Original Article

Anti-diabetic effects of lactic acid bacteria in normal and type 2 diabetic mice

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The antidiabetic effects of lactic acid bacteria were investigated using mice. In Experiment 1, normal ICR mice were loaded with sucrose or starch with or without viable *Lactobacillus rhamnosus* GG cells. GG significantly inhibited postprandial blood glucose levels when administered with sucrose or starch. In Experiment 2, KK-A^y mice, a model of genetic type 2 diabetes, were given a basal diet containing viable GG cells or viable *Lactobacillus delbrueckii* subsp. *bulgaricus* cells for 6 weeks. Viable GG cells significantly inhibited fasting blood glucose, postprandial blood glucose in a glucose tolerance test and HbA1c. Such effects were not shown by viable *L. bulgaricus* cells. In Experiment 3, the KK-A^y mice were given a basal diet containing viable GG cells or heat-treated GG cells for 3 weeks. The viable GG cells significantly suppressed fasting blood glucose and impaired glucose tolerance, but the heat-treated GG showed no effects. These results demonstrated that GG decreased the postprandial blood glucose in ICR mice, and that the antidiabetic activity of lactic acid bacteria on the KK-A^y mice differed depending on the bacterial strain and whether the bacterium is viable when it arrives in the intestine. In the present study, we conclude that the antidiabetic activity may result from continuous inhibition of the postprandial blood glucose through suppression of glucose absorption from the intestine. These findings indicate that specific strains of lactic acid bacterium can be expected to be beneficial for the management of type 2 diabetes.

***Key Words*:** **lactic acid bacterium, antidiabetic effects, *Lactobacillus rhamnosus* GG, glucose tolerance test, KK-A^y mouse**

Lactic acid bacteria are widely distributed in the natural world. Various species including *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*), used for the production of fermented milk in many countries for thousands of years, are used in the field of food processing. Recently, the functionality of probiotic lactic acid bacteria has received a great deal of attention worldwide. Probiotics are defined as "living microorganisms and products containing microbial metabolites which, *in vivo*, affect the normal intestinal bacterial flora by improving its balance, and which are consequently biologically beneficial".[1,2]

Lactobacillus rhamnosus GG (GG) is isolated from the feces of healthy humans, and has been shown to survive passage through the human gastrointestinal tract.[3] Some beneficial effects of GG have been reported, and it is considered one of the probiotic lactic acid bacterium.[4–7] We previously showed that the daily administration of viable GG cells decreased the blood glucose level in a genetic type 2 diabetes model, KK-A^y mice.[8] However, the effect of GG on the postprandial blood glucose level is not known. Antidiabetic effects of lactic acid bacteria on KK-A^y mice have been reported for *Lactobacillus casei*,[9] but an insufficient number of studies have been undertaken on other species of lactic acid bacteria.

In this study, we performed three experiments. We examined the effect of single administration of viable GG cells on normal ICR mice in Experiment 1. In Experiment 2, we investigated the effect of daily administration of viable GG cells or *L. bulgaricus* cells on blood glucose levels of KK-A^y mice. In Experiment 3, we examined the effect of daily administration of viable and heat-treated GG cells on KK-A^y mice.

Materials and Methods

Preparation of lyophilized cells. The *Lactobacillus rhamnosus* GG (GG) and *Lactobacillus delbrueckii* subsp. *bulgaricus* LB3 (*L. bulgaricus*) used in this study were obtained from Takanashi Milk Products (Yokohama, Japan). Each strain was cultured in MRS broth (Oxoid Ltd., Basingstoke, Hampshire, England) and incubated at 37°C for 24 h. After cultivation, the cells were harvested by centrifugation at 3,000 × *g* for 15 min and washed twice with sterile distilled water. The cells were lyophilized and stored at −80°C. Heat-treated GG cells were prepared by heating lyophilized GG cells at 121°C for 15 min, and were kept at −80°C.

Animals. Eight-week-old male ICR normal mice and 4-week-old male KK-A^y male mice, a model of genetic type 2 diabetes, were obtained from Clea Japan (Tokyo, Japan). The animals were maintained in accordance with the guidelines of governmental legislation in Japan (1980, 2006) and were kept in an air-conditioned room at 23 ± 1°C with 55 ± 5% humidity on a cycle of 12 h light-dark cycle (lights on from 7:00 to 19:00). They were provided standard laboratory chow (CE-2; Clea Japan Inc., Tokyo, Japan) and tap water *ad libitum* during the period prior to the experiments.

Experiment 1. Single administration in carbohydrate tolerance test on normal mice. Normal ICR mice that had been fasting overnight for 18 h were divided into 2 groups ($n = 6$), a control group and a viable GG (V-GG) group. The control group was given 0.2 ml of carbohydrate solution (1 g/kg body weight), and the V-GG group was given 0.2 ml of a mixture of carbohydrate (1 g/kg body weight) and viable GG cells. Sucrose or soluble starch was used as the carbohydrate. Viable GG cells at concentrations of 3.984 g per 100 g of the sucrose or 13.33 g per 100 g of the starch were suspended in the carbohydrate solution. These ratios are the same as those of the experimental diet when we previously administered GG to KK-A^y mice.[8] Blood samples were collected from the tail vein at 0, 15, 30, 60 and 120 min after administration and the glucose levels determined by the glucose

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oxidase method (Glutest Ace, Sanwa Kagaku Kenkyusho, Nagoya, Japan).

Experiment 2. Continuous administration of GG or *L. bulgaricus* in KK-A^y mice. The KK-A^y mice were divided on the basis of their mean body weight and blood glucose level into 3 groups: the control, GG, and bulgaricus groups (n = 7 each). Each group was given an experimental diet as shown in Table 1. The control group received a basal diet, the GG group received a basal diet containing 0.5% viable GG cells and the bulgaricus group received a basal diet containing 0.5% viable *L. bulgaricus* cells. All groups were fed their diets *ad libitum* for 6 weeks. Body weight and fasting blood glucose were measured every other week. Blood samples were collected from the tail vein. Fasting blood glucose levels were measured after 6 h fasting by Glutest Ace. During the final week of the experimental period, a glucose tolerance test was performed, and HbA1c was measured after 18 h fasting. In the glucose tolerance test, 0.2 ml of a glucose solution (1 g/kg body weight) was administered orally. Glucose levels were measured at 0, 30, 60 and 120 min after administration. HbA1c was determined by the DCA-2000 system (Bio Medical, Tokyo, Japan).

Experiment 3. Continuous administration of viable GG or heat-treated GG on KK-A^y mice. The KK-A^y mice were divided on the basis of their mean body weight and blood glucose level into 3 groups: control, viable GG (V-GG), and heat-treated GG (HT-GG). Each group was given an experimental diet as shown in Table 2. The control group received a basal diet, the V-GG group received a basal diet containing 2% viable GG cells and the HT-GG group received a basal diet containing 2% heat-treated GG cells. All groups were fed their diets *ad libitum* for 3 weeks. Body weight and fasting blood glucose were measured weekly. During the final week of the experimental period, a glucose tolerance test was performed after 18 h fasting, and glucose levels were measured at 0, 15, 30, 60 and 120 min after administration. In addition, the fasting plasma insulin level was measured with a mouse insulin ELISA kit (Shibayagi Co., Gunma, Japan).

Statistical analysis. Experimental data are presented as means ± SEMs. Differences between the control and treatment groups were considered significant at $p<0.05$ by Student's t test and Dunnett's test. All statistical analyses were performed with SPSS Statistics ver. 19.0.

Table 1. Composition of experimental diets in Experiment 1 (%)

	Control	GG	Bulgaricus
Casein	20	20	20
DL-Methionine	0.3	0.3	0.3
Corn starch	15	15	15
Sucrose	50.2	50.2	50.2
Corn oil	5	5	5
Mineral mixture†	3.5	3.5	3.5
Vitamin mixture†	1	1	1
Cellulose	5	4.5	4.5
Viable *L.* GG cells	0	0.5	0
Viable *L. bulgaricus* cells	0	0	0.5
Total	100	100	100

†AIN-76; Oriental Yeast Co. Ltd., Tokyo, Japan.

Table 2. Composition of experimental diets in Experiment 2 (%)

	Control	V-GG	HT-GG
Casein	20	20	20
DL-Methionine	0.3	0.3	0.3
Corn starch	15	15	15
Sucrose	50.2	50.2	50.2
Corn oil	5	5	5
Mineral mixture†	3.5	3.5	3.5
Vitamin mixture†	1	1	1
Cellulose	5	3	3
Viable *L.* GG cells	0	2	0
Heat-treated *L.* GG cells	0	0	2
Total	100	100	100

†AIN-76; Oriental Yeast Co. Ltd., Tokyo, Japan.

