

Anti-obesity Effect of Yogurt Fermented by *Lactobacillus plantarum* Q180 in Diet-induced Obese Rats

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Abstract

This study aimed to investigate the anti-obesity effects of yogurt fermented by *Lactobacillus plantarum* Q180 in diet-induced obese rats. To examine the effects, male Sprague-Dawley rats were fed on six different diets, as follows: Group A was fed an ND and orally administrated saline solution; Group B, an HFD and orally administrated saline solution; Group C, an HFD and orally administrated yogurt fermented by ABT-3 and *L. plantarum* Q180; Group D, an HFD and orally administrated yogurt with added *Garcinia cambogia* extract, fermented by ABT-3 and *L. plantarum* Q180; Group E, an HFD and orally administrated yogurt fermented by *L. plantarum* Q180; and Group F, an HFD and orally administrated yogurt with added *Garcinia cambogia* extract, fermented by *L. plantarum* Q180 for eight weeks. After eight weeks, the rate of increase in bodyweight was 5.14%, 6.5%, 3.35% and 10.81% lower in groups C, D, E and F, respectively, compared with group B; the epididymal fat weight of groups E and F was significantly lower than that of group B; and the level of triglyceride and leptin was significantly reduced in groups C, D, E and F compared to group B. In addition, the level of AST was reduced in group C compared to the other groups. To examine the effects of yogurt on the reduction of adipocyte size, the adipocyte sizes were measured. The number of large-size adipose tissue was less distributed in groups A, C, D, E and F than in group B.

Keywords: *Lactobacillus plantarum*, anti-obesity activity, adipocyte size, yogurt, diet-induced rats

Received April 30, 2015; Revised January 4, 2016; Accepted January 7, 2016

Introduction

Obesity is a serious medical condition in which excess body fat accumulates to the extent that it may have a negative effect on health, leading to reduced life expectancy and/or increased health problems (Kopelman, 2000; Plessis *et al.*, 2010). Obesity causes various kinds of metabolic syndrome such as diabetes, insulin resistance, hypertension, arteriosclerosis, cardiovascular diseases, type-2 diabetes, certain cancers, and sleep apnea/sleep-disordered breathing (Haslam and James, 2005; Hirose *et al.*, 2013).

Most probiotic products such as yogurts contain lactic acid bacteria (Adolfsson *et al.*, 2004). Each type of lactic acid bacteria has individual characteristics and various health effects such as stimulation of the immune system and stabilization of gut microflora (Axelsson, 1998; Gibson *et al.*, 1997; Parvez *et al.*, 2006; Perdigon *et al.*, 1995). Recently, the anti-obesity effect of probiotics has attracted the attention of the public (Pereira and Gibson, 2002; Us-

man and Hosono, 2000). Although the relationship between probiotics and obesity has not been extensively studied, additional beneficial effects of some probiotics or food products containing probiotics on bodyweight control and metabolic disorders related with obesity have been reported recently (Bhathena *et al.*, 2009; Lee *et al.*, 2007; Lye *et al.*, 2009).

Obesity is associated with an increase in the size and amount of adipose tissue (Cani *et al.*, 2007). Adipose tissue secretes a number of signaling molecules commonly referred to as adipocytokines (Koerner *et al.*, 2005). Leptin and adiponectin are the important adipocytokines produced by adipocytes.

In this study, we investigated the effects of yogurt fermented by *Lactobacillus plantarum* Q180 on bodyweight, serum lipid, adipocytokine concentrations and adipocyte size in the epididymal fat pad of rats with a view to developing new functional food products.

Materials and Methods

Bacterial strains used in this study

Lactobacillus plantarum Q180 was isolated from the

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feces of healthy adults. In several *in vitro* studies, it was demonstrated that the strain has potential as a probiotic with anti-obesity effects (Park, 2014). The culture was maintained in MRS broth (Difco, USA). ABT-3, a commercial probiotic start culture obtained from Chr. Hansen's Laboratory (Denmark), was used for lowering pH of the yogurt because of slow acid production rate of *Lactobacillus plantarum* Q180. Also, to increasing of anti-obesity effect, *Garcinia cambogia* was added in some of yogurts.

Animals and treatment

Male Sprague-Dawley rats aged 7 wk were purchased from Hanlim Animal Co. (Korea). All of the rats were individually housed at constant temperature and humidity (20±2°C, 55±5%) under a 12 h light/dark cycle. After acclimatization for 1 wk on a normal chow diet, they were then randomized according to their bodyweights and divided into six groups of six rats. The rats were fed either a high-fat diet (HFD; n=30) or a normal diet (ND; n=6) for 8 wk. In calorific terms, the high-fat diet consisted of 34.3% fat, 27.3% carbohydrate, and 34.3% protein (5.1 kcal/g), whereas the normal diet contained 4.2% fat, 64.7% carbohydrate, and 18.6% protein (3.7 kcal/g). The compositions of HFD and ND are shown in Table 1. The group of rats were fed as follows: Group A was fed an ND and orally administrated saline solution; Group B, an HFD and orally administrated saline solution; Group C, an HFD and orally administrated yogurt fermented by ABT-3, a commercial probiotic start culture obtained from Chr. Hansen's Laboratory (Denmark), and *L. plantarum* Q180; Group D, an HFD and orally administrated yogurt with added extract of *Garcinia cambogia*, a plant popularly used as an ingredient of dietary supplements

for weight loss (Saito *et al.*, 2005), and fermented by ABT-3 and *L. plantarum* Q180; Group E, an HFD and orally administrated yogurt fermented by *L. plantarum* Q180; and Group F, an HFD and orally administrated yogurt with added *Garcinia cambogia* extract, and fermented by *L. plantarum* Q180. Food and water were freely available. Yogurt or only saline solution was orally administrated once a day for eight weeks (1 mL/200 g bodyweight of rat). Each yogurt was fermented by 1% of probiotics for 18 h at 37°C. To the yogurt for groups D and F was added 0.15% *Garcinia cambogia* extract. After fermentation, the amount of lactic acid bacteria in the yogurt was over 10⁹ CFU/mL. Bodyweight and food intake were measured once a week. Before being sacrificed, the rat were fasted for 12 h and anesthetized with diethyl ether. All animal experimentation was performed after obtaining the approval of the Institutional Animal Care and Use Committee (IACUC) of Seoul National University College of Medicine.

Analysis of blood serum and organ weight

At the end of the 8-wk experimental period, blood samples were collected from all the rats (taken from the eyes) and immediately placed in sterile tubes. Serum was collected by centrifugation at 2000 × g for 15 min at 4°C. The serum samples were analyzed for total cholesterol (TC), triglyceride (TG) concentration, total protein, glucose, aspartate transaminase (AST) and alanine transaminase (ALT) activity by Green Cross (Korea). Serum adiponectin and leptin concentrations were measured using a quantikine ELISA kit (R&D system, USA). The detection ranges of adiponectin and leptin were 0.062-4 ng/mL and 0.156-10 ng/mL, respectively. Also, the rats' epididymal fat pads, visceral fat, kidney fat, liver, spleen, and kidney were quickly isolated after the sacrifice and weighed.

Table 1. Formula for the normal diet and the high-fat diet

Formula	g/kg	
	Normal diet	High-fat diet
Casein	210.0	265.0
L-Cystine	3.0	4.0
Maltodextrin	50.0	160.0
Sucrose	325.0	90.0
Lard	20.0	310.0
Soybean Oil	20.0	30.0
Cellulose	37.15	65.5
Mineral Mix, AIN-93G-MX(94046)	35.0	48.0
Calcium Phosphate, dibasic	2.0	3.4
Vitamin Mix, AIN-93-VX(94047)	15.0	21.0
Choline Bitartrate	2.75	3.0
Food Color	0.1	0.1

Measurement of adipocyte size

The left and right epididymal fat pads were quickly isolated after the animals were sacrificed, and fixed in 10% formaldehyde solution. According to the method of Hirsch and Gallian (1968), the samples were passed through a 250-m nylon filter in order to remove fibrous tissue and intact shreds of fixed adipose tissue. The tissue shreds were flushed thoroughly with PBS. The filtrate was then passed through a 25-m nylon filter to trap fixed adipocytes, and the cells were washed thoroughly with PBS. Cell suspensions were applied to glass slides and then stained with hematoxylin and eosin solutions (H&E staining), as previously described (van Goor *et al.*, 1986). The

cells on the glass slides were observed with a microscope and the size of the adipocytes was measured using Image J Software (National Institute of Health, USA).

Statistical analysis

The results are expressed as the mean±standard deviation (SD). Statistical analysis was performed with a statistical analysis system (SAS, SAS Institute Inc., USA). The significance of the differences was analyzed by conducting a one-way analysis of variance (ANOVA) with Duncan's multiple range tests. Values of $p < 0.05$ were considered statistically significant.

Results and Discussion

Bodyweight, organ weight, fat weight and food intake

Fig. 1 and Table 2 show the changes in bodyweight and food intake of the groups during the experiment. After eight weeks, group B's bodyweight increased by 23.96 percent, whereas the bodyweight of groups C, D, E and F increased by 17.59, 15.91, 19.79 and 10.56 percent, respectively. Notably, the rate of increase in bodyweight was 10.81 percent lower in group F compared with group B. However, there were no significant differences in bodyweight gains between B group and the other groups fed with yogurt fermented by probiotics. Also, no significant differences in food efficiency ratio (FER) were observed for the groups fed with yogurt fermented by probiotics in comparison with group B.

Organ weights and fat weights are shown in Table 3.

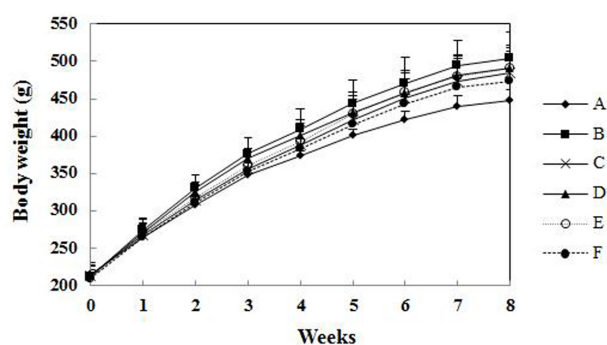


Fig. 1. Changes in bodyweight gain of SD rats fed on experimental diets for 8 wk. All values are the mean ± SD of 6 rats per group. ¹⁾Group A, fed an ND and orally administrated saline solution; B, fed an HFD and orally administrated saline solution; C, fed an HFD and orally administrated yogurt fermented by ABT-3 and *L. plantarum* Q180; D, fed an HFD and orally administrated yogurt with added *Garcinia cambogia* extract and fermented by ABT-3 and *L. plantarum* Q180; E, fed an HFD and orally administrated yogurt fermented by *L. plantarum* Q180; F, fed an HFD and orally administrated yogurt with added *Garcinia cambogia* extract and fermented by *L. plantarum* Q180.

There were no significant differences in the liver, kidney, spleen, and kidney fat weight between group B and the other groups fed with yogurt fermented by probiotics. However, epididymal fat weights were reduced in the groups fed yogurt fermented by probiotics. The epididymal fat weights of groups E and F (9.44 ± 2.08 g and 9.02 ± 2.26 g, respectively) were significantly lower than that of B group (12.33 ± 2.71 g). It was also reduced in groups C and D (9.89 ± 2.09 g and 9.80 ± 1.58 g, respectively), but no signi-

Table 2. Bodyweight changes, food intakes and food efficiency ratio of rats fed on experimental diets for 8 wk

	A ¹⁾	B	C	D	E	F
Bodyweight(g/rat)						
Initial	215.01±12.75 ^a	210.93±19.31 ^a	212.62±8.63 ^a	213.72±12.48 ^a	209.93±8.36 ^a	209.57±7.50 ^a
Final	447.61±14.12 ^b	504.39±35.34 ^a	485.18±36.28 ^a	490.91±22.23 ^a	491.07±27.56 ^a	473.98±12.69 ^{ab}
Wt. gain	232.60±15.15 ^b	293.46±27.68 ^a	272.56±29.25 ^a	277.18±26.91 ^a	281.14±19.24 ^a	264.40±8.71 ^a
Wt. gain rate (%) ²⁾	0	23.96	17.59	15.91	19.79	10.56
Wt. lose rate (%) ³⁾	19.33	0	5.14	6.50	3.36	10.81
Food intake						
Total (g/rat)	1049.14±27.60 ^a	978.94±51.74 ^b	902.73±84.89 ^c	894.20±31.28 ^c	904.04±66.64 ^c	849.67±64.87 ^c
Weekly (g/wk/rat)	130.63±11.34 ^a	120.79±14.51 ^b	113.64±16.93 ^{cd}	111.37±16.92 ^{cd}	113.80±17.55 ^{bc}	105.13±16.13 ^d
FER ⁴⁾	22.20±1.85 ^b	30.09±4.50 ^a	30.22±2.29 ^a	31.05±3.53 ^a	31.17±2.33 ^a	31.24±2.26 ^a

¹⁾A, normal diet; B, high-fat diet; C, high-fat diet with yogurt fermented by ABT-3 and *L. plantarum* Q180; D, high-fat diet with yogurt with added *Garcinia cambogia* extract and fermented by ABT-3 and *L. plantarum* Q180; E, high-fat diet with yogurt fermented by *L. plantarum* Q180; F, high-fat diet with yogurt added *Garcinia cambogia* extract and fermented by *L. plantarum* Q180.

²⁾Weight gain rate compared to ND group (group A)

³⁾Weight loss rate compared to HFD group (group B)

⁴⁾FER: Food efficiency ratio, Wt. gain(g)/Food intake(g) × 100

Values are the mean±SD of 6 rats per group.

^{a-d)}Means with the same letter within the same line are not significantly different ($p < 0.05$).

Table 3. Organ weight and fat weight of rats fed on experimental diets for 8 wk (g)

	Liver	Kidneys	Spleen	Epididymal fat pad	Visceral fat	Kidney fat
A ¹⁾	11.29±1.06 ^b	2.75±0.15 ^a	0.97±0.04 ^{ab}	7.07±1.20 ^c	2.58±0.26 ^b	7.80±1.42 ^b
B	12.72±1.30 ^a	2.80±0.12 ^a	0.85±0.15 ^b	12.33±2.71 ^a	4.63±0.83 ^a	13.97±2.43 ^a
C	12.59±1.18 ^a	2.77±0.18 ^a	0.92±0.07 ^{ab}	9.89±2.09 ^{ab}	3.97±0.99 ^a	12.68±2.29 ^a
D	12.62±0.40 ^a	2.80±0.23 ^a	1.07±0.15 ^a	9.80±1.58 ^{ab}	3.59±0.86 ^{ab}	11.87±1.57 ^a
E	12.74±0.70 ^a	2.87±0.21 ^a	0.96±0.12 ^{ab}	9.44±2.08 ^{bc}	3.61±1.25 ^{ab}	12.02±3.92 ^a
F	12.62±1.04 ^a	2.75±0.06 ^a	0.92±0.19 ^{ab}	9.02±2.26 ^{bc}	3.46±0.95 ^{ab}	11.80±3.05 ^a

¹⁾A, normal diet; B, high-fat diet; C, high-fat diet with yogurt fermented by ABT-3 and *L. plantarum* Q180; D, high-fat diet with yogurt with added *Garcinia cambogia* extract and fermented by ABT-3 and *L. plantarum* Q180; E, high-fat diet with yogurt fermented by *L. plantarum* Q180; F, high-fat diet with yogurt with added *Garcinia cambogia* extract and fermented by *L. plantarum* Q180.

Values are the mean±SD of 6 rats per group.

