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# Effects of soy isoflavone supplementation on plasma glucose, lipids, and antioxidant enzyme activities in streptozotocin-induced diabetic rats

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**Abstract**

This study was designed to examine the effects of feeding diets containing different levels of isoflavone on plasma glucose, insulin concentrations, and lipid profiles as well as tissue antioxidant enzyme activities in diabetic rats. Diabetes was induced in the rats with streptozotocin. Diabetic rats were further assigned to 1 control group and 3 experimental groups (ISO-1, ISO-2, and ISO-8). The control group received a casein-based diet without isoflavone, whereas the ISO-1, ISO-2, and ISO-8 groups received a similar diet but supplemented with 1, 2 and 8 times of isoflavone equivalent of normal human consumption as suggested by the manufacturer. All diets were adjusted to contain identical nutrients and were maintained for 24 days. Fasted and non-fasted blood was drawn after feeding for 21 and 24 days, respectively, and blood chemistry was analyzed. The liver, lung, and kidney were excised after sacrifice, and antioxdiant enzyme activities and lipid peroxidation products were measured. The results demonstrate that there were no differences in plasma glucose or insulin levels among groups, irregardless of whether rats had fasted or not. However, hemoglobin $A_{1c}$ tended to be lower in the ISO-2 group than in the control and the ISO-1 groups. Plasma total cholesterol and low-density lipoprotein-cholesterol were significantly lower in the ISO-8 group than in the other groups. No differences in plasma triglyceride or high-density lipoprotein-cholesterol were observed among groups in the non-fasting state. There were no significant differences in superoxide dismutase, glutathione peroxidase activities, and malondialdehyde concentrations in liver, lung, and kidney homogenates among groups. These results suggest that 3 doses of isoflavone supplementation had no favorable effect on plasma glucose or insulin concentrations, nor had any influence on attenuating

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oxidative stress in diabetic rats. However, the ISO-2 group tended to have better chronic glycemic control than did the control and the ISO-1 group. In addition, a larger amount of isoflavone supplementation had beneficial effects on reducing plasma total cholesterol and low-density lipoprotein-cholesterol levels. 

*Keywords:* Isoflavone; Diabetes; Plasma lipid profiles; Antioxidant enzymes; Lipid peroxidation products

## 1. Introduction

Isoflavones are present in relatively large amounts in virtually all products containing soy protein. Many studies have shown that isoflavones have various physiological effects which are relevant to human health [1]. A number of human and animal studies have shown that consumption of products containing soy protein reduces plasma total cholesterol and low-density lipoprotein (LDL)-cholesterol concentrations [2–5]. Recently, some studies have suggested that the components of soy protein that lower lipid concentrations are the isoflavones—genistein and daidzein [2,4]. In addition to the hypocholesterolemic effect, flavonoids in legumes and soybean products have demonstrated antioxidant activity in a variety of in vitro assay systems [6]. Hodgson et al. [7] demonstrated that the major isoflavonoids, genistein and daidzein, and the metabolic products of daidzein metabolism inhibit lipoprotein oxidation in vitro, and Vinson et al. [8] reported that genistein can inhibit the oxidative modification of isolated LDL.

Diabetes mellitus (DM) is a major source of morbidity in developed countries. It is a metabolic disorder caused by an absolute or relative lack of insulin. The metabolism of all fuels including carbohydrates, fats, and proteins are altered, and patients with diabetes have lipoprotein disorders and an increased risk of coronary heart disease, peripheral vascular disease, and cerebrovascular disease [9–11]. Many studies have shown that increased lipid peroxides and/or oxidative stress are present in diabetic subjects, and may underlie their increased risk of cardiovascular disease [12–16]. Attention has been focused on possible interventions to decrease levels of oxidative stress, such as improved glycemic control, and drug or antioxidant ($\alpha$-tocopherol) therapy [17–19]. To our knowledge, there is no study investigating the effects of isoflavone supplementation on glycemic control and antioxidant status in DM. Therefore, the present investigation was undertaken with the objective of examining the effects of feeding diets containing different levels of isoflavone on plasma glucose, lipid profiles, and insulin concentrations as well as tissue antioxidant enzyme activities in DM rats. STZ-induced diabetic rats were used in this study. This model is frequently used to simulate non-insulin-dependent DM in animal studies [20,21].