Results

Effects of single administration of viable GG. Postprandial changes in blood glucose in mice loaded with carbohydrates are presented in Fig. 1. At 60 min after the sucrose administration, the amount of change in the blood glucose level of the V-GG group was significantly lower than that of the control group, and a tendency for lower levels in the V-GG group continued throughout the experimental period. Changes in blood glucose levels of the V-GG group were significantly lower than the control group at 30, 60 and 120 min after starch administration as well.

Effects of continuous administration of GG or *L. bulgaricus*. Fig. 2 shows the change of body weight and fasting blood glucose with the administration of GG or *L. bulgaricus*. There were no significant changes in body weight during the experiment. The fasting blood glucose level of the GG group was significantly

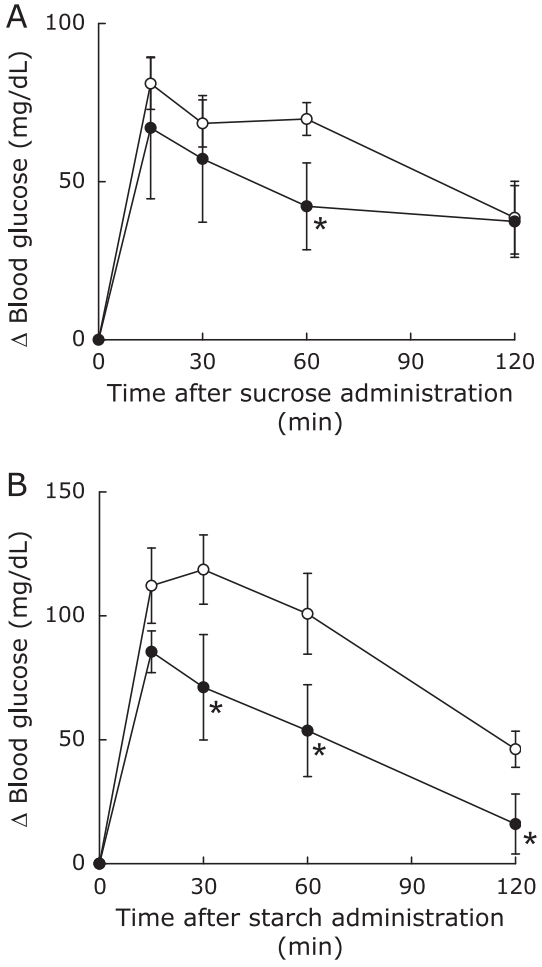

Fig. 1. Effect of administration of viable GG cells (V-GG) on sucrose (A) and starch (B) tolerance test. Each point represents the mean ± SE (n = 6). ○, control; ●, V-GG. *Values with asterisks are significantly different from the control-group values at $p<0.05$ by Student's t test.

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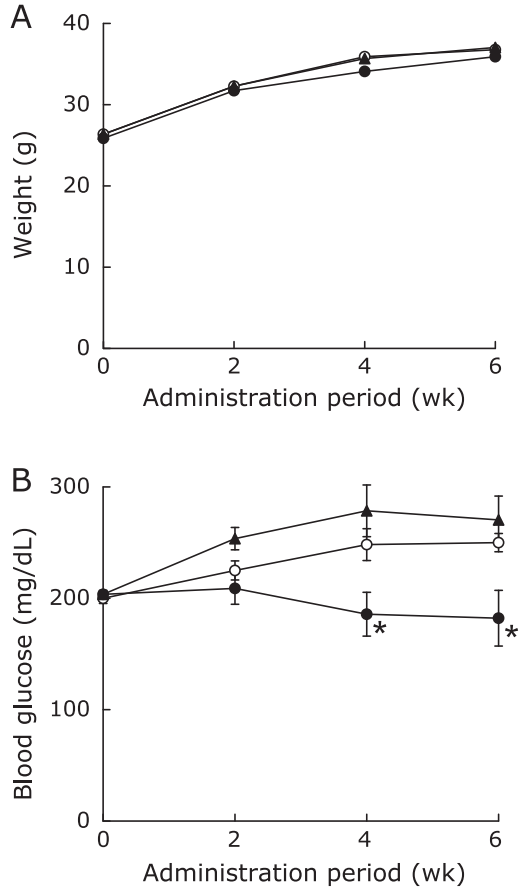

Fig. 2. Effect of administration of *L.* GG or *L. bulgaricus* on body weight (A) and fasting blood glucose (B) in KK-A^y mice. Each point represents the mean ± SE (n = 7). ○, control; ●, GG; ▲, bulgaricus. *Values with asterisks are significantly different from control-group values at p<0.05 by Dunnett's test.

