

# Okara ameliorates glucose tolerance in GK rats

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**Okara, a food by-product from the production of *tofu* and soy milk, is rich in three beneficial components: insoluble dietary fiber,  $\beta$ -conglycinin, and isoflavones. Although isoflavones and  $\beta$ -conglycinin have recently been shown to improve glucose tolerance, the effects of okara have not yet been elucidated. Therefore, we herein investigated the effects of okara on glucose tolerance in Goto-Kakizaki (GK) rats, a representative animal model of Japanese type 2 diabetes. Male GK rats were fed a 10% lard diet with or without 5% dry okara powder for 2 weeks and an oral glucose tolerance test was performed. Rats were then fed each diet for another week and sacrificed. The expression of genes that are the master regulators of glucose metabolism in adipose tissue was subsequently examined. No significant differences were observed in body weight gain or food intake between the two groups of GK rats. In the oral glucose tolerance test, increases in plasma glucose levels were suppressed by the okara diet. The mRNA expression levels of PPAR $\gamma$ , adiponectin, and GLUT4, which up-regulate the effects of insulin, were increased in epididymal adipose tissue by the okara diet. These results suggest that okara provides a useful means for treating type 2 diabetes.**

**Key Words:** okara, PPAR $\gamma$ , adiponectin, diabetes mellitus, animal model

The incidence of type 2 diabetes is increasing worldwide, especially in Asia. Individuals with diabetes are at an increased risk of developing a number of serious health problems due to diabetic complications. Chronic hyperglycemia may lead to neuropathy, retinopathy, nephropathy, and macrovascular disease. Furthermore, individuals with uncontrolled diabetes are also more susceptible to infections. Diabetic complications may be delayed or prevented by maintaining blood glucose levels, blood pressure, and cholesterol close to normal ranges. Therefore, individuals with diabetes need to undergo lifestyle modifications such as diet and exercise, and, if indicated, receive drug therapy.

Scientifically validated food-based interventions are a practical means of addressing the epidemic of type 2 diabetes. Okara is a pulp that consists of the insoluble parts of the soybean, which is a by-product of the production of soy milk and *tofu*. It is considered a functional food in Asia and contains three beneficial components: insoluble dietary fiber, soy proteins, and isoflavones. Although isoflavones and soy proteins have been shown to improve glucose tolerance, the effects of okara on diabetes have not yet been elucidated in detail.<sup>(1,2)</sup>

One of the characteristics of type 2 diabetes is a selective impairment in the insulin secretory response of pancreatic  $\beta$  cells to glucose.<sup>(3)</sup> Glucose-stimulated insulin secretion was previously shown to be selectively impaired in Goto-Kakizaki (GK) rats, a polygenetic model of type 2 diabetes mellitus.<sup>(4)</sup> Thus, GK rats are an insulinopenic diabetic model that may represent the pathophysiology of Japanese type 2 diabetes.

Adipocytes and  $\beta$  cells play pivotal roles in regulating glucose metabolism. The growth and differentiation of adipocytes are

regulated by PPAR $\gamma$ , which is highly expressed in adipose tissue. The activation of PPAR $\gamma$  by agonists such as thiazolidinediones has been shown to increase the number of small adipocytes, thereby elevating the amount of the insulin-sensitizing hormone, adiponectin.<sup>(5,6)</sup> Adiponectin is known to decrease blood glucose levels using several mechanisms, including the suppression of hepatic gluconeogenesis, stimulation of  $\beta$  oxidation in the liver, and stimulation of glucose uptake in skeletal muscle.<sup>(7)</sup> Adiponectin receptor agonists such as AdipoRon were recently found to ameliorate glucose tolerance in mice.<sup>(8)</sup> Previous studies reported that thiazolidinediones improved glucose tolerance in patients with type 2 diabetes.<sup>(9–13)</sup> Rosiglitazone, a synthetic PPAR $\gamma$  agonist, has been shown to increase the mRNA expression levels of GLUT4 in adipose tissue in a diabetic animal model and in patients with type 2 diabetes.<sup>(14,15)</sup> Since GLUT4 is rate-limiting for insulin-stimulated glucose uptake, the up-regulated expression of the GLUT4 gene in adipose tissue may play a role in the insulin-sensitizing actions of PPAR $\gamma$  agonists. In the present study, we investigated the effects of okara on glucose tolerance and the gene expression of important regulators of glucose metabolism in adipose tissue. We fed GK rats a dietary okara-containing diet and performed glucose tolerance tests. We also examined the effects of okara on the mRNA expression levels of PPAR $\gamma$ , adiponectin, insulin receptor, and GLUT4 in white adipose tissue. Our results revealed that okara up-regulated the mRNA expression of PPAR $\gamma$  and adiponectin in GK rats, and exerted anti-diabetic effects.

## Materials and Methods

**Chemicals.** Dry okara powder, which was provided by Kyowa Co. (Osaka, Japan), was stored at 4°C until use. All other chemicals were reagent grade and obtained mostly from Wako Pure Chem. Ind., Ltd. (Osaka, Japan).

