Dietary β -Conglycinin Modulates Insulin Sensitivity, Body Fat Mass, and Lipid Metabolism in Obese Otsuka Long-Evans Tokushima Fatty (OLETF) Rats

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Abstract: The physiological effects of dietary β -conglycinin (β -CON), one of the major components of soy protein (SOY), were examined in an obese animal model. Prior studies show that β -CON intake decreases plasma triglycerides and visceral adipose tissue weight, and increases plasma adiponectin in rodents. Since plasma adiponectin is known to affect both lipid and glucose metabolism, feeding a diet containing β -CON could modulate insulin sensitivity. Therefore, we examined the effects of dietary β -CON on insulin sensitivity and blood glucose levels, as well as lipid metabolism in obese Otsuka Long-Evans Tokushima Fatty (OLETF) rats (pre-symptomatic stage of type 2 diabetes mellitus). Male OLETF rats (6 weeks old) were fed diets containing 20% protein such as casein (CAS), CAS replaced with soy protein (SOY), or β -CON at a proportion of 50% for 13 weeks. Fasting blood glucose levels were measured every 3 weeks, and an insulin tolerance test (ITT; 0.75 IU/kg body weight) was conducted at week 12. During the feeding period, fasting blood glucose was comparable among the groups. Insulin sensitivity measured by the ITT revealed that the SOY and β -CON diets decreased blood glucose levels at 30 min after intraperitoneal insulin injection (vs. CAS diet). In addition, the β -CON diet increased plasma adiponectin concentrations, hepatic gene expression of insulin receptor substrate (IRS) 2, and muscle gene expression of adiponectin receptor 1 (AdipoR1) and IRS1, and with a decrease in plasma insulin concentration. Finally, the β -CON diet decreased the mesenteric adipose tissue weight and liver triglyceride concentration compared to the CAS diet. These results suggest that the metabolic effects of dietary β -CON are mediated by increasing plasma adiponectin to increase insulin sensitivity and influence the hepatic lipid metabolism in obese OLETF rats.

Key words: β -conglycinin, Otsuka Long-Evans Tokushima Fatty rats, insulin sensitivity, adiponectin, adipose tissue

1 INTRODUCTION

Dietary soy protein (SOY) decreases plasma and hepatic lipid concentrations in normal and obese rodents¹⁻³. β -Conglycinin (β -CON), one of the major components of SOY⁴, is reported to lower body fat mass and plasma and hepatic triglyceride concentrations in rodents to a greater extent compared to SOY^{1, 2, 5-7}. The effect of β -CON may be due in part to decreased fatty synthesis^{2, 3, 5} and increased fatty acid β -oxidation in the liver^{2, 3}. These pre-clinical studies suggest that β -CON may be useful as an anti-obesity food component. However, the mechanisms of action underlying the impact of β -CON on obesity and me-

tabolism are not well-understood.

β -CON is known to increase the serum/plasma adiponectin concentration in rodents⁸⁻¹⁰. Adiponectin is mainly secreted from adipocytes and enhances fatty acid β -oxidation and increases insulin sensitivity in rodents^{11, 12}. Tachibana *et al.* reported that dietary β -CON increased the plasma adiponectin concentration and insulin sensitivity accompanied with a decrease in plasma very-low-density-lipoprotein triglyceride levels in Wistar rats⁵. Insulin regulates lipid and glucose metabolism in the liver¹³. Because obesity may induce dyslipidemia and insulin resistance¹⁴, it is important to understand the underlying mechanism by which

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β -CON can influence whole body insulin sensitivity, body fat mass, and lipid metabolism.

Otsuka Long-Evans Tokushima Fatty (OLETF) rats lack the cholecystokinin receptor, and are a pre-clinical model for obesity and type 2 diabetes. Because of hyperphagia, OLETF rats become chronically obese (30–40% heavier than their control counterparts Long-Evans-Tokushima-Otsuka (LETO) rats) and exhibit high plasma triglyceride concentration and lipid accumulation in islets at 20 weeks of age^{15, 16}. By 25 weeks, OLETF rats demonstrate hyperglycemia and hyperinsulinemia and reach the early phase of type 2 diabetic mellitus¹⁶.