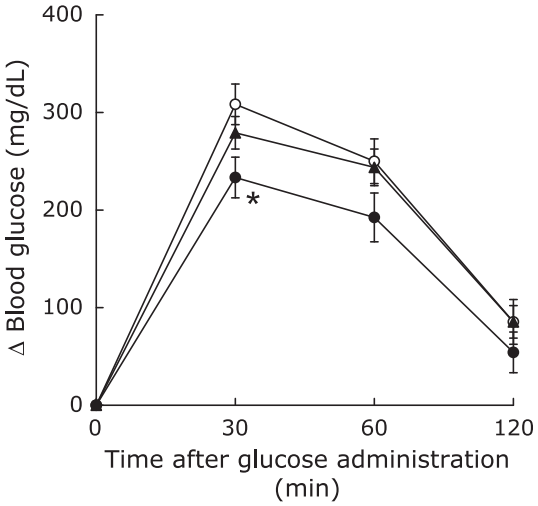

Fig. 3. Effect of administration of *L.* GG or *L. bulgaricus* on glucose tolerance test in KK-A^y mice at 6 weeks after the experiment began. Each point represents the mean ± SE (n = 7). ○, control; ●, GG; ▲, bulgaricus. *Values with asterisks are significantly different from control-group values at p<0.05 by Dunnett's test.

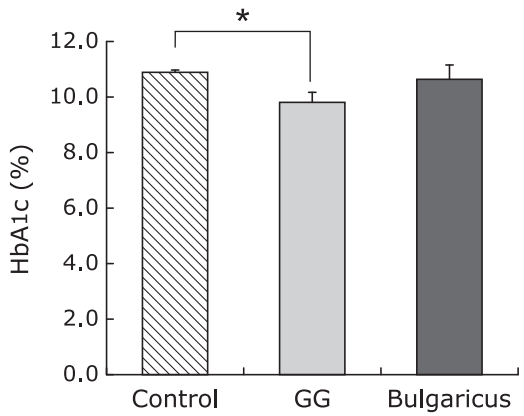

Fig. 4. Effect of administration of *L.* GG or *L. bulgaricus* on HbA1c in KK-A^y mice at 6 weeks after the experiment began. Each point represents the mean ± SE (n = 7). *Values with asterisks are significantly different from control-group values at p<0.05 by Dunnett's test.

lower than that of the control group, but those of the bulgaricus group and control group were similar. Fig. 3 shows the blood glucose level changes in the glucose tolerance test. The postprandial change in blood glucose levels of the GG group was significantly lower than that in the control group at 30 min after administration. However, those of the bulgaricus group and control group were almost the same. HbA1c levels in mice fed the experimental diet for 6 weeks are presented in Fig. 4. The HbA1c level of the GG group was significantly lower than that of the control group, while those of the bulgaricus group and control group were similar.

Effects of continuous administration of viable GG or heat-treated GG. Fig. 5 shows the changes of body weight and the fasting blood glucose levels with the administration of viable GG cells (V-GG group) or heat-treated GG cells (HT-GG group). There were no significant changes in body weight during the experiment. Two weeks after the experiment began, the fasting blood glucose level in the V-GG group was significantly lower than that in the control group. In the HT-GG group, the fasting blood glucose level increased, as in the control group. The results of the glucose tolerance test performed at 3 weeks of treatment are presented in Fig. 6. The amount of change of the blood glucose levels in the V-GG group tended to be lower than those of the control group during the experimental period. In addition, at 120 min after the administration, that of the V-GG group was significantly lower than that of the control group. The plasma insulin level at 3 weeks of treatment tended to be lower in the V-GG group than the control group, but levels in the HT-GG group and control group did not differ (Fig. 7).