**Animals.** Male GK rats (4–6 weeks old) were purchased from Japan SLC Co. (Shizuoka, Japan), and individually housed in an air-controlled room (24°C) with a 12-h light/dark cycle (8:00–20:00). Food and water were provided *ad libitum*. Age-matched male Wistar rats (origin of GK) were used as controls.

**Experimental protocol.** After 4 weeks of acclimation to normal chow, male GK rats were fed a 10% lard diet with or without 5% dry okara powder for 2 weeks. An oral glucose tolerance test was performed 2 weeks after beginning the study. Rats were then fed each diet for an additional week. The composition of the lard diet was (g/100 g)  $\beta$ -starch, 39.52;  $\alpha$ -starch, 12.55; sucrose, 10; casein, 20; lard, 10; cellulose, 5.0; salt mixture,<sup>(16)</sup> 2.73; choline chloride, 0.1; and vitamin mixture,<sup>(16)</sup> 0.1. The major fatty acid compositions of the lard used were (%) 14:0, 1.7; 16:0, 25.1; 18:0, 14.4; 16:1, 2.5; 18:1, 43.2; 18:2, 9.6, and 18:3, 0.5. Five grams of okara powder was added to 100 g of the lard diet.

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**Table 1.** Composition of okara (per 100 g)

Water	6.1 g
Protein	25.1 g
β-conglycinin	17%
glycinin	39%
lipoprotein	44%
Fat	13.2 g
Ash	3.4 g
Carbohydrate	52.2 g
Fiber	46.2 g
Energy	336 kcal
Sodium	12.6 mg
Calcium	210 mg
Thiamin	0.38 mg
Trypsin inhibitor	2.4 TIU/mg
Phytic acid	500 mg
Soybean saponin	150 mg
Soy isoflavones	0.19 g

GK rats treated with okara were pair-fed to the food intake of those not treated with okara. Wistar rats treated with okara were also pair-fed to the food intake of those not treated with okara. Body weights were measured twice a week. The ingredient composition of okara was shown in the Table 1.

Rats were anesthetized with isoflurane using an anesthetic vaporizer (MK Vapo, Muromachi Kikai Co., Japan). Blood was collected using a heparinized syringe from the inferior vena cava while under anesthesia and plasma was obtained by centrifuging heparinized blood at 4°C for 10 min at 1,200 × g. The liver, plasma, and adipose tissues were quickly removed, frozen with liquid nitrogen, and stored at -80°C to extract RNA and total lipids. All experiments were performed in the laboratories of the Tezukayama-gakuin University in accordance with the Declaration of Helsinki. The Animal Care Committee of Tezukayama-gakuin University approved animal care and procedures.

**Measurements of glucose, insulin, and triacylglycerol concentrations.** An oral glucose tolerance test (OGTT) was performed using orally loaded glucose (2 g/kg body weight). Plasma glucose levels were measured using the Glucose II test (Wako Pure Chem. Ind.). Plasma insulin levels were measured using the rat insulin RIA kit (EMD Millipore Corporation, Billerica, MA). The areas under the curve (AUC) of glucose and insulin were calculated using the trapezoidal rule. Plasma and

liver triacylglycerols (TAG) were enzymatically quantified using the TAG E-Test Wako (Wako Pure Chem. Ind.).

**Total RNA extraction, cDNA synthesis, and real-time RT-PCR.** Total RNA was extracted from adipose tissue using Trizol reagent (Invitrogen, Carlsbad, CA) and cDNA was synthesized using the High Capacity RNA-to-cDNA kit (Applied Biosystem, Foster, CA). A TaqMan probe (Applied Biosystems) was then used to ensure the mRNA specificities of the primers and probes. The selected target genes were as follows: PPAR $\gamma$  (Rm00440945\_m1, 105 bp), adiponectin (Rn00595250\_m1, 63 bp), GLUT4 (Rn01752377\_m1, 89 bp), the insulin receptor (Rn01637243\_m1, 140 bp), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Rn01775763\_g1, 174 bp) as the endogenous control genes. Amplification was conducted using the Step One Real time PCR system (Applied Biosystems) under the following conditions: initial denaturation at 95°C for 10 s, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s.

**Statistical analysis.** The results are shown as the mean  $\pm$  SEM ( $n = 7-10$  rats). The main effects were analyzed by a two- or three-way ANOVA followed by the Tukey post hoc test.<sup>(17)</sup> Mean comparisons were conducted using a Bonferroni  $t$  test when the main effect was significant. If an interaction was found between the main effects, a mean comparison was conducted conditionally. All analyses were performed with SPSS software (ver. 22.0, IBM). Significance was set at  $p < 0.05$ .