Although a number of animal studies show that β -CON affects plasma and hepatic lipid concentrations, limited evidence is available for the physiological effects of β -CON on insulin sensitivity and lipid metabolism in obese animal models. The present study examined how dietary β -CON affects insulin sensitivity as well as lipid metabolism in obese OLETF rats in the pre-symptomatic stage of type 2 diabetic mellitus.

2 EXPERIMENTAL

2.1 Animals and diets

Six-week-old male OLETF rats were purchased from Japan SLC, Inc. (Shizuoka, Japan). The animals were housed individually in steel cages under controlled conditions ($22 \pm 1^\circ\text{C}$, $55 \pm 5\%$ humidity, 08:00–20:00 light and dark cycle). After acclimatization for 7 days, 18 rats were randomly assigned to 3 groups with 6 animals each, according to the source of dietary protein. As a nitrogen source, we used CAS (Wako Pure Chemical Industries Ltd.,

Osaka, Japan), SOY (FUJIPRO, >90% purity, Fuji Oil Co., Osaka, Japan), and β -CON (RIPOFU-700, >90% purity, Fuji Oil Co.). Since SOY was previously reported to contain 23% of β -CON⁴, SOY used in the present study was considered to contain approximately the same level of β -CON. The control diet was prepared according to the AIN-93G formula, containing 20% of CAS¹⁷, as shown in Table 1. For the experimental diet, 50% of CAS was replaced with either SOY or β -CON. Rats were given free access to the diets for 13 weeks.

During the feeding period, fasting blood glucose levels (6 h-fasting) were measured every 3 weeks. Blood was collected from the tail vein, and glucose levels were measured using a glucometer (Accu-Check Aviva, Roche DC Japan K. K., Tokyo, Japan). At week 12, an insulin tolerance test (ITT) was conducted. After 6 h fasting, rats were injected intraperitoneally with 0.75 IU/kg body weight insulin (Humulin R, Eli Lilly Japan K.K., Kobe, Japan). Blood glucose levels were measured immediately before the insulin injection (time 0) and at 15, 30, 60, 120, and 180 min after the insulin injection. Five days after the ITT, rats were fasted (03:00–09:00) and anesthetized with isoflurane and pentobarbital. Then, blood (9 mL) was collected from the abdominal aorta in a syringe containing 1.0 mL of 5 mM EDTA- Na_2 (pH 7.0). The liver, muscle, and adipose tissues were excised, weighed, frozen immediately under the liquid nitrogen, and stored at -80°C until analysis. Plasma was prepared by centrifugation ($1,000 \times g$ for 15 min at 4°C), and stored at -80°C until analysis. The experimental procedures were in accordance with the guidelines for animal experiments of the University of Nagasaki.

Table 1 Diet composition (g/kg diet).

	Groups		
	CAS	SOY	β -CON
Casein	200	100	100
Soy protein isolate	—	100	—
β -Conglycinin	—	—	100
Cornstarch	200.5	200.5	200.5
Pregelatinized cornstarch	132	132	132
Sucrose	300	300	300
Soybean oil	70	70	70
Cellulose	50	50	50
Mineral mixture (AIN-93G) ¹⁷	35	35	35
Vitamin mixture (AIN-93) ¹⁷	10	10	10
Choline bitartrate	2.5	2.5	2.5
<i>t</i> -Butylhydroquinone	0.014	0.014	0.014

CAS, casein; SOY, soy protein isolate; β -CON, β -conglycinin.

2.2 Plasma analysis

Plasma concentrations of total cholesterol, triglycerides, free fatty acids, and glucose were measured using commercially available assay kits (Cholesterol E test Wako, Triglyceride E test Wako, NEFA C test Wako and Glucose CII test, Wako Pure Chemical Industries, Ltd.). Plasma concentrations of insulin, adiponectin, and leptin were measured by enzyme-linked immunosorbent assays using commercial kits (Rat Insulin ELISA kit, Morinaga Institute of Biological Science, Kanagawa, Japan; Mouse/Rat Adiponectin ELISA kit, Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan; and Mouse/Rat Leptin ELISA kit, Morinaga Institute of Biological Science). All measurements were performed in accordance with the respective manufacturers' instruc-

tions. The index of insulin resistance was calculated according to the following equation¹⁸⁾:

$$\text{Index of insulin resistance} = \text{plasma glucose (mg/dL)} \times \text{plasma insulin (ng/mL)} / 405$$

2.3 Liver lipid analysis

Total lipids were extracted from the liver according to the method of Folch *et al.*¹⁹⁾. Triglyceride and cholesterol concentrations were determined using assay kits as described elsewhere²⁰⁾, with minor modifications. Phospholipid concentrations were measured according to the method of Rouser *et al.*²¹⁾.

Table 2 Sequence of quantitative reverse transcription-polymerase chain reaction primers.

Gene	Accession no	Primer sequence
LXR α	NM_031627.2	F 5'-AGAGCATCACCTTCCTCAAGA-3 R 5'-GGAGAACTCAAAGATGGGGTTA-3
FAS	NM_017332.1	F 5'-GGTAGGCTTGGTGAAGTGTCTC-3 R 5'-TCTAACTGGAAGTGACGGAAGG-3
SREBP-1 c	XM_213329	F 5'-CATCAACAACCAAGA CAGTG-3 R 5'-GAAGCAGGAGAAGAG AAGC-3'
IRS2	NM_001168633.1	F 5'-GTCGTTGTCTCCACCACCG-3 R 5'-AGGGACCCACCTGACTTC-3
IRS1	NM_012969.1	F 5'-CAAGACTGCCCCCTCTCAACA-3 R 5'-CGGTCCTCTGGTTGCTTCTG-3
GLUT4	XM_006246596.3	F 5'-AGATGCCGTCGGGTTTCC-3 R 5'-CCAAACTGAAGGGAGCCAAG-3
AdipoR1	XM_006249852.3	F 5'-TCTTCCGCATCCACACAGAA-3 R 5'-ATATTGCTCTGAGCATGGTCAAG-3
PGC-1 α	XM_017599391.1	F 5'-CGGTACAGTGAGTGTCTGG-3 R 5'-TGAGGACTTGCTGAGTTGTG-3
CPT1b	NM_013200.1	F 5'-CGAGGTGGGATTGGAGTGG-3 R 5'-GCACCTAAGGGCTGAGAGAG-3
Adiponectin	NM_144744.3	F 5'-AATCCTGCCCAGTCATGAAG-3 R 5'-CATCTCCTGGGTCACCCCTTA-3
ATGL	XM_017604198.1	F 5'-CCAACGCCACTCACATCTA-3 R 5'-CCTCCTTGACACCTCAATA-3
PPAR γ	XM_006237009.3	F 5'-CCCTTTACCACGGTTGATTTCTC-3 R 5'-GCAGGCTCTACTTTGATCGCACT-3
HSL	NM_012859.1	F 5'-CGATTGTGGAAAGATGTCAGGAT-3 R 5'-CCCTTTACCACGGTTGATTTCTC-3
36b4	NM_022402.2	F 5'-GGTGTTTGACAATGGCAGCAT-3 R 5'-ATTGCGGACACCCTCTAGGA-3

2.4 Preparation of hepatic subcellular fractions and measurement of enzyme activities

Hepatic subcellular fractions (mitochondria/peroxisomes, cytosols, and microsomes) were prepared by differential centrifugation as described earlier²²⁾. All subcellular fractions were stored at -80°C until used.

The cytosolic activities of malic enzyme, glucose-6-phosphate dehydrogenase (G6PDH), and fatty acid synthase (FAS) were measured according to the methods reported by Ochoa²³⁾, Kelly and Kletzien²⁴⁾, and Kelley *et al.*²⁵⁾, respectively. The microsomal activity of phosphatidate phosphohydrolase (PAP) was measured according to the method reported by Surette *et al.*²⁶⁾. Using the mitochondrial/peroxisomal fraction, the activities of carnitine palmitoyltransferase (CPT) and acyl-CoA oxidase (ACO) were measured as described by Bieber *et al.*²⁷⁾ and Hashimoto *et al.*²⁸⁾, respectively.

Interscapular brown adipose tissue was homogenized in 3 volumes of 10 mM Tris-HCl buffer and CPT activity was measured.

The protein concentrations of subcellular fractions were measured according to the method of Lowry *et al.*²⁹⁾.

