Milk fermented by *Lactobacillus gasseri* SBT2055 influences adipocyte size via inhibition of dietary fat absorption in Zucker rats

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We have demonstrated previously that a diet containing skimmed milk (SM) fermented by *Lactobacillus gasseri* SBT2055 (LGSP) reduces adipocyte size in Sprague–Dawley rats. Two experiments were conducted to extend these observations in order to elucidate the mechanism involved. In experiment 1, lean and obese Zucker rats were fed a diet containing SM or LGSP for 4 weeks. The LGSP diet, compared with the SM diet, resulted in lowering of the mesenteric adipose tissue weight (23 %; $P < 0.05$), adipocyte sizes (28 %; $P < 0.001$) and serum leptin concentration (36 %; $P < 0.05$) in lean rats. Obese Zucker rats did not display such dietary effects. Only the number of smaller adipocytes was increased ($P < 0.05$) by the LGSP diet in the subcutaneous adipose tissue of obese rats. The LGSP diet significantly reduced the serum and hepatic cholesterol in rats. In addition, the LGSP diet led to an increased excretion of faecal fatty acids and total neutral faecal sterols in both rat strains. In experiment 2, Sprague–Dawley rats with permanent cannulation of the thoracic duct were fed either the SM or LGSP diets and their lymph was collected. The LGSP diet lowered the maximum transport rate of TAG and phospholipids. These results indicate that fermented milk regulates adipose tissue growth through inhibition at the stage of dietary fat absorption in lean Zucker rats.

Lactobacillus gasseri: Zucker rats: Adipocyte size: Lymph lipids: Faecal fatty acids

Recently, the use of probiotic strains (particularly lactobacilli and bifidobacteria) has been promoted because of their potential therapeutic effects, which have been studied extensively[1,2]. *Lactobacillus gasseri*, previously classified as *L. acidophilus*, is considered as a major species of human flora. There are many reports revealing the potential beneficial effects of *L. gasseri*, including immunomodulatory properties[3], enhancement of intestinal functions[4], hypocholesterolaemic effect[5] and inhibitory action against undesirable bacteria[6].

L. gasseri SBT2055 is one of the strains that was isolated at the Technical Research Institute of Snow Brand Milk Products Co., Ltd (Kawagoe, Japan) from human infant or adult faeces[7]. This strain has the ability to reach and establish in the intestine in both man and mice[8]. Therefore, this strain was chosen as an excellent probiotic strain due to its suitability as a starter for preparing fermented milk[9].

The adipose tissue expansion that occurs during the development of obesity is initially characterised either by an increase in fat cell size or by an increase in fat cell number. A recent study in our laboratory has shown that a fermented milk product containing *L. gasseri* SBT2055 (LGSP) exerts a beneficial effect on the onset of obesity by lowering adipocyte size and the level of leptin in Sprague–Dawley rats[10]. Although the mechanism

of this effect of fermented milk on adipocyte size has not been elucidated, it is likely that LGSP may affect adipocyte size through inhibition of energy input. In fact, it was found that inhibitors of lipid absorption affect the body fat mass through increased excretion of faecal lipids[11]. Furthermore, *L. gasseri* strains have been reported to bind micellar lipids, thereby raising a possibility of inhibiting the absorption of fatty acids and cholesterol[12].

Because Sprague–Dawley rats are relatively lean, it is necessary to use an obese animal model for further studies. Zucker obese rats show early-onset, extreme obesity due to leptin receptor deficiency[13] and overeating[14,15]. Therefore, Zucker obese rats are often used as an animal model of hyperphagia and hyperlipidaemia. Accordingly, Zucker obese rats may provide a better model to further clarify the effects of *L. gasseri* SBT2055-fermented milk on adipocyte size.

In the present study, we studied whether LGSP has an effect on adiposity parameters in obese as well as lean Zucker rats. Simultaneously, faecal fatty acid excretion was determined in these Zucker rats in order to clarify the underlying mechanism of altered adiposity parameters. Moreover, we directly determined the effect of the LGSP diet on lymphatic lipid absorption by Sprague–Dawley rats with cannulated thoracic lymph ducts.

Materials and methods

Preparation of the milk product fermented by Lactobacillus gasseri SBT2055

LGSP was prepared by fermenting skimmed milk (SM) using *L. gasseri* SBT2055 (this strain was isolated and classified by DNA homology analysis at the Technical Research Institute of Snow Brand Milk Products Co., Ltd, Kawagoe, Japan[9]) as described previously[10,16]. Briefly, SM powder (Snow Brand Milk Products, Co., Ltd, Tokyo, Japan) was hydrated with deionised water, added to a yeast extract, and sterilised at 95°C for 30 min. After inoculation with *L. gasseri* SBT2055, the SM was incubated at 37°C for 16 h. The fermented product containing the bacterial cells was freeze dried (in order to retain bacterial viability[17]) and used for subsequent experiments. SM powder was also treated in a similar manner without inoculation and used as a control (non-fermented SM). The chemical composition of SM (34·7 % protein, 0·9 % fat, 52·6 % carbohydrate, 7·9 % ash, 3·9 % moisture) and fermented SM (35·4 % protein, 0·9 % fat, 52·6 % carbohydrate, 7·7 % ash, 3·4 % moisture) was almost the same. The latter also contained 11·8 g lactic acid/100 g. In addition, the concentration of viable *L. gasseri* in the final fermented milk-containing diet was 6×10^7 colony-forming units per g diet.

Animals and diets

Two experiments were carried out in accordance with the Guidelines for Animal Experiments of the Faculty of Agriculture and the Graduate Course of Kyushu University, Fukuoka, Japan, and law no. 105 and Notification no. 6 of the government of Japan. In both experiments, all rats were allowed free access to commercial chow for 1 week and then housed individually in stainless steel cages in an air-conditioned room (21–24°C, lights on from 08.00 to 20.00 hours).

Experiment 1

Zucker lean and obese rats, age 5 weeks, were obtained from Japan SLC, Inc. (Shizuoka, Japan). They were divided into two groups (eight rats per group) for each rat strain. One group in each rat strain was fed the SM-containing diet as a control group, and the other group was fed the *L. gasseri* STB2055-fermented milk-containing diet. Experimental diets were prepared according to the AIN-76 formula[18] with some modifications, containing (g/kg): 70 fat (maize oil), 200 SM powder or fermented milk powder, 125 casein, 150 α-maize starch, 50 cellulose, 3 DL-methionine, 35 mineral mixture (AIN-76), 10 vitamin mixture, and sucrose to 1000 g. The diets containing SM powder and fermented milk powder were designated as the SM and LGSP diets, respectively. The quantity of protein supplied from the fermented and non-fermented SM powder was 70·8 and 69·4 g/kg diet, respectively. The rats were allowed free access to the experimental diets and water for 4 weeks, and their body weight was monitored. The rats were killed by withdrawing blood from the abdominal aorta under diethyl ether anaesthesia. Faeces were collected for the last 3 d of the feeding period and kept at −20°C until analysis. The liver and white adipose tissues from mesenteric, perirenal, retroperitoneal, epididymal, and subcutaneous (depots extended from the dorsolumbar area to the gluteal with an intermediate region located in the inguinal area) regions were excised and weighed. The liver was kept at −20°C until analysis.

Experiment 2

Male Sprague–Dawley rats, aged 5 weeks, were obtained from Japan Kyudo Co. Ltd (Saga, Japan). The animals were divided into two groups (six rats per group). One group was fed the SM diet as a control group, and the other group was fed the LGSP diet. The rats were trained to consume the experimental diets twice per d from 10.00–11.00 and 16.00–17.00 hours, respectively, for 1 week. