[3] found that isoflavones play an important role in the regulation of glucose homeostasis in type 1 diabetic mice. Cross-sectional study showed that the body mass index (BMI) and fasting insulin level are lower in postmenopausal women who consume a high soy diet than in those who consume no soy diet[4].

Several longitudinal studies examined the relationship between soy isoflavone supplementation and CVD risk factors, such as body weight (BW), lipid and glucose metabolism, but their results are controversial. Double-blind, cross-over, placebo-controlled studies showed that the BW, fasting blood glucose and insulin level are lower in postmenopausal women taking soy isoflavone supplementation than in those who take no soy isoflavone supplementation.

Our prior study displayed that soy isoflavone crude extract (SIF) supplementation can reduce BW, total abdominal fat, food intake, food utilization rate and plasma insulin level in healthy ovariectomized rats[5]. However, the effect of SIF supplementation on CVD risk factors is unknown in insulin-resistant ovariectomized rats. This study was to determine the effect of SIF supplementation on IR using a well-established animal model of menopause and to assess its other effects on menopause.

In this study, high fat diet (HFD) was used to induce IR as previously described. As shown in Figure 1, HFD increased the BW in all groups. The BW was significantly higher in ovarietomized groups than in sham operation group (*P*<0.05). However, no significant difference was found in BW between SIF supplementation groups and OVX control group. Similar trends were found in parametrial and perirenal fat weight (data not shown). Previous study revealed that 2-week genistein treatment (250 mg/kg BW/d) can significantly decrease the BW and fat pad weight, and increase the insulin levels in OVX SD rats[6]. However, such phenomina are not found in the present study because the dos- age in this study was too low to reduce the BW[5,7], a SIF

doi: 10.3967/ bes2014.014

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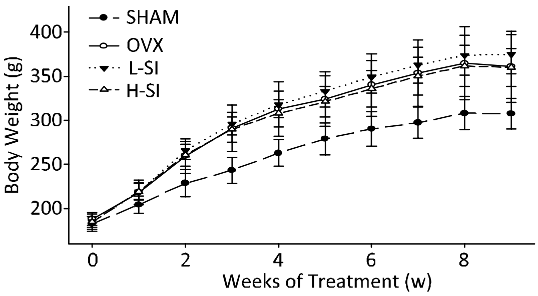

Figure 1. Effect of SIF on BW of ovariectomized adult rats with their age >9 weeks.

Table 1. Effect of SIF Supplementation on Blood Lipid Levels (X±S, mmol/L)

Groups	TC	TG	LDL	HDL
SHAM	0.99±0.17[a]	0.62±0.09[a]	0.06±0.07[a]	0.88±0.12[a]
OVX	1.55±0.11[b]	0.56±0.09[a,c]	0.21±0.05[b]	1.05±0.12[b]
L-SI	1.30±0.17[c]	0.48±0.08[b,c]	0.08±0.07[a]	0.90±0.13[a,b]
H-SI	1.39±0.10[c]	0.45±0.07[b]	0.18±0.07[b]	0.95±0.09[a,b]

Note. Values in the same column with different superscripts are significantly different at $P<0.05$ by LSD test.

mixture containing 60% of other components might attenuate the effect of SIF on BW, and Wistar rats rather than SD rats might lead to differences in strain.

The daily food intake and food utilization rate were significantly higher whereas the uterine weight was significantly lower in OVX groups than in sham operation group ($P<0.05$). No significant difference was found in daily food intake and food utilization rate between SIF supplementation groups and OVX control group and in uterine weight between OVX control group, L-SI and H-SI groups, indicating that isoflavone has no estrogen-like effect in insulin-resistant ovariecto- mized rats. One-way ANOVA indicated that SIF supplementation had no effect on weights of other organs, such as liver, kidney, spleen, and brain ($P>0.05$, data not shown).