## Results

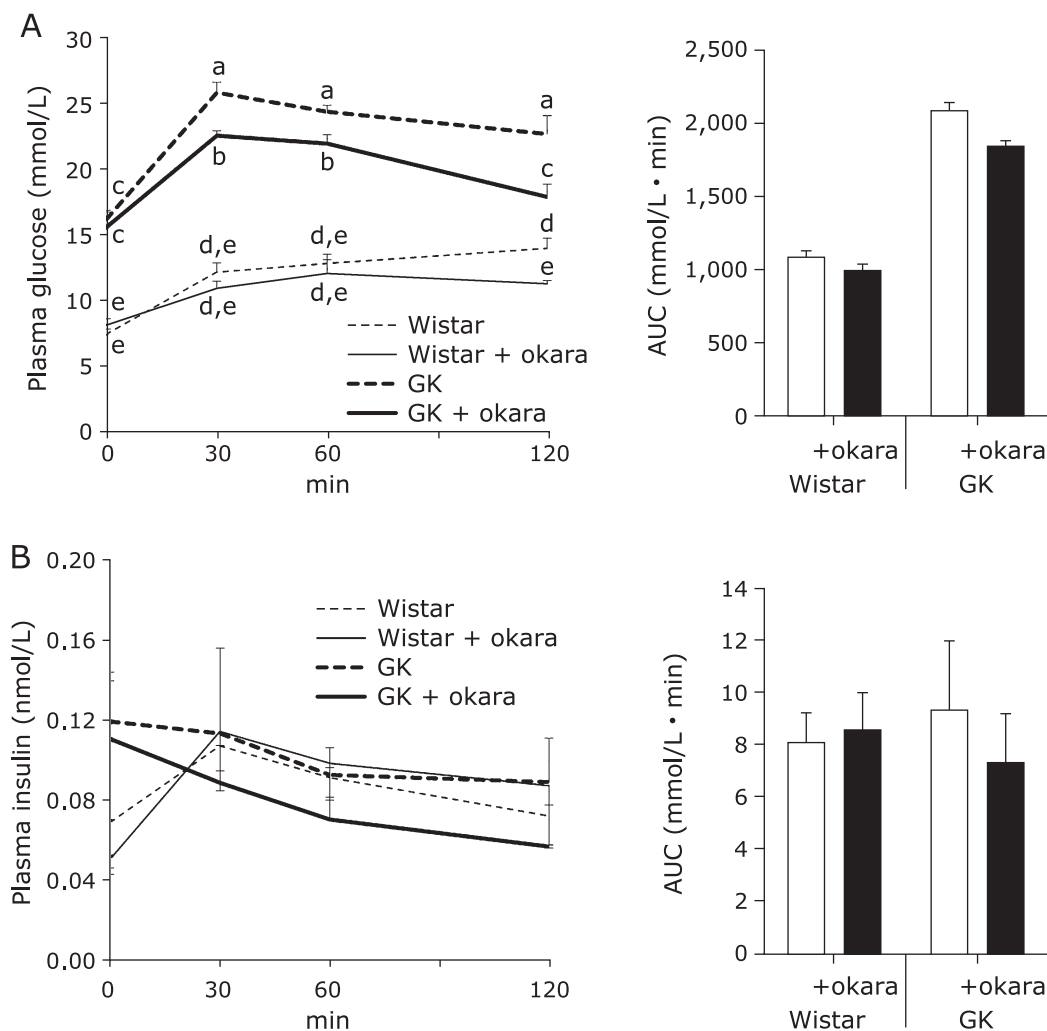
### Effects of okara on body and tissue weights and TAG levels in plasma and the liver.

Wistar and GK rats were fed a 10% lard diet with or without okara for 3 weeks. Body and tissue weights at the end of the experiment were shown in Table 2. No significant differences were observed in body weight gain between okara-treated and non-treated GK rats. Body weight gain was less in Wistar rats treated with okara than in non-treated rats. The amount of food consumed by Wistar rats with or without okara feeding was similar ( $5.67 \pm 0.18$  vs  $5.83 \pm 0.14$  g/day/100 g body weight, respectively). The energy intake of Wistar rats with or without okara feeding were also similar ( $23.5 \pm 0.8$  vs  $24.3 \pm 0.6$  kcal/day/100 g body weight, respectively). The amount of food consumed by GK rats with or without okara feeding was also similar ( $6.80 \pm 0.32$  vs  $6.95 \pm 0.25$  g/day/100 g body weight, respectively). No significant differences were observed in energy intake by GK rats with or without okara feeding ( $28.2 \pm 1.3$  vs  $29.1 \pm 1.0$  kcal/day/100 g body weight, respectively). Cecum weights at the end of the experiment were shown as g/100 g