## 2. Materials and methods

### *2.1. Animals and operation procedures*

Thirty-four male Wistar rats were used in this study. All rats were housed in temperature- and humidity-controlled rooms, and allowed free access to a standard rat chow for 1 week

Table 1
Composition of the Experimental Diet (g/100g)

| Component | Control | ISO-1 | ISO-2 | ISO-8 |
|---|---|---|---|---|
| Casein | 20 | 19.5 | 19 | 16.1 |
| Corn starch | 62.0 | 61.6 | 61.3 | 59.3 |
| Soybean oil | 10 | 9.9 | 9.7 | 9.0 |
| Cholesterol | 0.1 | 0.1 | 0.1 | 0.1 |
| Salt mixture[a] | 4.0 | 4.0 | 4.0 | 4.0 |
| Vitamin mixture[b] | 1.0 | 1.0 | 1.0 | 1.0 |
| Methyl cellulose | 3.5 | 3.5 | 3.5 | 3.5 |
| Choline chloride | 0.1 | 0.1 | 0.1 | 0.1 |
| DL-methionine | 0.3 | 0.3 | 0.3 | 0.3 |
| Soylife[#] | 0 | 12 | 24 | 96 |
| Isoflavone (mg) | 0 | 240 | 480 | 1920 |

[a] The salt mixture contains the following (mg/g): calcium phosphate diabasic 500, sodium chloride 74, potassium sulphate 52, potassium citrate monohydrate 220, magnesium oxide 24, manganese carbonate 3.5, ferric citrate 6, zinc carbonate 1.6, cupric carbonate 0.3, potassium iodate 0.01, sodium selenite 0.01, and chromium potassium sulphate 0.55.

[b] The vitamin mix contains the following (mg/g): thiamin hydrochloride 0.6, riboflavin 0.6, pyridoxine hydrochloride 0.7, nicotinic acid 3, calcium pantothenate 1.6, D-biotin 0.02, cyanocobalamin 0.001, retinyl palmitate 1.6, DL-(-tocopherol acetate 20, cholecalciferol 0.25, menaquinone 0.005.

[#] Soylife contains: crude protein 0.405 g/g, fat 0.112 g/g, carbohydrate 0.282 g/g, fiber 0.039 g/g, total isoflavone 20.8 mg/g, and $\alpha$-tocopherol 0.081 mg/g according to the manufacturer.

prior to the experiment. After that, diabetes was induced in the rats by a single tail vein injection of streptozotocin (STZ, Sigma Chemical, St Louis, MO) at a dose of 60 mg/kg BW [22]. STZ was dissolved immediately before use in 0.05 mol/L sodium citrate (pH 4.5) [21]. Three days later, blood was drawn from the tail vein, and rats were considered diabetic only if their blood glucose levels exceeded 250 mg/dl [23]. Diabetic rats were not treated with insulin in this study.

The diabetic rats were assigned to 4 groups according to the weight and blood glucose of each animal to make average weights and blood glucose levels among groups as similar as possible. The control group received a casein-based control diet without isoflavone, whereas animals belonging to the ISO-1, ISO-2, and ISO-8 groups received a similar diet but supplemented with different levels of isoflavone. Rats in the ISO-1 group were supplemented with an amount that was equivalent to normal human consumption as suggested by the manufacturer. Rats in the ISO-2 and ISO-8 groups were supplemented with 2 and 8 times the isoflavone used in the ISO-1 group to evaluate the possible additive or adverse effect of isoflavone supplementation. All diets were adjusted to contain identical nutrients so that the effect of increasing amounts of isoflavone could be evaluated (Table 1).

Diets were fed to the animals ad libitum for 24 days. Records of daily food intake and weekly body weight changes of individual animals were maintained throughout the experiment. After 3 weeks, rats were fasted overnight, and blood was drawn from a tail vein after anesthetization with ether, to measure differences in blood chemistry among groups under a fasting condition. Three days later, non-fasting rats were sacrificed by drawing arterial blood from the aorta of the abdomen to compare plasma glucose, lipid profiles, and blood

Table 2
Initial and final body weight of the control and experimental groups

| | Control (10)[a] | ISO-1 (9) | ISO-2 (7) | ISO-8 (8) |
|---|---|---|---|---|
| Initial weight (g) | 171.2 ± 17.7 | 177.3 ± 14.0 | 178.1 ± 24.1 | 175.9 ± 19.9 |
| Final weight (g) | 215.3 ± 30.8 | 226.5 ± 26.7 | 219.7 ± 27.6 | 225.5 ± 24.7 |

[a] Number of rats in the groups.

hemoglobin (Hb) $A_{1c}$ among groups in a non-fasting state. The entire liver, lung and kidney were rapidly excised. Samples for the analysis of Hb $A_{1c}$ were freshly prepared. Specimens of plasma and tissues were stored at −70 °C for measurement of blood chemistry, lipid peroxidation products, and antioxidant enzyme activities.