SIF supplementation improved the blood lipid level in insulin-resistant ovariectomized rats. The TC level was lower in SIF supplementation groups than in OVX control group ($P<0.05$). The TG level was 14.3% and 19.6% lower in SIF supplementation groups than in L-SI group and H-SI group ($P<0.05$). The LDL level was significantly lower in SIF supplementation groups than in OVX control group ($P<0.05$, Table 1). It has been generally accepted that dyslipidemia is one of the major risk factors for CVD and atherosclerosis. Many studies investigated the effect of soy isoflavone supplementation on blood lipid levels, but their findings are controversial. It was reported that SIF supplementation has little or no effect on serum TC, TG, HDL, and LDL levels[8]. However, it has been shown that SIF supplementation can decrease the serum TC and LDL levels in humans[9]. In the present study, either low- or high-dose SIF supplementation significantly reduced the serum TC, TG, and LDL levels, indicating that SIF supplementation can effectively reduce the blood lipid level. Furthermore, SIF supplementation did not reduce the LDL level in a dose-dependent manner. Soybean isoflavone is a plant estrogenic hormone. Active estrogenic hormone has a bidirectional regulatory effect and can thus balance the estrogenic hormone, suggesting that low dose SIF supplementation can more effectively reduce the LDL level in ovariectomized rats. Future study is needed to determine the effect of SIF supplementation on blood lipid levels in men and women.

The effect of SIF supplementation on fasting blood glucose and insulin levels in insulin-resistant ovariectomized rats is shown in Table 2. Although no difference was found in baseline fasting plasma glucose levels, substantial differences occurred at week 9. The blood glucose level was significantly higher in OVX control group than in sham operation group at week 9 ($P<0.05$). Furthermore, the blood glucose level was significantly lower in L-SI group and H-SI group than in the OVX control group ($P<0.05$). The plasma insulin level measured by radioimmunoassay was higher in the rats 9 weeks after ovariectomy than before ovariectomy. The plasma insulin level was 3.70% and 14.8% lower in L-SI group and H-SI group than in OVX control group ($P=0.867$, $P=0.608$).

Since the glucose metabolism decreases in ovariectomized rats with their age, the prevalence of diabetes mellitus and impaired glucose tolerance increases gradually[10]. It has been shown that the fasting glucose and insulin levels are significantly improved in postmenopausal women taking 54 mg genistein daily 12 and 24 months after treatment[11]. In the present study, the blood glucose level was lower in SIF supplementation group than in OVX control group after fed with HFD for 9 weeks, suggesting that SIF supplementation may mitigate

Table 2. Effects of SIF Supplementation on Blood Glucose and Insulin Levels (X±S)

Groups	Glucose (0W, mmol/L)	Glucose (9W, mmol/L)	Insulin (ng/mL)
SHAM	5.5±0.5[a]	5.8±0.4[a]	0.31±0.11[a]
OVX	5.7±0.4[a]	6.3±0.2[b]	0.54±0.16[a]
L-SI	5.6±0.4[a]	6.0±0.2[a]	0.52±0.36[a]
H-SI	5.5±0.2[a]	5.9±0.3[a]	0.46±0.20[a]

***Note*.** Values in the same column with different superscripts are significantly different at *P*<0.05 by LSD test.

HFD-induced IR in postmenopausal or ovariectomized animals.

IGTT was performed in the animals after overnight fasting with an intraperitoneal injection of glucose (2 g/kg BW) 9 weeks after fed with HFD or HFD plus isoflavone, showing impaired glucose/insulin tolerance in the rats. The effect of SIF supplementation was assessed. IGTT also revealed that the blood glucose level after glucose injection was elevated rapidly and reached its peak at 30 min, then returned gradually to its baseline level. The blood glucose level after injection of glucose was higher in ovariectomized groups than in sham operation group, indicating that the glucose tolerance is lower in ovariectomized rats than in normal controls. Furthermore the area under the AUC was smaller in SIF supplementation groups than in OVX control group. Although the difference was not significant, SIF supplementation tended to protect rats against IR.

[*]This work was supported by the National Natural Science Foundation of China (30972439), Bureau of Health Foundation, Heilongjiang Province (2006-300), (2012-768) and Youth Fund of School of Public Health, HMU.

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Received: July 27, 2013;

Accepted: October 24, 2013

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