**Table 2.** Body and tissue weights and triacylglycerol levels in plasma and the liver

Okara	Wistar		GK		$p$ values		
	-	+	-	+	Rat	Diet	R $\times$ D
Body (g)	404 $\pm$ 11.3	396 $\pm$ 16.2	280 $\pm$ 16.8	297 $\pm$ 11.5	0		
Increase ratio	1.41 $\pm$ 0.03 <sup>a</sup>	1.2 $\pm$ 0.04 <sup>b</sup>	1.07 $\pm$ 0.06 <sup>c</sup>	1.15 $\pm$ 0.03 <sup>c</sup>	0		0.002
Cecum (g/100 g bw)	0.85 $\pm$ 0.03	1 $\pm$ 0.04	0.51 $\pm$ 0.02	0.73 $\pm$ 0.04	0	0	
Liver (g/100 g bw)	3.55 $\pm$ 0.08 <sup>a</sup>	3.17 $\pm$ 0.04 <sup>a</sup>	3.94 $\pm$ 0.26 <sup>b</sup>	4.31 $\pm$ 0.13 <sup>b</sup>	0		0.016
Adipose tissues (g/100 g bw)							
Epididymal	1.17 $\pm$ 0.04	1.28 $\pm$ 0.07	1.27 $\pm$ 0.07	1.31 $\pm$ 0.04			
Perirenal	1.57 $\pm$ 0.09	1.66 $\pm$ 0.2	1.94 $\pm$ 0.2	2.38 $\pm$ 0.13	0.002		
Mesenteric	0.65 $\pm$ 0.03	0.68 $\pm$ 0.06	0.65 $\pm$ 0.04	0.58 $\pm$ 0.04			
Liver TAG (mg/g)	75.8 $\pm$ 6.9 <sup>a</sup>	47.6 $\pm$ 4.1 <sup>b</sup>	19.4 $\pm$ 1.1 <sup>c</sup>	15.6 $\pm$ 0.6 <sup>d</sup>	0	0.001	0.005
Plasma TAG (mg/dl)	161 $\pm$ 13.4	111 $\pm$ 9.6	243 $\pm$ 18	195 $\pm$ 9.2	0	0.004	

Wistar and GK rats were fed a 10% lard diet with or without 5% okara for 3 weeks. The body weights of rats at the end of the experiment are shown as the ratio to those at the start. The weights of the cecum, liver, and adipose tissues at the end of the experiment are shown as g/100 g body weight. Plasma and liver triacylglycerols were enzymatically quantified. Values are means  $\pm$  SE ( $n = 7-10$ ). Two-way ANOVA for body and tissues weights and triacylglycerol. Means with different letters are significantly different ( $p < 0.05$ ).



**Fig. 1.** The oral glucose tolerance test. Left: Plasma glucose levels (A) and insulin levels (B) after oral glucose loading following 2 weeks of the 10% lard diet with or without 5% okara are shown. Right: area under the curve (AUC) 0–120 for the time lines on the left. Values are means  $\pm$  SEM ( $n = 7-10$ ). Three-way ANOVA for plasma glucose: rat (R),  $p < 0.001$ ; diet (D),  $p < 0.001$ ; time (T),  $p < 0.001$ ; R  $\times$  D,  $p < 0.05$ ; R  $\times$  T,  $p < 0.001$ ; D  $\times$  T,  $p < 0.05$ . Means with different letters are significantly different ( $p < 0.05$ ). Two-way ANOVA for glucose AUC: rat (R),  $p < 0.001$ ; diet (D),  $p < 0.001$ . Plasma insulin levels were not significantly different. AUCs of plasma insulin were not significantly different.

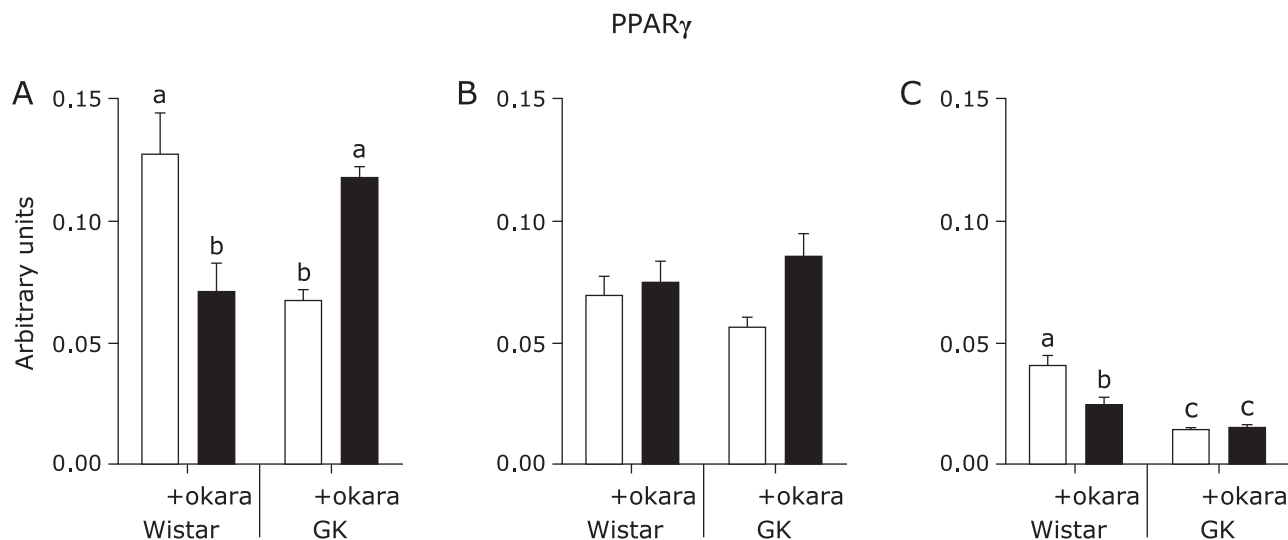
body weight in Table 2. The supply of okara markedly increased cecum weights in Wistar and GK rats, reflecting an increase in the uptake of dietary fiber. Liver weights were significantly lower in Wistar rats treated with okara than in non-treated rats. Liver weights were not lower in GK rats treated with okara than in those not fed okara. No significant differences were observed in the weights of the epididymal, perirenal, and mesenteric fat pads between Wistar and GK rats fed and not fed okara. Okara feeding significantly suppressed plasma and hepatic TAG levels in Wistar rats (Table 2). It also significantly decreased hepatic TAG levels, but not plasma TAG levels in GK rats.

**Glucose tolerance and insulin secretion *in vivo*.** The results of the oral glucose tolerance test are shown in Fig. 1. Blood glucose levels were higher in GK rats than in Wistar rats at 0, 30, 60, and 120 min regardless of the okara treatment (Fig. 1A). However, the blood glucose levels of okara-treated GK rats were significantly lower than non-treated GK rats 30, 60, and 120 min after oral glucose loading. The overall change in blood glucose levels in okara-treated GK rats, measured as AUC, was significantly lower than that in non-treated GK rats. In Wistar rats, the okara treatment did not significantly decrease blood glucose levels after oral glucose loading.

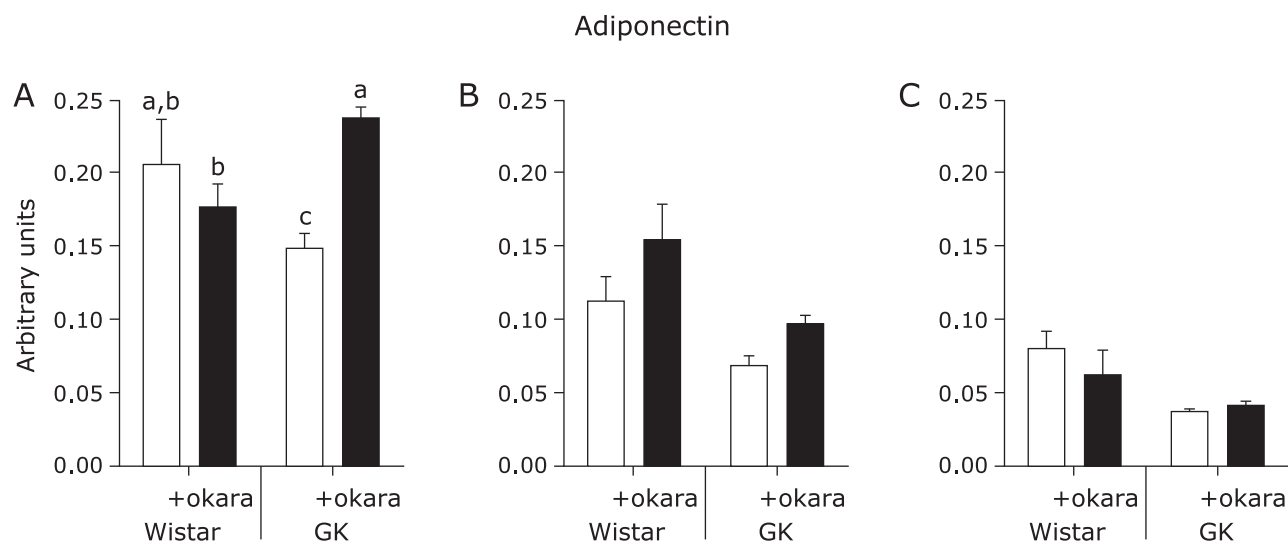
Fasting insulin levels were slightly higher in GK rats than in Wistar rats (Fig. 1B). The insulin response after oral glucose loading was severely impaired in GK rats regardless of the okara treatment. In Wistar rats, the insulin response peaked 30 min after oral glucose loading and the okara treatment did not affect insulin secretion during the test. Insulin secretion was slightly lower in okara-treated GK rats than in non-treated GK rats.

**Effects of okara on PPAR $\gamma$  mRNA expression levels in WAT (white adipose tissue).** In the epididymal fat pads of GK rats, mRNA expression levels of PPAR $\gamma$  were significantly higher in okara-treated rats than in non-treated rats (Fig. 2). In the epididymal and mesenteric fat pads of Wistar rats, mRNA expression levels of PPAR $\gamma$  were significantly lower in okara-treated rats than in non-treated rats. In the perirenal fat pads of Wistar rats, mRNA expression levels of PPAR $\gamma$  were similar in okara-treated and non-treated rats. In the epididymal and mesenteric fat pads, the expression levels of PPAR $\gamma$  mRNA were significantly lower in control GK rats than in control Wistar rats.

**Effects of okara on adiponectin mRNA expression levels in WAT (white adipose tissue).** In the epididymal fat pads of GK rats, mRNA expression levels of adiponectin were significantly higher in okara-treated rats than in non-treated rats (Fig. 3).



**Fig. 2.** mRNA expression levels of PPAR $\gamma$  in epididymal adipose tissue (A), perirenal adipose tissue (B), and mesenteric adipose tissue (C). mRNA expression levels in adipose tissues of Wistar and GK rats fed the 10% lard diet with or without 5% okara for 3 weeks. The open column represents rats fed the diet with okara and the closed column shows the diet without okara. Values are means  $\pm$  SEM ( $n = 7-11$ ). Two-way ANOVA for PPAR $\gamma$ : R  $\times$  D,  $p < 0.001$  in (A); R,  $p < 0.001$ ; D,  $p < 0.05$ ; R  $\times$  D,  $p < 0.05$  in (C), respectively. Means with different letters are significantly different ( $p < 0.05$ ).



**Fig. 3.** mRNA expression levels of adiponectin in epididymal adipose tissue (A), perirenal adipose tissue (B), and mesenteric adipose tissue (C). mRNA expression levels in adipose tissues of Wistar and GK rats fed the 10% lard diet with or without 5% okara for 3 weeks are shown. The open column represents rats fed the diet with okara and the closed column shows the diet without okara. Values are means  $\pm$  SEM ( $n = 7-11$ ). Two-way ANOVA for adiponectin: R  $\times$  D,  $p < 0.05$  in (A); R,  $p < 0.01$  in (B); R,  $p < 0.05$  in (C), respectively. Means with different letters are significantly different ( $p < 0.05$ ).

In the mesenteric fat pads of control GK rats, mRNA expression levels of adiponectin were similar to those of okara-treated GK rats. In the epididymal, perirenal, and mesenteric fat pads of control Wistar rats, mRNA expression levels of adiponectin were similar to those of okara-treated Wistar rats.

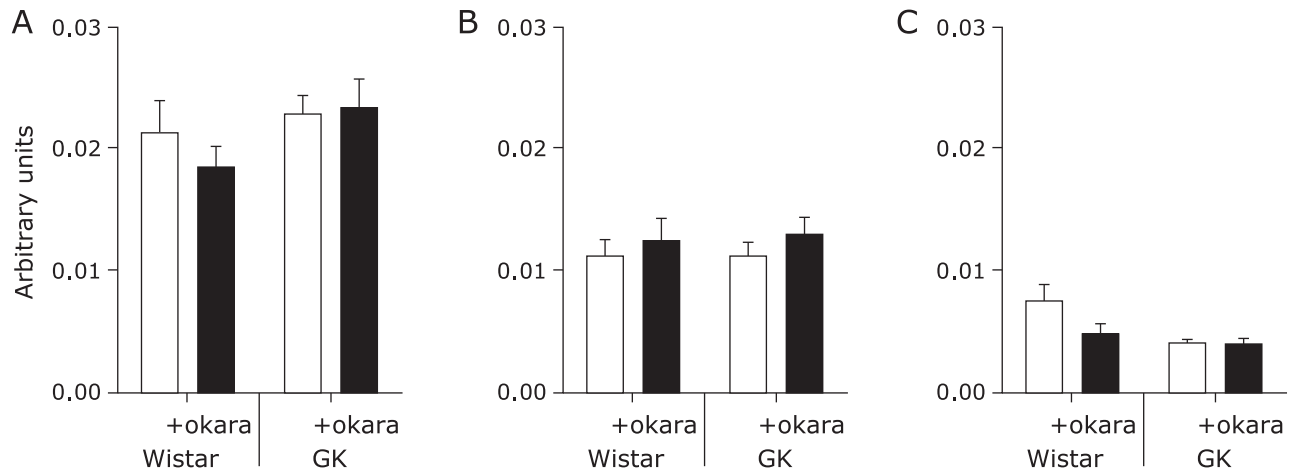
**Effects of okara on insulin receptor mRNA expression levels in WAT (white adipose tissue).** mRNA expression levels of the insulin receptor in the epididymal, perirenal, and mesenteric fat pads of GK and Wistar rats were not affected by okara feeding (Fig. 4). In the epididymal and perirenal fat pads of control GK rats, mRNA expression levels of the insulin receptor were similar to those of control Wistar rats.

**Effects of okara on GLUT4 mRNA expression levels in WAT (white adipose tissue).**

In the epididymal fat pads of GK rats, mRNA expression levels of GLUT4 were significantly higher in okara-treated rats than in controls (Fig. 5). In the epididymal fat pads of control GK rats, mRNA expression levels of GLUT4 were lower than those of control Wistar rats.

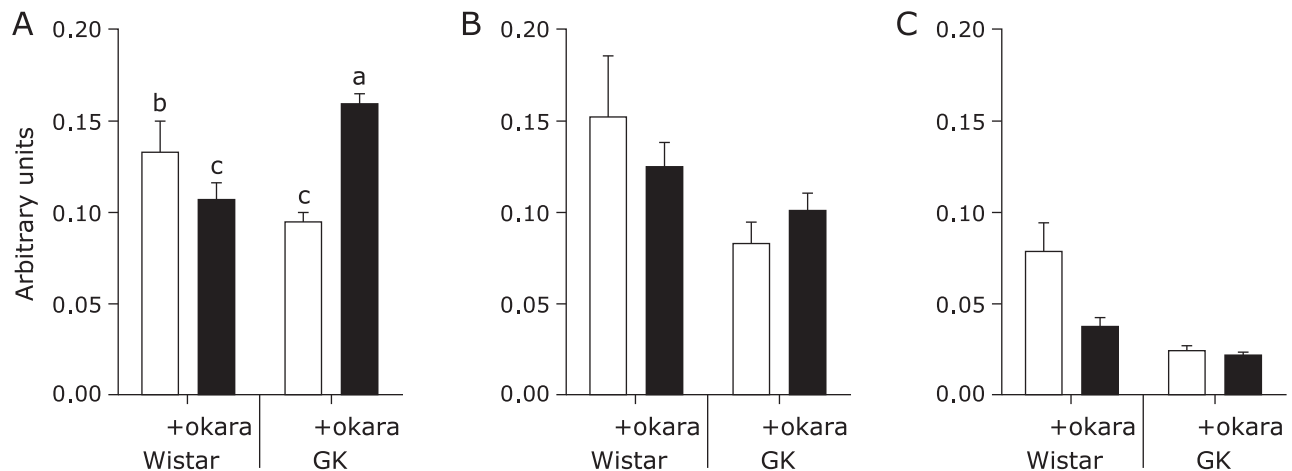
**Plasma glucose and insulin levels.** At the end of the experiment, rats were sacrificed and blood samples were collected from the inferior vena cava. Casual glucose levels were not decreased by okara feeding in Wistar or GK rats (Fig. 6). Plasma insulin levels in control GK rats were similar to those in control Wistar rats. Plasma insulin levels in Wistar and GK rats were not changed by okara feeding.

## Insulin receptor



**Fig. 4.** mRNA expression levels of the insulin receptor in epididymal adipose tissue (A), perirenal adipose tissue (B), and mesenteric adipose tissue (C). mRNA expression levels in adipose tissues of Wistar and GK rats fed the 10% lard diet with or without 5% okara for 3 weeks are shown. The open column represents rats fed the diet with okara and the closed column shows the diet without okara. Values are means  $\pm$  SEM ( $n = 7-11$ ). Two-way ANOVA for the insulin receptor revealed no significant differences.

## GLUT4



**Fig. 5.** mRNA expression levels of GLUT4 in epididymal adipose tissue (A), perirenal adipose tissue (B), and mesenteric adipose tissue (C). mRNA expression levels in adipose tissues of Wistar and GK rats fed the 10% lard diet with or without 5% okara for 3 weeks. The open column represents rats fed the diet with okara and the closed column shows the diet without okara. Values are means  $\pm$  SEM ( $n = 7-11$ ). Two-way ANOVA for GLUT4: R  $\times$  D,  $p < 0.01$  in (A); R,  $p < 0.01$ ; D,  $p < 0.05$  in (C), respectively. Means with different letters are significantly different ( $p < 0.05$ ).

## Discussion

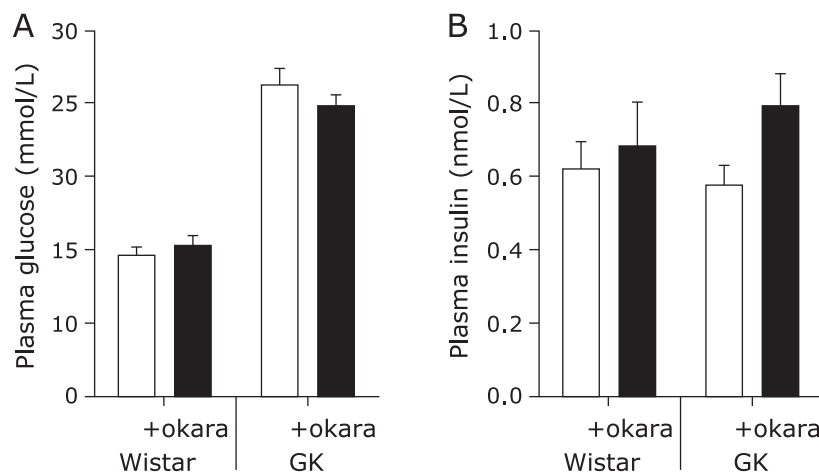
This is the first study to demonstrate that an okara treatment ameliorated glucose tolerance in a diabetic animal model. It is generally accepted that the two main causes of diabetes are increased insulin resistance and impaired insulin secretion. Impaired insulin secretion is well known in GK rats, whereas insulin resistance caused by chronic inflammation in adipose tissue has only recently been reported.<sup>(18)</sup>

Furthermore, the expression of adiponectin mRNA in adipose tissue was shown to be decreased in GK rats.<sup>(19)</sup> In the present study, we found that the okara treatment significantly increased the mRNA expression levels of PPAR $\gamma$  and adiponectin in the white adipose tissues of GK rats. The activation of PPAR $\gamma$  is

known to induce apoptosis in enlarged adipocytes, and decrease the secretion of inflammatory adipocytokines such as TNF- $\alpha$ , thereby increasing insulin sensitivity. The activation of PPAR $\gamma$  has also been shown to increase the number of small adipocytes, which, in turn, increases the amount of insulin-sensitizing hormone, adiponectin.<sup>(5,6)</sup>

In the oral glucose tolerance test on GK rats, we found that plasma glucose levels were suppressed by the okara diet without changing insulin levels, and this may have reflected an increase in insulin sensitivity.

GLUT4 is the key effector molecule in insulin-stimulated glucose uptake into adipocytes and myocytes. The overexpression of GLUT4 by genetic engineering in diabetic mice has been reported to improve insulin resistance.<sup>(20,21)</sup> Wang *et al.*<sup>(22)</sup> demon-



**Fig. 6.** Casual glucose levels (A) and insulin levels (B) in the plasma of Wistar and GK rats fed the diets for 3 weeks. The open column represents rats fed the diet with okara and the closed column shows the diet without okara. Values are means  $\pm$  SEM ( $n = 7-10$ ). Two-way ANOVA for plasma glucose: rat (R),  $p < 0.001$ . No significant differences were observed in plasma insulin levels.

strated that an increase in PPAR $\gamma$  inhibited the expression of TNF- $\alpha$ , which, in turn, enhanced that of GLUT4 in adipose tissue. We showed that the okara treatment significantly increased the mRNA expression levels of GLUT4 in the white adipose tissue of GK rats, suggesting the alleviation of insulin resistance. Consequently, okara may exert anti-diabetic effects, in part at least, through the PPAR $\gamma$ -mediated pathway in GK rats.

Regarding triglyceride metabolism, adiponectin is known to act on hepatocytes and increase the  $\beta$  oxidation of fatty acids in mitochondria in the liver, thereby decreasing plasma TAG. We showed that the okara diet decreased plasma and hepatic TAG levels, which is consistent with previous findings.<sup>(2)</sup>

In the present study, okara decreased the mRNA expression levels of PPAR $\gamma$ , but not those of adiponectin in the adipose tissue of Wistar rats. Okara feeding did not affect normal glucose tolerance in Wistar rats. These results in Wistar rats suggest that as yet undetermined components in okara may be involved in decreasing the mRNA expression levels of PPAR $\gamma$  without affecting glucose tolerance *in vivo*. Therefore, further investigations are warranted.

Okara contains three beneficial components: insoluble dietary fiber,  $\beta$ -conglycinin, and isoflavones. In the present study, the okara diet contained isoflavones (9.5 mg/100 g diet) and  $\beta$ -conglycinin (0.21 g/100 g diet). Tachibana *et al.*<sup>(2)</sup> previously reported that a diet containing a higher amount of  $\beta$ -conglycinin (21.3 g/100 g diet), a soy protein, increased the mRNA expression of adiponectin in the white adipose tissue of GK rats. Mezei *et al.*<sup>(1)</sup> also found that a diet containing a higher amount of isoflavones (116 mg/100 g diet) improved glucose tolerance in Obese Zucker rats, another diabetic rodent. A recent meta-analysis of randomized controlled trials showed that an increase in fiber intake improved glycemic control in type 2 diabetes.<sup>(23)</sup> Our okara diet (7.3 g/100 g diet) and non-okara diet (5.0 g/100 g diet) both contained fiber. A major fiber in okara is cellulose. A higher cellulose

diet (10 g/100 g diet) was shown to alleviate hyperglycemia in diabetic rats.<sup>(24)</sup> Our okara diet contained smaller amounts of active ingredients (isoflavones,  $\beta$ -conglycinin, and fiber) than previously tested diets. Nevertheless, the combination of these ingredients may have had synergistic or additive effects on improving glucose tolerance.

A limitation of this study must be considered. Fecal TAG levels were not measured in the present study. To know the balancing of TAG, the fecal excretion may be one of important points. We previously measured the amount of TAG excretion in feces in Wistar rats which were fed in 10% lard diet containing 5% cellulose or fed in 10% lard diet not-containing fiber (unpublished data). As a result, the fecal TAG levels in Wistar rats fed in the 5% cellulose diet were  $1.27 \pm 0.18$  mg/day, while lard intake were  $1.64 \pm 0.11$  g/day ( $n = 3$ ). The fecal TAG levels in those fed the fiber-free diet were  $1.35 \pm 0.23$  mg/day, while lard intake was  $1.32 \pm 0.10$  g/day ( $n = 4$ ). These results suggest that very small amount of TAG was excreted in feces when compared to the large amount of ingested lipid in the both diet (<1%). Therefore, the effects of fecal TAG on liver TAG levels, if any, are considered to be minor. Similar to these data in Wistar rats, those in GK rats may show that small amount of TAG is excreted in feces when compared to the large amount of ingested lipid.

Collectively, the results of the present study suggest that okara provides a useful means for treating type 2 diabetes.

## Acknowledgments

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## Conflict of Interest

No potential conflicts of interest were disclosed.

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