### *2.2. Measurements and analytical procedures*

Blood samples were collected into tubes containing heparin and were immediately centrifuged. High-density lipoprotein (HDL) was precipitated from plasma by a modification of the dextran-sulphate method, as described by Warnick et al. [24]. Plasma glucose, non-esterified fatty acids (NEFA), total cholesterol (TC), HDL-cholesterol (HDL-C), and triglyceride (TG) were determined by colorimetric methods after enzymatic reaction with peroxidase (Randox, Antrim, Ireland). Low-density lipoprotein-cholesterol (LDL-C) was estimated by the Friedewald formula which is reliable when TG levels are under 400 mg/dl [25]. Plasma insulin was measured by a commercially available enzyme-linked immunosorbent assay in a microtiter plate. Antibody specific for rat insulin was coated onto the wells of the microtiter strips provided (Mercodia AB, Uppsala, Sweden). Blood $HbA_{1c}$ was estimated using a commercial kit (Helena BioSciences, Sunderland, UK). Whole blood (collected with heparin) was mixed with lysing reagent to prepare a hemolysate. This was then mixed with cation-exchange resin. The nonglycosylated Hb binds to the resin, leaving glycosylated Hb free in the supernatant. The glycosylated Hb level (%) was determined by measuring the absorbance of the glycosylated Hb and total Hb at 415 nm. Fifteen percent tissue homogenates were prepared at 4 °C in a 250-mM sucrose solution, containing 10 mM-Hepes (pH 7.4) using a homogenizer. The homogenates were centrifuged to discard cell debris and mitochondria [26]. The supernatant was used for analysis of superoxide dismutase

Table 3
Fasting plasma glucose and lipid profiles of the 4 groups after consuming the experimental diets for 4 weeks

| | Control (10)[a] | ISO-1 (9) | ISO-2 (7) | ISO-8 (8) |
|---|---|---|---|---|
| Glucose (mg/dL) | 435.5 ± 136.0 | 443.4 ± 108.5 | 461.9 ± 53.3 | 479.3 ± 128.6 |
| Insulin (ng/mL) | 0.52 ± 0.51 | 0.30 ± 0.29 | 0.39 ± 0.39 | 0.45 ± 0.43 |
| TC (mg/dL) | 127.4 ± 35.5 | 142.6 ± 33.1 | 132.4 ± 34.6 | 115.5 ± 38.3 |
| TG (mg/dL) | 224.4 ± 77.8 | 270.2 ± 80.3 | 179.0 ± 43.9 | 190.1 ± 154.1 |
| NEFA (mmol/L) | 1.27 ± 0.28 | 1.44 ± 0.29 | 1.28 ± 0.3 | 1.05 ± 0.22 |

[a] Number of rats in the groups.
Abbreviation: TC, total cholesterol; TG, triglyceride; NEFA, nonesterified fatty acids

Table 4
Non-fasting plasma glucose, insulin and hemoglobin $A_{1c}$ concentrations of the 4 groups after consuming the experimental diets for 4 weeks

| | Control | ISO-1 | ISO-2 | ISO-8 |
|---|---|---|---|---|
| Glucose (mg/dL) | 716.8 ± 105.3 | 726.1 ± 93.3 | 739.2 ± 239.5 | 729.2 ± 117.7 |
| Insulin (ng/mL) | 0.57 ± 0.55 | 0.42 ± 0.32 | 0.41 ± 0.08 | 0.40 ± 0.27 |
| $HbA_{1c}$ (%) | 8.63 ± 0.55 | 8.70 ± 0.43 | 7.52 ± 2.22* | 7.86 ± 0.67 |

* $p = 0.12$ compared with the control group and $p = 0.055$ compared with the ISO-1 group.

(SOD) and glutathione peroxidase (GSHPx) activities (enzyme kits from Randox). Protein concentrations of supernatants were measured by Lowry's method [27]. The production of thiobarbituric acid-reactive substances (TBARS, assumed to be mainly malondialdehyde and its precursors) in rat tissue homogenates was determined by the method of Uchiyama and Mihara [28]. The molar extinction coefficient of malondialdehyde was assumed to be 156,000 [29].