^{a-c} Means with the same letter within the same row are not significantly different ($p < 0.05$).

ficant differences in group B were observed. The visceral fat weights in groups C, D, E and F (3.97±0.99 g, 3.59±0.86 g, 3.61±1.25 g and 3.46±0.95 g, respectively) were lower than that of group B (4.63±0.83 g). However, no significant differences were observed between the groups, except for group A. According to the results, with the exception of group A, the mean values of the fat weights (epididymal, visceral and kidney fat) of group F were generally the lowest.

In another study, SD rats were fed on two different diets, as follows: the Control group was fed a high-carbohydrate diet and administered PBS; BNR17 was fed a high-carbohydrate diet and administered *Lactobacillus gasseri* BNR17 isolated from breast milk. PBS and the probiotics orally administered twice a day. After twelve weeks, the average bodyweight was 9.58 percent lower in the BNR group compared to the control group. In addition, the weights of the mesenteric fat pads, perirenal fat pads, and epididymal fat pads were lower in the BNR group compared to the control group (Kang *et al.*, 2010). The tendency toward weight increase in that study is similar to the findings of our study.

Analysis blood serum levels

The blood serum levels are shown in Tables 4 and 5. No significant differences were observed in the levels of TC, HDL-C, LDL-C, total protein, glucose and ALT between group B, the HFD control group, and the other groups fed with yogurt fermented by probiotics. However, the TG level of the groups fed with yogurt fermented by probiotics was lower than that of the HFD control group, which suggests that probiotics help to reduce the accumulation of triglyceride in rats. The AST level was lower in group C than in the other groups. It was also lower in groups D, E and F than in the control group, although no significant differences were observed. The level of adiponectin, a physiologically active polypeptide hormone secreted by adipose tissue, did not show a significant difference between groups. However, the average value of the adiponectin level in groups A, C, E and F was higher than that of group B. Generally, the amount of diponectin is reduced in obese and diabetic mice, and the plasma levels of adiponectin are lower in obese than lean humans (Arita *et al.*, 1999; Hu *et al.*, 1996). The level of leptin, a hormone produced predominantly in adipocytes, was sig-

Table 4. Blood serum levels of rats fed on experimental diets for 8 wk

	A ¹⁾	B	C	D	E	F
TC (mg/dL)	83.17±11.96 ^a	91.33±18.93 ^a	80.83±12.19 ^a	87.00±15.81 ^a	94.67±14.53 ^a	79.50±7.42 ^a
TG (mg/dL)	108.00±17.31 ^b	144.33±40.71 ^a	104.67±18.15 ^b	96.00±7.85 ^b	90.50±7.26 ^b	93.83±22.57 ^b
LDL-C (mg/dL)	9.33±3.08 ^c	12.50±2.07 ^{abc}	10.00±1.67 ^{bc}	13.17±4.07 ^{ab}	15.67±2.94 ^a	11.33±1.86 ^{bc}
HDL-C (mg/dL)	73.00±10.47 ^a	73.33±16.61 ^a	68.17±10.61 ^a	74.17±14.77 ^a	80.33±11.62 ^a	69.50±7.79 ^a
Total protein (g/dL)	6.90±0.13 ^a	6.75±0.16 ^a	6.78±0.12 ^a	6.85±0.10 ^a	6.57±0.08 ^b	6.77±0.10 ^a
Glucose (mg/dL)	104.17±3.37 ^b	121.00±7.95 ^a	117.83±6.34 ^a	116.00±9.12 ^a	122.50±4.75 ^a	120.83±7.44 ^a
AST (U/L)	119.33 30.36 ^a	108.17±31.33 ^a	76.00±3.41 ^b	92.67±9.54 ^{ab}	86.83±6.55 ^{ab}	83.50±11.59 ^{ab}
ALT (U/L)	54.17±47.34 ^a	48.17±9.85 ^a	35.00±6.72 ^a	38.17±3.13 ^a	36.17±4.75 ^a	39.83±3.60 ^a

¹⁾A, normal diet; B, high-fat diet; C, high fat-diet with yogurt fermented by ABT-3 and *L. plantarum* Q180; D, high-fat diet with yogurt with added *Garcinia cambogia* extract and fermented by ABT-3 and *L. plantarum* Q180; E, high-fat diet with yogurt fermented by *L. plantarum* Q180; F, high-fat diet with yogurt with added *Garcinia cambogia* extract and fermented by *L. plantarum* Q180.

Values are the mean±SD of 6 rats per group.

^{a-c} Means with the same letter within the same line are not significantly different ($p < 0.05$).

Table 5. Adipocytokine levels of plasma in rats fed on experimental diets for 8 wk

	A	B	C	D	E	F
adiponectin (ng/mL)	3.26±0.55 ^a	2.99±0.39 ^a	3.10±0.24 ^a	2.83±0.32 ^a	3.04±0.58 ^a	3.25±0.84 ^a
leptin (pg/mL)	315.05±20.71 ^c	532.76±135.92 ^a	426.55±46.14 ^b	408.16±61.19 ^b	426.61±64.87 ^b	413.76±42.19 ^b

¹⁾A, normal diet; B, high-fat diet; C, high-fat diet with yogurt fermented by ABT-3 and *L. plantarum* Q180; D, high-fat diet with yogurt with added *Garcinia cambogia* extract and fermented by ABT-3 and *L. plantarum* Q180; E, high-fat diet with yogurt fermented by *L. plantarum* Q180; F, high-fat diet with yogurt with added *Garcinia cambogia* extract and fermented by *L. plantarum* Q180.

Values are the mean±SD of 6 rats per group.

^{a-c}Means with the same letter within the same line are not significantly different ($p < 0.05$).

nificantly reduced in groups A, C, D, E and F compared with group B. Unlike adiponectin, plasma leptin concentrations are generally higher in obese than lean humans or mice (Considine *et al.*, 1996; Frederich *et al.* 1995).

Adipocyte size

The epididymal fat pads of the rat were quickly isolated and the adipocyte size was observed and measured. Each cell was photographed on the microscope five times and

the adipocyte size was measured using Image J Software. The distribution of various sizes of adipocytes and a picture of adipocytes are shown in Fig. 2. Large adipocytes were more distributed in group B than in the other groups; whereas small adipocytes were relatively more distributed in groups A, C, D, E and F. In many previous studies, it has been demonstrated that an increase in the size of adipocyte cells is associated with obesity (Guo *et al.*, 2004; Weisberg *et al.*, 2003). Meanwhile, a decrease in the