2.5 Total RNA extraction and real-time polymerase chain reaction

Total RNA was isolated from tissues (liver, gastrocnemius muscle and mesenteric adipose tissue) using the TriPure isolation reagent (Roche Diagnostics GmbH, Mannheim, Germany)³⁰⁾. RNA was reverse-transcribed to cDNA using Prime Script RT Master Mix (Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions. After cDNA

synthesis, real-time PCR analysis was performed using the Applied Biosystems 7300 Real-time system (Life Technologies Japan Ltd, Tokyo, Japan) with a SYBR green system (Thunderbird[®] SYBR[®] qPCR Mix, Toyobo Co. Ltd., Osaka, Japan). Primer sequences were designed and checked for specificity using NCBI BLAST³¹⁾. The sequences for the primer pairs used in the present study are listed in Table 2. Target gene expression was normalized to the expression of 36B4 and was expressed as $2^{-\Delta\Delta\text{CT}}$ relative to the CAS group³²⁾.

2.6 Statistical Analysis

Results are expressed as mean $\pm$ SE. Data were analyzed by one-way ANOVA followed by the Turkey-Kramer test to determine the protein-dependent difference. Differences were considered statistically significant at $p < 0.05$. Statistical analysis was performed using the Super ANOVA software (Abacus Concepts, Berkeley, CA, USA).

3 RESULTS

3.1 Growth parameters

No significant difference was observed in food intake and body weight among the groups (Table 3).

The mesenteric adipose tissue weight was significantly lower in rats fed the SOY and β -CON diets than in those fed the CAS diet, which was consistent with the previous report¹⁰⁾. The protein-dependent effect was not evident in epididymal and perirenal adipose tissue weights. The interscapular brown adipose tissue weight tended to be heavier

Table 3 Effect of dietary β -conglycinin on growth parameters and tissue weights in OLETF rats.

	Groups		
	CAS	SOY	β -CON
Body weight (g)			
Initial	178 $\pm$ 5	178 $\pm$ 4	177 $\pm$ 5
Final	642 $\pm$ 18	650 $\pm$ 17	615 $\pm$ 14
Food intake (g/day)	28.2 $\pm$ 0.7	26.6 $\pm$ 0.6	26.4 $\pm$ 0.5
Food efficiency (BW gain/g diet)	0.193 $\pm$ 0.003	0.203 $\pm$ 0.005	0.189 $\pm$ 0.003
Relative tissue weights (g/100g BW)			
Liver	3.42 $\pm$ 0.06	3.54 $\pm$ 0.25	3.54 $\pm$ 0.09
Epididymal adipose	3.69 $\pm$ 0.24	3.59 $\pm$ 0.16	3.49 $\pm$ 0.12
Perirenal adipose	4.89 $\pm$ 0.21	4.89 $\pm$ 0.11	4.56 $\pm$ 0.12
Mesenteric adipose	3.50 $\pm$ 0.21 ^a	2.86 $\pm$ 0.10 ^b	2.58 $\pm$ 0.01 ^b
Interscapular brown adipose	0.169 $\pm$ 0.090	0.182 $\pm$ 0.020	0.198 $\pm$ 0.027

CAS, casein; SOY, soy protein isolate; β -CON, β -conglycinin; BW, body weight.

Data are expressed as mean $\pm$ SE of 6 rats.

Values without sharing a common superscript letter are significantly different at $p < 0.05$.

Fig. 1 Effect of dietary β -conglycinin on fasting blood glucose level (A) and insulin tolerance (B) in OLETF rats.

At week 12, rats received 0.75IU/kg body weight insulin by intraperitoneal injection after a 6 h fast. Blood glucose was measured at the intervals indicated over 3h. Values are expressed as means $\pm$ SE of 5 rats. Values without sharing a common letter are significantly different at $p < 0.05$. —●—, CAS group; —▲—, SOY group; and —■—, β -CON group.

in rats fed the SOY and β -CON diets than in those fed the CAS diet, although the trend was not statistically significant.

3.2 Fasting blood glucose levels and ITT

During the feeding period, fasting blood glucose levels were comparable among the groups (Fig. 1A). At week 12, an ITT was conducted to assess the response to insulin (Fig. 1B). The insulin-dependent decrease of blood glucose levels in the β -CON group was found to be significantly larger and that of the SOY group tended to be larger

than that of the CAS group at 30 min after insulin injection.

3.3 Plasma biochemical analysis

The plasma concentrations of triglycerides, phospholipids, free fatty acids, and cholesterol were comparable among the groups (Table 4). Plasma leptin concentrations tended to be lower in the SOY and β -CON groups than in the CAS group. On the other hand, plasma adiponectin concentration was approximately 45% higher in the β -CON group than in the CAS group, though the difference did not reach significance. However, a SOY-dependent increase in plasma adiponectin concentration was not observed in the present study. Plasma glucose concentration was not affected by the diets (Table 4). Plasma insulin concentration was approximately 29% lower in the β -CON group, whereas it was approximately 20% lower in the SOY group than in the CAS group, though the difference was not statistically significant. The index of insulin resistance was approximately 38% lower in the β -CON group, whereas it was approximately 35% lower in the SOY group than that in the CAS group, even though the difference was not statistically significant.

3.4 Liver lipids

The concentration of triglycerides in the liver was significantly lower in the SOY and β -CON groups than in the CAS group (Table 5). The concentration of cholesterol and phospholipids did not differ among the groups.

3.5 Lipogenic and β -oxidation enzyme activities

Liver cytosolic FAS activity was low in the order, β -CON < SOY < CAS, and the difference between the CAS and β -CON groups was statistically significant (Table 6). A similar trend was observed in the activities of liver cytosolic malic enzyme and G6PDH. The effect of dietary protein on the activity of liver microsomal PAP was not clear. In contrast, the activities of mitochondrial CPT and peroxisomal ACO were comparable among the groups.

The CPT activity in brown adipose tissue was comparable among the groups (data not shown).

3.6 Gene expression related to lipid metabolism in the liver

In accordance with the specific activity, gene expression of hepatic FAS was the lowest in the β -CON group followed by that in the SOY and CAS groups (Fig. 2). The expression of sterol regulatory element-binding protein (SREBP)-1c mRNA also tended to be low in the order, β -CON < SOY < CAS, and its expression in the β -CON group was on average half compared to that of the CAS group, though the trend was not statistically significant (Fig. 2). The expression of liver X receptor α (LXR α) mRNA tended to be lower in the β -CON group than in the CAS and SOY groups, and the difference was just below the statistically significant.

Table 4 Effect of dietary β -conglycinin on plasma concentrations of lipids, glucose and hormones in OLETF rats.

	Groups		
	CAS	SOY	β -CON
Cholesterol (mmol/L)	3.07 $\pm$ 0.16	2.69 $\pm$ 0.17	2.70 $\pm$ 0.31
Triglyceride (mmol/L)	1.62 $\pm$ 0.16	1.63 $\pm$ 0.13	1.59 $\pm$ 0.15
Phospholipid (mmol/L)	2.49 $\pm$ 0.15	2.39 $\pm$ 0.15	2.32 $\pm$ 0.25
Free fatty acid (mmol/L)	0.673 $\pm$ 0.039	0.586 $\pm$ 0.047	0.639 $\pm$ 0.073
Glucose (mg/dL)	230 $\pm$ 19	200 $\pm$ 12	202 $\pm$ 7
Insulin (ng/mL)	13.2 $\pm$ 1.4	10.6 $\pm$ 1.7	9.38 $\pm$ 1.16
Index of insulin resistance	7.56 $\pm$ 1.08	4.88 $\pm$ 0.72	4.70 $\pm$ 0.61
Adiponectin (μ g/mL)	2.75 $\pm$ 0.11	2.63 $\pm$ 0.41	3.98 $\pm$ 0.83
Leptin (ng/mL)	32.0 $\pm$ 3.7	26.8 $\pm$ 2.2	26.9 $\pm$ 2.0

CAS, casein; SOY, soy protein isolate; β -CON, β -conglycinin.

Index of insulin resistance = plasma glucose (mg/dL) $\times$ plasma insulin (ng/mL)/405.

Data are expressed as mean $\pm$ SE of 6 rats.

Table 5 Effect of dietary β -conglycinin on liver lipid concentrations in OLETF rats.