### *2.3. Statistical analysis*

Data are expressed as the mean ±SD. Student's *t*-test was used to analyze the significance of differences between mean values, and different groups were analyzed by analysis of variance using Duncan's multiple range test. A $p$ value of less than 0.05 was considered statistically significant.

## 3. Results

There were no significant differences in initial body weights and body weight gain during the experiment among all groups (Table 2). Plasma concentrations of TC, TG, NEFAs, glucose, and insulin in a fasting state did not differ among all groups (Table 3).

There were no significant differences in plasma glucose or insulin concentrations among groups in a non-fasting state. However, blood $HbA_{1c}$ tended to be lower in the ISO-2 group

Table 5
Non-fasting plasma lipid profiles of the 4 groups after consuming the experimental diets for 4 weeks

| | Control | ISO-1 | ISO-2 | ISO-8 |
|---|---|---|---|---|
| TC (mg/dL) | 136.7 ± 21.9 | 149.2 ± 33.2 | 146.3 ± 39.8 | 101.8 ± 10.8* |
| TG (mg/dL) | 203.7 ± 83.3 | 245.9 ± 88.1 | 323.0 ± 168.0 | 190.8 ± 141.7 |
| HDL-C (mg/dL) | 81.0 ± 13.9 | 82.2 ± 14.5 | 81.2 ± 6.5 | 71.6 ± 9.0 |
| LDL-C (mg/dL) | 30.5 ± 12.9 | 38.6 ± 16.1 | 39.8 ± 27.6 | 16.0 ± 3.8* |
| HDL-C/LDL-C | 2.97 ± 1.40 | 2.30 ± 1.10 | 2.56 ± 0.95 | 4.50 ± 0.84* |

Abbreviations: TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol.

* Significantly different from other groups.

Table 6
Superoxide dismutase activities in liver, lung, and kidney homogenates of the 4 groups

| | Control | ISO-1 | ISO-2 | ISO-8 |
|---|---|---|---|---|
| Liver | 14.73 ± 5.12 | 17.33 ± 2.22 | 14.0 ± 4.01 | 17.86 ± 4.12 |
| Lung | 5.52 ± 1.96 | 5.59 ± 1.03 | 6.26 ± 1.73 | 4.87 ± 1.47 |
| Kidney | 12.05 ± 3.37 | 13.55 ± 5.3 | 15.49 ± 4.45 | 12.96 ± 4.79 |

Data are expressed as the mean ± SD (U/g protein).

than in the control group ($p = 01.2$) and the ISO-1 group ($p = 0.55$) (Table 4). Plasma TC and LDL-C were significantly lower whereas the HDL-C/LDL-C ratio was higher in the ISO-8 group than in the other groups. No differences in plasma TG or HDL-C were observed among groups in the non-fasting state (Table 5).

SOD activities in liver, lung, and kidney homogenates did not differ among all groups (Table 6). Also, no difference in GSHPx activities of tissue homogenates were observed among groups (Table 7). There were no significant differences in malondialdehyde (MDA) concentrations in tissue homogenates among groups (Table 8).

## 4. Discussion

Although many in vitro studies have shown that flavonoids in legumes and soybean products inhibit the oxidation of lipoprotein, there is, however, no clear evidence to date of free radical-scavenging antioxidant effects of isoflavonoids or their metabolites in vivo. In this study we investigated the effect of isoflavone supplementation on preventing oxidative damage in DM rats. We did not include a non-diabetic group, because numerous studies have shown that DM is a disease with high oxidative stress, and lipid peroxide or conjugated diene are elevated in diabetes [12–19]. Several reports have indicated a relationship between fasting blood glucose and lipid peroxide levels [12,14,15]. SOD and GSHPx are enzymes that protect tissues from the effects of free radicals and lipid peroxides, and the activities of both SOD and GSHPx increase after free-radical-mediated injury and lipid peroxidation [30]. The results of our study show that SOD and GSHPx activities as well as MDA concentrations in liver, lung, and kidney homogenates did not differ between the experimental and control groups. These results suggest that isoflavone supplementation for 3 weeks in diabetic rats had no effect on reducing oxidative stress. However, this result is consistent with our previous

Table 7
Glutathione peroxidase activities in liver, lung, and kidney homogenates of the 4 groups

| | Control | ISO-1 | ISO-2 | ISO-8 |
|---|---|---|---|---|
| Liver | 1.12 ± 0.22 | 0.99 ± 0.17 | 0.93 ± 0.21 | 1.04 ± 0.26 |
| Lung | 0.46 ± 0.12 | 0.4 ± 0.14 | 0.41 ± 0.04 | 0.37 ± 0.05 |
| Kidney | 1.57 ± 0.32 | 1.69 ± 0.19 | 1.79 ± 0.45 | 1.77 ± 0.40 |

Data are expressed as the mean ± SD (U/g protein).