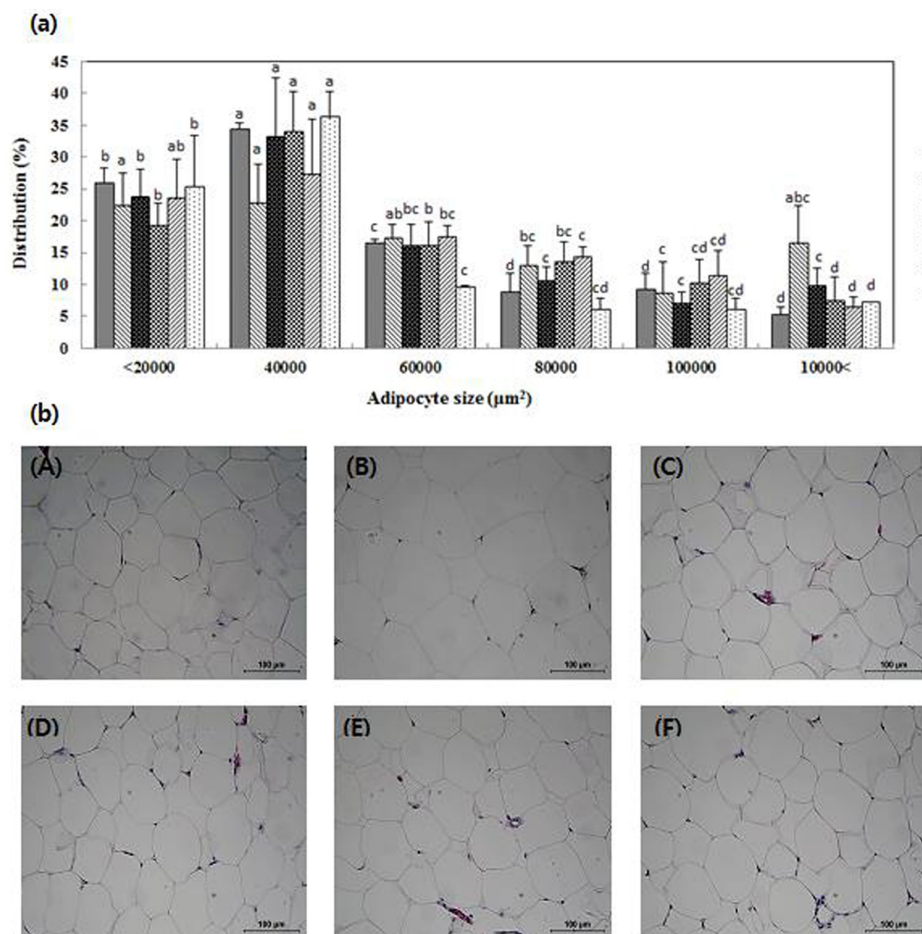


Fig. 2. Adipocyte size of the epididymal fat pads in rats. (a) Percentage of cell number. All values are the mean±SD of 6 rats per group. ^{a-d}Means that the values with different superscripts within the same group are significantly different ($p < 0.05$). (b) Photograph of the epididymal fat pads in rats (original magnification ×200).

size of adipocytes is considered to help in preventing obesity due to the inhibition of hypertrophy and hyperplasia (Cariou *et al.* 2006; Faust, 1984). Furthermore, Marques *et al.* (1998) suggested that enlarged adipocytes secrete growth factors that trigger adipogenesis through preadipocyte differentiation. According to Sato *et al.* (2008), they measured the size of adipocytes in SD rats fed on a diet containing skimmed milk fermented by *Lactobacillus gasseri* SBT2055 for 4 wk, and observed a significant reduction in the size of the rats' adipocytes, as well as in increase in the number of small adipocytes. Therefore, they suggested that the fermented milk product containing *L. gasseri* SBT2055 may have a beneficial effect on the onset of obesity by influencing the size of the cells from visceral adipose tissues. Thus, the results of our study indicate that *L. plantarum* Q180 has an anti-obesity effect because the size of the cells taken from epididymal adipose tissues was observed to decrease.

Conclusion

Bodyweight gain was reduced in diet-induced SD rats treated with yogurt fermented by *Lactobacillus plantarum* Q180. Compared with the HFD control group, the rate of increase in the bodyweight of group F, the rats fed with yogurt with added *Garcinia cambogia* extract and fermented by *L. plantarum* Q180, was the lowest, being some 10.81% lower than that of the HFD control group. The epididymal fat weight of groups E and F was also significantly lower than that of the HFD control group. Furthermore, the amounts of triglyceride and leptin were significantly reduced in groups C, D, E and F compared to the HFD control group. In addition, the percentage of large-size adipose tissue was less distributed in groups A, C, D, E and F than in the HFD control group. When used only *L. plantarum* Q180 for yogurt fermentation, there was small effects in bodyweight loss compared with other groups except control group. It seems because the growth rate of *L. plantarum* Q180 was slower than commercial strain. However, the decrease effect of fat weight and TG and the increase effect of HDL level were good in group E, the rats fed with yogurt fermented by *L. plantarum* Q180. Taken together, these findings suggest that yogurt fermented by *Lactobacillus plantarum* Q180 might prevent obesity, at least in rats. In particular, the anti-obesity effect was greatest when the rats were fed with yogurt with added *Garcinia cambogia* extract and fermented by *L. plantarum* Q180.

Acknowledgements

This research was supported by the High Value-added Food Technology Development Program (Project No. 111151-3) of the Ministry of Agriculture, Food and Rural Affairs.

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