	Groups		
	CAS	SOY	β -CON
Triglyceride (μ mol/g)	38.9 $\pm$ 1.3 ^a	33.2 $\pm$ 1.7 ^b	29.7 $\pm$ 1.0 ^b
Cholesterol (μ mol/g)	10.8 $\pm$ 0.8	10.3 $\pm$ 1.8	10.1 $\pm$ 1.4
Phospholipid (μ mol/g)	21.1 $\pm$ 1.6	22.5 $\pm$ 1.0	22.8 $\pm$ 0.2

CAS, casein; SOY, soy protein isolate; β -CON, β -conglycinin.

Data are expressed as mean $\pm$ SE of 6 rats.

Values without sharing a common superscript letter are significantly different at $p < 0.05$.

Table 6 Effect of dietary β -conglycinin on hepatic enzyme activities in OLETF rats.

	Groups		
	CAS	SOY	β -CON
	nmol/min/mg protein		
Cytosolic FAS	14.3 $\pm$ 2.1 ^a	13.5 $\pm$ 0.6 ^b	10.5 $\pm$ 0.6 ^b
Cytosolic malic enzyme	57.5 $\pm$ 7.2	63.6 $\pm$ 2.6	40.0 $\pm$ 5.6
Cytosolic G6PDH	79.0 $\pm$ 12.3	71.5 $\pm$ 8.7	53.1 $\pm$ 12.6
Microsomal PAP	6.97 $\pm$ 0.43	8.10 $\pm$ 0.47	7.67 $\pm$ 1.33
Mitochondrial CPT	5.14 $\pm$ 0.43	4.57 $\pm$ 0.58	5.32 $\pm$ 0.70
Peroxisomal ACO	2.64 $\pm$ 0.18	2.32 $\pm$ 0.23	2.11 $\pm$ 0.29

CAS, casein; SOY, soy protein isolate; β -CON, β -conglycinin. FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; PAP, phosphatidate phosphohydrolase; CPT, carnitine palmitoyltransferase; ACO, acyl CoA oxidase.

Data are expressed as mean $\pm$ SE of 6 rats.

Values without sharing a common superscript letter are significantly different at $p < 0.05$.

cant level ($p = 0.086$). Gene expression of insulin receptor substrate (IRS) 2 was significantly higher in the β -CON group than in the CAS and SOY groups.

3.7 Gene expression related to glucose metabolism in the gastrocnemius muscle

In the gastrocnemius muscle, the mRNA expression of peroxisome proliferator-activated receptor- γ coactivator-1 α

Fig. 2 Effect of dietary β -conglycinin on hepatic gene expressions in OLETF rats. Data are expressed as mean $\pm$ SE of 6 rats. Values without sharing a common letter are significantly different at $p < 0.05$. FAS, fatty acid synthase; SREBP 1c, sterol regulatory element-binding protein 1c; LXR, liver X receptor; IRS, insulin receptor substrate. Open bar, CAS group; hatched bar, SOY group; solid bar, β -CON group.

Fig. 3 Effect of dietary β -conglycinin on gastrocnemius muscle gene expressions in OLETF rats. Data are expressed as mean $\pm$ SE of 6 rats. Values without sharing a common letter are significantly different at $p < 0.05$. PGC1- α , peroxisome proliferator-activated receptor- γ coactivator-1 α ; CPT1b, carnitine palmitoyl-transferase 1b; AdipoR, adiponectin receptor; IRS, insulin receptor substrate; GLUT, glucose transporter. Open bar, CAS group; hatched bar, SOY group; solid bar, β -CON group.

(PGC1- α) and CPT1b was significantly higher in the β -CON and SOY groups than that in the CAS group (Fig. 3). The expressions of IRS1 and adiponectin receptor (AdipoR) 1 tended to be high in the order, β -CON > SOY > CAS. The expression of glucose transporter (GLUT) 4 mRNA tended to be higher in the β -CON group than in the CAS and SOY groups, though the trend was not statistically significant.

3.8 Gene expression related to lipid metabolism in the mesenteric white adipose tissue

In the mesenteric white adipose tissue, the mRNA level of peroxisomes proliferator-activated receptor (PPAR) γ in the SOY group was significantly higher, whereas the level in the β -CON group was much higher than that in the CAS group (Fig. 4). The mRNA expression of adiponectin tended to be high in the order, β -CON > SOY > CAS, and the expression in the β -CON group was approximately 1.9

Fig. 4 Effect of dietary β -conglycinin on mesenteric adipose gene expressions in OLETF rats.

Data are expressed as means $\pm$ SE of 6 rats. Values without sharing a common letter are significantly different at $p < 0.05$. PPAR γ , peroxisome proliferator-activated receptor γ ; HSL, hormone sensitive lipase; ATGL, adipose triglyceride lipase. Open bar, CAS group; hatched bar, SOY group; solid bar, β -CON group.

times higher on average compared with that in the CAS group, though the difference was not statically significant.

Gene expression of hormone-sensitive lipase (HSL) was significantly higher in the β -CON group than in the CAS and SOY groups. A similar trend was also observed in the mRNA level of adipose triglyceride lipase (ATGL) and GLUT 4, and the expressions in the β -CON group were approximately three times higher on average compared with that in the CAS group.

4 DISCUSSION

In the present study, we examined the effects of β -CON on insulin sensitivity as well as on body fat mass and on lipid metabolism in obese OLETF rats (at the pre-symptomatic stage of type 2 diabetes mellitus). Visceral adipose tissue weight was lower, whereas plasma adiponectin levels tended to be higher in rats fed the β -CON diet than in those fed the CAS diet. These results are consistent with those of previous reports^{5, 9, 10, 33}, and indicate that β -CON increases plasma adiponectin concentration and exhibits an anti-obesity effect. In the present study, dietary SOY also decreased the visceral adipose tissue weights, but the effect of SOY on plasma adiponectin concentration was not clear. These results suggest that SOY contains components other than β -CON, which possibly affect body fat mass such as glycinin³⁴ and the indigestible hydrolysate fraction (high molecular fraction) derived from SOY³⁵. The underlying mechanism for these components is not necessarily clear at this point, and is an issue for possible further research.

In the mesenteric adipose tissue, gene expression of HSL and ATGL was higher in rats fed the β -CON diet than in

those fed the CAS and SOY diets in the present study. Schweiger *et al.* demonstrated that both ATGL and HSL are enzymes responsible for more than 95% of triglyceride hydrolysis in mice adipose tissue³⁶. Therefore, it was suggested that dietary β -CON suppressed adipocyte hypertrophy through enhancement of lipolysis. In the present study, we observed that the gene expression of both adiponectin and PPAR γ in mesenteric adipose tissue was significantly higher in rats fed the β -CON diet than in those fed the SOY and CAS diets. PPAR γ agonists enhance the gene expression of adiponectin in adipose tissue and increase plasma adiponectin concentration in obese mice³⁷. Therefore, the results in the present study suggest that dietary β -CON increases plasma adiponectin concentration through upregulation of PPAR γ followed by adiponectin in adipose tissue. Since adiponectin is demonstrated to be an insulin-sensitizing adipokine^{38, 39}, a β -CON-dependent increase in plasma adiponectin concentration possibly affected insulin sensitivity in the OLETF rats.

Although the fasting blood glucose level was comparable among the groups over the feeding period in the present study, results from the ITT and the calculated index of insulin resistance show that dietary β -CON exhibited high insulin sensitivity compared to CAS. In this regard, dietary β -CON exhibited high expression of IRS2 in the liver, with high expression of AdipoR1 and IRS1 in the gastrocnemius muscle in the present study. Tachibana *et al.* suggested that dietary β -CON increases insulin sensitivity and improves glucose tolerance, accompanied with decreased plasma insulin levels in rats⁵. They also reported that dietary β -CON significantly decreases both plasma insulin and glucose concentrations in non-obese type 2 diabetic Goto-Kakizaki rats⁹. All the evidence supports the idea that dietary β -CON increased insulin sensitivity in obese

OLETF rats in the present study.

Although, the protein-dependent effect on plasma triglyceride concentration was not clear in the present study, the liver triglyceride concentration was significantly decreased by β -CON intake. The result was consistent with a previous study¹⁰. The β -CON-dependent effect could partly be due to a decrease in liver cytosolic FAS activity, which was associated with downregulation of FAS expression controlled by SREBP-1c (Fig. 3)⁴⁰. These results were also consistent with previous studies. They demonstrated that β -CON decreases fatty acid synthesis through transcriptional suppression of SREBP-1c signaling^{2, 5, 6}. As, circulating adiponectin has been shown to decrease the hepatic gene expression of SREBP-1c⁴¹, the β -CON-dependent decrease in hepatic fatty acid synthesis could be attributed to the enhancement of plasma adiponectin concentration. On the other hand, dietary β -CON did not affect the liver mitochondrial CPT activity, an index of mitochondrial fatty acid β -oxidation, in the present study. Therefore, modulation of fatty acid synthesis rather than fatty acid β -oxidation could be responsible for the β -CON-dependent decrease in liver triglyceride concentrations in the present study.

β -CON-dependent downregulation of hepatic SREBP-1c expression may not only result in decreased fatty acid synthesis, but may also modulate insulin sensitivity in the liver. High expression of hepatic SREBP-1c is known to contribute to hepatic insulin resistance through decreased expression of IRS2, a main mediator of insulin signaling, in mice⁴². It is also reported that SREBP-1c represses the transcription of IRS2 in the liver⁴². Indeed, the hepatic gene expression of SREBP-1c was lower, whereas that of IRS2 was higher, along with insulin sensitivity, which seemed to be higher in rats fed the β -CON diet than in those fed the CAS diet in the present study. Increased hepatic insulin sensitivity in rats fed the β -CON diet could be a factor for the decreased plasma insulin concentration. It is known that insulin induces SREBP-1c with the requirement of LXR α , a nuclear receptor, in the insulin signaling pathway^{43, 44}. Therefore, lower plasma insulin concentration in rats fed the β -CON diet might not induce SREBP-1c, but may instead contribute to decreasing fatty acid synthesis in the liver. Thus, the results in the present study suggest that β -CON-dependent increase in plasma adiponectin downregulates hepatic SREBP-1c, and modulates fatty acid synthesis and insulin sensitivity in the liver.

Skeletal muscle is a major organ for glucose and energy consumption. PGC-1 α , a nuclear receptor coactivator, is known to play an important role in regulating glucose metabolism⁴⁵ and fatty acid β -oxidation^{46, 47} in the muscle. PGC-1 α is reported to be upregulated by adiponectin and its receptor, AdipoR1 in mouse muscle⁴⁸. Consistent with the evidence, β -CON intake increased the adiponectin level in plasma and the AdipoR1 gene expression in the gastroc-

nemius muscle, and then increased the muscle gene expression of PGC-1 α in the present study. It was demonstrated that PGC-1 α could stimulate the gene expression of CPT and increase its enzyme activity^{45, 49}. It was also suggested that overexpression of CPT in skeletal muscle upregulated IRS1, and in turn improved insulin resistance⁵⁰. Indeed, β -CON intake induced the gene expression of CPT1 and IRS1 in the gastrocnemius muscle in the present study. β -CON might also promote serine phosphorylation of IRS1, through a protein-dependent increase in plasma adiponectin levels⁵¹. PGC-1 α has been shown to induce gene expression of CPT1 as well as GLUT4 expression in mouse cells⁴⁸. Consistent with these references, β -CON tended to increase the muscle gene expression of GLUT4 in the present study. Tachibana *et al.* also suggested that dietary β -CON induced the translocation of GLUT4 into the plasma membrane¹¹. Therefore, the β -CON-derived increase in insulin sensitivity could be in part due to an increase in gene expression and phosphorylation of IRS1 and the gene expression and translocation of GLUT4 in the muscle. The results of the present study suggest that dietary β -CON regulates lipid and glucose metabolism partly through an increased plasma adiponectin level, and modulates insulin sensitivity in OLETF rats.

5 CONCLUSIONS

Dietary β -CON ameliorated insulin sensitivity and decreased the mesenteric adipose tissue weight and liver triglyceride concentration in obese OLETF rats. This effect could be attributed at least in part to an increase in plasma adiponectin concentration and upregulation of insulin receptor substrate in the liver and muscle. Therefore, the β -CON-dependent increase in plasma adiponectin concentration and the resulting increase in insulin sensitivity seem to cause a decrease in lipogenesis in the liver and an increase in fatty acid β -oxidation in the muscle, accompanied by a low plasma insulin level. To our knowledge, this is the first report to identify a mechanism for the dietary β -CON-mediated modulation of insulin sensitivity and lipid metabolism in OLETF rats.

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Conflict of interests

The authors declare no conflicts of interest associated

with this manuscript.

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