β-conglycinin Lowers Very-Low-Density Lipoprotein-Triglyceride Levels by Increasing Adiponectin and Insulin Sensitivity in Rats

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The relationship between insulin sensitivity and the plasma triglyceride-lowering effect induced by β-conglycinin was investigated. Male Wistar rats (19 weeks old) were fed diets containing casein, soy protein isolate, or β-conglycinin for 4 weeks. In oral glucose administration, the β-conglycinin-fed rats showed a significant decrease in the area under the glucose curve (0–60 min) as compared with the casein-fed rats. The hypoglycemic effect was significantly higher in the β-conglycinin-fed rats than in the casein-fed rats at 30 min after intraperitoneal insulin injection. The liver sterol regulatory element-binding-protein-1 mRNA expression level was significantly lower and the plasma sterol regulatory element-binding-protein-1 mRNA expression in the liver of β-conglycinin-fed rats than in the casein-fed rats. The hypotriglyceridemic effect of β-conglycinin depended on a significant decrease in the concentration of very-low-density-lipoprotein triglycerides. These results indicate that β-conglycinin increases adiponectin levels and improves glucose tolerance. The ability of β-conglycinin to lower plasma lipid levels might be due to increased insulin sensitivity of the liver.

Key words: β-conglycinin; oral glucose tolerance test; insulin tolerance test; insulin resistance; very-low-density lipoprotein

Life style-related diseases such as diabetes and dyslipidemia are serious problems. Many researchers report that impaired glucose disorder mediates between various diseases and obesity.1–4) Obesity leads to the production of inflammatory cytokines and induces insulin resistance.5,6) Currently, in addition to the development of medical therapies, improvements in life style and exercise are considered important for corpulence patients.7,8) Many researchers have shown that caloric restriction and exercise can improve insulin sensitivity.9)

Many dietary nutrients are effective in reducing the risk of disease. The indigestible dextrin is known to be a suppressor of postprandial blood glucose concentra-

tion.10) Soy protein isolate (SPI) has been consumed for a long time and is recognized as having a hypocholes-
terolemic effect.11–13) In a recent report, Nagasawa et al. reported that SPI intake decreased blood glucose in diabetic kk-Ay mice.14)

Recently, Samoto et al. found that 11S globulin (glycinin), lipophilic protein (LP), and 7S globulin (β-conglycinin) are the major protein components of SPI.15) They reported that the ratio of β-conglycinin in SPI is 23%. Previous experiments indicated a new physiological function of β-conglycinin: consumption of β-conglycinin results in a significant decrease in blood triglyceride (TG) concentration in rodents and humans.16–18) Although enzymes related to fatty acid metabolism have been shown to be activated by β-conglycinin,19) it remains unclear how β-conglycinin causes these effects.

In this study, a β-conglycinin diet was fed to normal mature rodents, and the regulators of plasma triglyceride concentration were investigated. Measurement of glucose tolerance19,20) and mRNA expression in the liver allowed us to identify several key factors involved in the decrease in triglyceride levels caused by β-conglycinin.

Materials and Methods

Animals and diets. All animals were treated in accordance with the guidelines established by the Japanese Society for Nutrition and Food Science (Law no. 105 and Notification no. 6 of the Japanese government). Vitamin-free casein (Oriental Yeast, Tokyo), SPI (Fujipro, Fuji Oil, Osaka, Japan), and β-conglycinin (Lipoff, Fuji Oil) were provided as dietary proteins. The experimental diets were based on the AIN-93G formula, and are described in Table 1.21) Twenty-four specific pathogen-free male Wistar rats, 19 weeks old, were purchased from Japan Crea (Tokyo). All the rats were housed individually in stainless steel cages under controlled conditions (temperature, 23 ± 1°C; humidity, 55 ± 5%; light, 0700–1900 h). After acclimation to commercial food (CRF-1; Oriental Yeast) for 5 d, the rats were divided into three groups with similar average body weights. The experimental diets and water were given ad libitum for 4 weeks. Food intake was recorded daily, and body weight was measured twice a week.

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Abbreviations: SPI, soy protein isolate; OGTT, oral glucose tolerance test; ITT, insulin tolerance test; AUC, area under curve; SREBP1, sterol regulatory element-binding protein 1; LP, lipophilic protein; FAS, fatty acid synthase; ELISA, enzyme-linked immunosorbent assay; Apo B, apolipoprotein B; SEM, standard error of the mean; LSD, the least significant difference; HPLC, high-performance liquid chromatography; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; XLR, liver X receptor; NIDDM, non-insulin dependent diabetes mellitus
Oral glucose tolerance test and insulin tolerance test. An oral glucose tolerance test (OGTT) was done 18 d after the beginning of the experiment. Briefly, each rat was fasted overnight, after which fasting blood samples were collected from the tail vein with a heparinized syringe. Then a 20% glucose solution (2 g of glucose per kg of body weight) was administered orally by sonde, and blood samples were taken at regular time intervals (0, 15, 30, and 60 min). Blood glucose concentrations were measured by Free Style Glucose Sensor (Nipro, Osaka, Japan). Plasma was separated by centrifugation (1,900 × g, 15 min, 4 °C) and stored at 4 °C until analysis. Insulin concentrations were measured using an enzyme-linked immunosorben assay (ELISA) kit (Ultra-high Sensitivity Rat Insulin ELISA kit; Otsuka Pharmaceutical, Tokyo) levels were measured. After plasma lipoprotein and plasma adiponectin (mouse/rat adiponectin ELISA kit; Otsuka and Yokohama, Japan). The areas under the glucose and insulin curves (the AUCs) were calculated by the trapezoidal rule using the glucose and insulin measurements at 0 (fasting), 15, 30, and 60 min after injection. Blood glucose concentrations were measured as described above.

Blood analysis. On day 28, after 6 h of food deprivation (0800–1400) blood was withdrawn from the abdominal aorta into a heparinized syringe under isoflurane anesthesia. Plasma was separated by centrifugation and stored at −80 °C pending analysis. Plasma leptin (rat leptin ELISA kit; Wako Pure Chemical Industries, Osaka, Japan) and plasma adiponectin (mouse/rat adiponectin ELISA kit; Otsuka Pharmaceutical, Tokyo) levels were measured. After plasma lipoprotein fractions were separated by high-performance liquid chromatography (HPLC), the triglyceride and cholesterol levels in each fraction were analyzed by Skylight-Biotech (Akita, Japan).22

Liver lipid analysis. Livers were excised, rinsed, weighed, and then stored at −80 °C until further analysis. Liver lipids were extracted by the method of Folch et al.,24 and were analyzed for triglyceride and cholesterol by the methods of Sperry and Webb and Fletcher.25,26

Gene expression analysis. Liver and mesenteric adipose tissue samples were dissected from each animal and stored at −80 °C for subsequent RNA isolation. Total RNA was isolated using ISOGEN (NipponGene, Tokyo) and purified using an RNeasy spin column and DNase I reagent (Qiagen, Valencia, CA) following the manufacturer’s instructions. The total RNA obtained was reverse-transcribed into cDNA using the PrimeScript™ RT reagent kit (Takara Bio, Tokyo).

Real-time semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed starting with 25 ng of cDNA, along with 50 pmol of sense primers and fluorescent-dye-labeled antisense primers (D-LUX SELECT pre-designed primers, Invitrogen, Carlsbad, CA), in an ABI PRISM 7700 sequence detection system (Applied Biosystems Japan, Tokyo). These samples were incubated for initial denaturation at 95 °C for 10 min, followed by 40 PCR cycles. Each cycle consisted of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 20 s. The following rat-gene-specific primers (D-LUX SELECT primer) were used: sterol regulatory element-binding-protein 1 (SREBP1) (Srebf1, RLXU3607120), and fatty acid synthase (FAS) (Fasn, RLXU3604630), adiponiprotein B (ApoB) (ApoB, RLXU3604723), with β-actin (Actb, RLXU3607934) as housekeeping gene. The specific primers are further described on the Invitrogen website (https://orf.invitrogen.com/lux/LUXSearch.jsp).

Table 2. Effects of β-Conglycinin Diet on Body Weight, Food Intake, Weight Gain, and Food Efficiency Ratio

<table>
<thead>
<tr>
<th>(n)</th>
<th>Casein</th>
<th>SPI</th>
<th>β-Conglycinin</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Initial weight (g)</td>
<td>449.4 ± 14.5</td>
<td>450.1 ± 14.7</td>
<td>449.9 ± 13.8</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>447.1 ± 12.6</td>
<td>439.9 ± 13.4</td>
<td>428.2 ± 13.1</td>
</tr>
<tr>
<td>Weight gain (g/d)</td>
<td>−0.1 ± 0.1a</td>
<td>−0.4 ± 0.1a</td>
<td>−0.8 ± 0.1b</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>18.1 ± 0.3</td>
<td>17.6 ± 0.4</td>
<td>18.4 ± 0.5</td>
</tr>
<tr>
<td>Food efficiency ratio</td>
<td>−0.005 ± 0.008a</td>
<td>−0.020 ± 0.004a</td>
<td>−0.042 ± 0.006b</td>
</tr>
</tbody>
</table>

Means in the same line not sharing a common superscript letter are significantly different at p < 0.05 by Fisher LSD test. Data are means ± SEM (n = 8).
0.27 ng/ml, and 0.97 ± 0.22 ng/ml in the casein, SPI, and β-conglycinin groups, respectively. In the casein group, plasma insulin showed a 3.15-fold increase from the initial level at 15 min after glucose administration. On the other hand, plasma insulin levels increased only 2.22-fold in the SPI group and 2.59-fold in the β-conglycinin group (Fig. 1C). The AUC of plasma insulin decreased in the following order: casein group > SPI group > β-conglycinin group (Fig. 1D).

**Insulin tolerance test**

Figure 2 shows the rate of decrease in blood glucose concentration. This decrease was more rapid in β-conglycinin-fed rats than in the casein-fed rats. In the casein group, blood glucose levels had decreased to 70.8% and 57.3% at 15 and 30 min respectively. On the other hand, in the β-conglycinin group, blood glucose levels were 56.5% and 46.0% at 15 and 30 min respectively. The blood glucose decrease level was significantly different between the β-conglycinin group and the casein group at 15 min after injection.

**Analysis of mRNA expression**

The gene expression levels of sterol regulatory element-binding protein 1 (SREBP1) mRNA in the liver were significantly lower in the β-conglycinin group and the SPI group than in the casein group (Fig. 3A). The fatty acid synthase (FAS) and apolipoprotein B (Apo B) mRNA expression levels in the β-conglycinin group were significantly lower than in the casein group and the SPI group (Fig. 3B).
**Fig. 3.** Effect of β-Conglycinin Diet on mRNA Expression Levels in Wistar Rats.

Total RNA extracted from liver samples was analyzed by real-time semi-quantitative RT-PCR. The casein group, the SPI group, and the β-conglycinin group were displayed by open column, striped column, and solid column respectively. A, Sterol regulatory element-binding protein 1 (SREBP1). B, Fatty acid synthase (FAS). C, Apolipoprotein B (Apo B). Areas are significantly different at \( p < 0.05 \) by the Fisher LSD test if they do not share a common letter. Data are mean ± SEM (\( n = 8 \)).

**Table 3.** Relative Liver Weights and Liver Lipid Analysis

<table>
<thead>
<tr>
<th></th>
<th>Casein (( n = 8 ))</th>
<th>SPI (( n = 8 ))</th>
<th>β-Conglycinin (( n = 8 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>444.1 ± 12.7</td>
<td>437.1 ± 13.3</td>
<td>426.1 ± 13.1</td>
</tr>
<tr>
<td>Liver (% )</td>
<td>2.65 ± 0.07 ( ^a )</td>
<td>2.56 ± 0.04 ( ^b )</td>
<td>2.41 ± 0.04 ( ^b )</td>
</tr>
<tr>
<td>Liver lipid contents ( ^d )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride mg/gLiver</td>
<td>16.62 ± 1.87 ( ^a )</td>
<td>9.77 ± 1.55 ( ^a )</td>
<td>13.24 ± 1.14 ( ^b )</td>
</tr>
<tr>
<td>Total cholesterol mg/gLiver</td>
<td>3.60 ± 0.11 ( ^a )</td>
<td>2.99 ± 0.07 ( ^b )</td>
<td>3.22 ± 0.12 ( ^b )</td>
</tr>
</tbody>
</table>

Means in the same line not sharing a common superscript letter are significantly different at \( p < 0.05 \), by Fisher LSD test. Data are means ± SEM (\( n = 8 \)).

**Table 4.** Plasma Characteristics and Lipid Profiles of Lipoprotein Fraction

<table>
<thead>
<tr>
<th></th>
<th>Casein (( n = 8 ))</th>
<th>SPI (( n = 8 ))</th>
<th>β-Conglycinin (( n = 7 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>202.1 ± 4.3</td>
<td>203.0 ± 6.2</td>
<td>195.3 ± 5.3</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>1.76 ± 0.34</td>
<td>1.52 ± 0.14</td>
<td>1.16 ± 0.12</td>
</tr>
<tr>
<td>Leptin (pg/ml)</td>
<td>1290.0 ± 136.9 ( ^a )</td>
<td>1102.2 ± 91.7 ( ^b )</td>
<td>877.6 ± 168.1 ( ^b )</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>4.36 ± 0.23 ( ^a )</td>
<td>4.53 ± 0.30 ( ^a )</td>
<td>7.01 ± 0.46 ( ^b )</td>
</tr>
<tr>
<td>Sum of total cholesterol mg/dl</td>
<td>70.47 ± 3.72 ( ^a )</td>
<td>60.79 ± 2.49 ( ^b )</td>
<td>50.24 ± 1.73 ( ^b )</td>
</tr>
<tr>
<td>Chylomicron (&gt;)80 nm (mg/dl)</td>
<td>1.06 ± 0.38 ( ^a )</td>
<td>0.46 ± 0.08 ( ^b )</td>
<td>0.31 ± 0.08 ( ^b )</td>
</tr>
<tr>
<td>VLDL ( ^1 ) (30–80 nm) (mg/dl)</td>
<td>10.55 ± 1.46 ( ^a )</td>
<td>6.63 ± 0.37 ( ^b )</td>
<td>5.54 ± 0.74 ( ^b )</td>
</tr>
<tr>
<td>LDL ( ^2 ) (16–30 nm) (mg/dl)</td>
<td>13.34 ± 0.75 ( ^a )</td>
<td>11.34 ± 0.63 ( ^b )</td>
<td>10.23 ± 0.39 ( ^b )</td>
</tr>
<tr>
<td>HDL ( ^3 ) (8–16 nm) (mg/dl)</td>
<td>45.52 ± 2.00 ( ^a )</td>
<td>42.37 ± 1.84 ( ^b )</td>
<td>34.15 ± 1.07 ( ^b )</td>
</tr>
</tbody>
</table>

Means in the same line not sharing a common superscript letter are significantly different at \( p < 0.05 \), by Fisher LSD test. Data are means ± SEM (\( n = 7 \), 8).

\( ^{1} \) Very-low-density lipoprotein

\( ^{2} \) Low-density lipoprotein

\( ^{3} \) High-density lipoprotein

**Relative liver weights and liver lipid analysis**

As Table 3 shows relative liver weight was significantly different in the β-conglycinin-fed rats as compared with the casein-fed rats. SPI and β-conglycinin intake caused a decrease in liver triglyceride level and total cholesterol content as compared with casein intake.

**Concentrations of plasma glucose and hormones and lipid content in lipoprotein fractions**

Table 4 presents the results of plasma analysis. Plasma leptin was significantly lowered in the β-
conglycinin group than in the casein group. Although blood glucose levels did not differ between the groups, plasma insulin levels were lower in the following order: casein > SPI > β-conglycinin. Plasma adiponectin increased significantly in the β-conglycinin group as compared with the other groups. The plasma triglyceride and cholesterol concentrations were lower in the rats fed SPI and β-conglycinin than in the casein-fed rats. In every group, approximately 75% of the plasma TG belonged to the very-low-density lipoprotein (VLDL) category. The average level of VLDL-triglyceride in the β-conglycinin group was half that of the casein group, a significant difference.

Discussion

This study indicates that β-conglycinin, which was obtained by the separation of soy protein, influenced the regulation of plasma glucose. The concentration of blood glucose is strictly regulated by highly complex mechanisms. Secretion of insulin responds quickly to a change in the blood glucose concentration. For instance, the improvement in glucose tolerance caused by cod induced adiponectin production. 14) The current study suggested that gluconeogenesis was suppressed by adiponectin in diabetes mellitus (NIDDM) patients, and pointed out reduced adiponectin levels decreased in non-insulin dependent diabetes mellitus (NIDDM) patients, and pointed out the regulation of plasma glucose. The concentration of plasma glucose in the β-conglycinin-fed rats tended to be lower than that in the casein-fed rats. Though insulin level might influence SREBP1 mRNA expression, the LXR mRNA expression level remained unclear in our further investigation is needed to clarify the effects to SREBP1 regulation caused by β-conglycinin.

Adiponectin, which is secreted from mesenteric adipose tissue, plays an important role in glucose metabolism and obesity. 31) Arita et al. reported a strong negative correlation between plasma adiponectin levels and body mass indices. 32) Hotta et al. found that adiponectin levels decreased in non-insulin dependent diabetes mellitus (NIDDM) patients, and pointed out that glucoseneogenesis was suppressed by adiponectin in the liver. 33) Nagasawa et al. reported that SPI intake induced adiponectin production. 14) The current study clearly indicates that β-conglycinin consumption strongly influences adiponectin levels. Furthermore, β-conglycinin causes a significant decrease in the plasma triglyceride concentration via a reduction in the VLDL-triglyceride concentration. β-Conglycinin can influence liver triglyceride synthesis and the concentration of VLDL by an increased dose of adiponectin and increased insulin action.

In conclusion, when compared with casein, β-conglycinin improved glucose tolerance and insulin sensitivity. A high dose of adiponectin had a hypoglycemic effect and might contribute to reduce the level of insulin. The current investigation indicates that β-conglycinin improved insulin resistance by activating crosstalk between the organs responsible for insulin action. Further studies are needed to clarify the influence of β-conglycinin in the various tissues.

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References