Table 8
Malondialdehyde concentrations in liver, lung, and kidney homogenates of the 4 groups

| | Control | ISO-1 | ISO-2 | ISO-8 |
|---|---|---|---|---|
| Liver | 2.11 ± 0.52 | 2.03 ± 0.46 | 1.94 ± 1.18 | 1.72 ± 0.38 |
| Lung | 5.46 ± 1.78 | 5.67 ± 1.07 | 4.52 ± 2.24 | 5.87 ± 1.27 |
| Kidney | 5.95 ± 0.87 | 7.18 ± 1.84 | 6.05 ± 0.74 | 6.94 ± 1.25 |

Data are expressed as the mean ± SD (nmol/g tissue).

report, which showed that erythrocyte antioxidant enzyme activities did not change after 6 months of isoflavone supplementation in postmenopausal women [31]. In addition, a study by Chai [32] showed that the inhibition of LDL oxidation by genestein and daidzein was pronounced in vitro but not in vivo. Tumbelaka and Sajuthi [33] also showed that isoflavones alone did not reduce the amount of TBARS when LDL from each individual was subjected to copper-mediated oxidative modification. It is possible that the concentrations of isoflavones in in vivo studies differ from those in in vitro studies, and the relative molar concentrations of the lipoproteins and the test compounds in in vitro assays may not be achieved in circulating plasma. Therefore, the results of in vitro studies may not accurately reflect the in vivo situation.

In this study, we observed that no significant differences in plasma glucose and insulin levels among groups, irregardless of the rats had fasted or not. This result suggests that isoflavone supplementation has no effect on attenuating blood glucose, nor was insulin secretion affected. However, blood $HbA_{1c}$ in the ISO-2 group had a tendency to be lower than those of the ISO-1 and control group ($p = 0.055$ and $p = 0.12$). $HbA_{1c}$ is a well-recognized marker of chronic glycemic control in DM. According to a prospective diabetic study, improved glycemic control reduced diabetes-related end points [34]. The finding in this study may indicate that long-term glycemic control tends to be better when 2 times the suggested amount of isoflavone is administered. Because the duration of this study was only 3 weeks, whether a longer duration would lead to more-obvious results needs to be confirmed with a longer experimental duration.

The results in this study show that plasma TC and LDL-C were significantly lower whereas the HDL-C/LDL-C ratio was higher in the ISO-8 group than in the other groups. Anthony et al. [2] assumed that the hypocholesterolemic effect is largely due to isoflavones contained in soy protein, especially genestein and daidzein. It is possible that the amount of isoflavone supplemented in the ISO-1 and ISO-2 groups was insufficient to lower plasma TC. On the other hand, a study by Potter et al. [35] showed that the amount of isoflavone consumed by postmenopausal women had little effect on blood lipid variables, a smaller amount of isoflavone showed significantly improved blood lipid profiles earlier than in the group receiving higher concentrations of isoflavone. Thus, they suggested that the cholesterol-lowering component of soy is not or is only partially related to the isoflavones. The isoflavone we used in this study contains small amounts of soy protein; in order to maintain identical protein content among the groups, 20% of casein was substituted by soy protein in the ISO-8 group. Therefore, the roles of other components in soy protein or an interaction between isoflavones and soy protein on the cholesterol-lowering effect can not be ruled out.

In conclusion, the results of this study show that 3 doses of isoflavone supplementation had no favorable effect on plasma glucose or insulin concentrations, nor had any influence on attenuating oxidative stress in diabetic rats. However, the ISO-2 group showed a tendency to have better chronic glycemic control than did the control and ISO-1 groups. In addition, larger amounts of isoflavone supplementation may have beneficial effects on reducing plasma TC and LDL-C levels. Determining whether the hypocholesterolemic effects resulted from isoflavone alone or soy protein ingested, or from an interaction between isoflavone and soy protein, requires further investigation.

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