Discussion

Previously, various foods including azuki beans extract have been reported to have antidiabetic effects in KK-A^y mice.[10–15] In addition, Itoh *et al.*[16] reported that postprandial blood glucose of normal mice was decreased by a single administration of azuki beans extract with carbohydrate through suppression of glucose absorption. There have been similar descriptions of the inhibition of postprandial blood glucose level in normal mice by other food materials for which the antidiabetic effects in KK-A^y mice have been reported.[17–20] Previously, we showed that daily administration of viable GG cells decreased the fasting blood glucose level in KK-A^y mice before the development of diabetes and in KK-A^y mice that developed severe diabetes.[8] Therefore, in our Experiment 1, the effect of a single administration of viable GG cells on postprandial blood glucose was investigated in normal ICR mice

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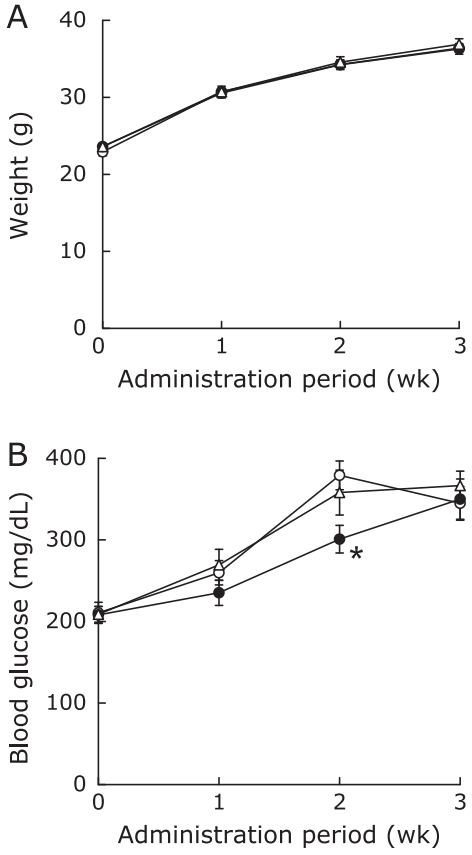

Fig. 5. Effect of administration of viable GG cells (V-GG) or heat-treated GG cells (HT-GG) on body weight (A) and fasting blood glucose (B) of KK-A^y mice. Each point represents the mean ± SE (n = 7). ○, control; ●, V-GG; △, HT-GG. *Values with asterisks are significantly different from control-group values at p<0.05 by Dunnett's test.

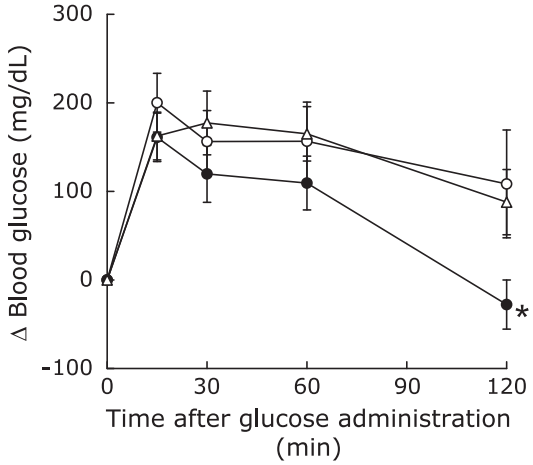

Fig. 6. Effect of administration of viable GG cells (V-GG) or heat-treated GG cells (HT-GG) on glucose tolerance test in KK-A^y mice at 3 weeks after the experiment began. Each point represents the mean ± SE (n = 7). ○, control; ●, V-GG; △, HT-GG. *Values with asterisks are significantly different from control-group values at p<0.05 by Dunnett's test.

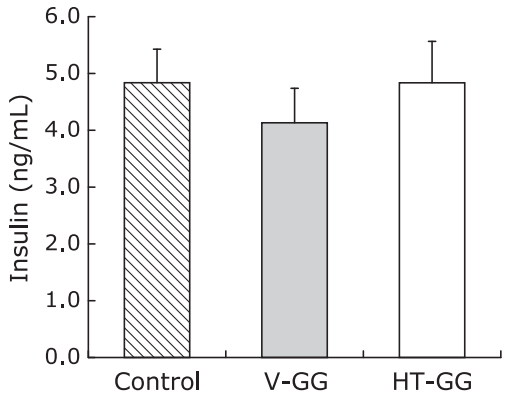

Fig. 7. Effect of administration of viable GG cells (V-GG) or heat-treated GG cells (HT-GG) on plasma insulin in KK-A^y mice at 3 weeks after the experiment began. Each point represents the mean ± SE (n = 7).

with sucrose or starch as the source of carbohydrates, as in the experimental diet of our previous study. As a result, viable GG cells were found to significantly inhibit postprandial blood glucose levels when administered with sucrose or starch (Fig. 1). We observed that viable GG cells were able to decrease glucose *in vitro* (data not shown); this result is similar to that reported by Tabuchi *et al.*[21] It is suggested that GG decreased postprandial blood glucose through suppression of glucose absorption by decreasing the glucose available from digestion of sucrose and starch in ICR mice.

Most lactic acid bacteria including GG use glucose as a nutrition source. Therefore, in Experiment 2, we investigated the effect on the blood glucose level in diabetic KK-A^y mice of daily administration of viable GG cells or *L. bulgaricus* cells, which differ in bacterial strain from GG. Viable GG cells significantly inhibited fasting blood glucose (Fig. 2), postprandial blood glucose in the glucose tolerance test (Fig. 3) and HbA1c (Fig. 4). However, such effects were not shown by viable *L. bulgaricus* cells. These results indicate that the antidiabetic effect of lactic acid bacteria differ according to bacterial strain. The survivability of *L. bulgaricus* in the intestinal tract is controversial. Elli *et al.*[22] reported that *L. bulgaricus* can survive the passage through the gastrointestinal tract, but Goldin *et al.*[3] and Rosa *et al.*[23] reported that it cannot survive. We previously confirmed that the resistance to gastric acid and bile of GG was stronger than that of *L. bulgaricus* LB3, which used in this study. Thus, we inferred that the antidiabetic effect might depend on whether or not the cells are viable when they reach the intestine.

On the other hand, Matsuzaki *et al.*[9] reported that the administration of heat-treated *Lactobacillus casei* cells had an antidiabetic effect on KK-A^y mice. Previous studies reported obtaining a beneficial effect even with HT-GG cells.[24–26] In addition, Li *et al.*[27] reported that both viable and heat-treated GG cells decreased the LPS-stimulated production of cytokine-induced neutrophil chemoattractant caused by cell components. Several other reports have also shown that viable and heat-treated GG cells demonstrate almost the same action, and assumed that the action is due to cell components and immunoregulations induced by the components.[28,29] Given this, we administered viable GG cells or heat-treated GG cells, which were derived by heat sterilization of viable GG cells, to KK-A^y mice in Experiment 2. Viable GG cells significantly suppressed fasting blood glucose (Fig. 5) and modulated the diabetic mice's impaired glucose tolerance (Fig. 6), but the heat-treated GG showed no such effects. These results suggested that the antidiabetic effect might depend on the cells being viable when they reached the intestine. Moreover, we speculated that the beneficial effects might not be caused by cell components and immunoregulation. The difference between viable GG cells and heat-treated GG cells is whether they have metabolic activity in the intestine. Accordingly, it is considered

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that GG continuously decreased the postprandial blood glucose through suppression of glucose absorption in intestine of KK-A^y mice during the experimental period. Furthermore, the antidiabetic effects in Experiment 2 and Experiment 3 might have resulted from the continuous inhibition of postprandial blood glucose. Future studies are needed to investigate the antidiabetic activity of other strains of lactic acid bacteria that are viable in the intestines.

Recently, intestinal environmental factors have been related to obesity in mice and humans.[30,31] Kondo *et al.*[32] have reported the antiobesity effects of *Bifidobacterium breve* B-3 through modulation of intestinal microbiota in high-fat diet-induced obese mice. It was shown that GG can change the intestinal environment by affecting the metabolic activity of the resident micro flora[3] and fermentation of food-derived indigestible carbohydrates.[26] Although there was no difference in body weight in Experiments 2 and 3, it is possible that the change in intestinal condition by GG is the reason for the antidiabetic activity.

In addition, hyperinsulinemia appeared as compensation for hyperglycemia on KK-A^y mouse.[33] Chronic high blood glucose in diabetes results from imbalances of insulin resistance and insulin secretion.[34] In Experiment 3, hyperinsulinemia in KK-A^y mice tended to be improved with the administration of viable GG cells (Fig. 7). This result suggests the possibility that GG contributed to the improvement of insulin imbalances.

In conclusion, the results of this study demonstrated that GG decreased the postprandial blood glucose in ICR mice, and that the antidiabetic activity of lactic acid bacteria on KK-A^y mice differed depending on the bacterial strain and whether the bacteria is viable when it arrives in the intestine. The activity appears to result from continuous inhibition of the postprandial blood glucose through suppression of glucose absorption in the intestines of KK-A^y mice during the experimental period. These findings indicate that a specific strain of lactic acid bacteria is beneficial for the management of type 2 diabetes. Further studies are needed to determine the underlying mechanisms and to identify other such strains of lactic acid bacteria.

Conflict of Interest

No potential conflicts of interest were disclosed.

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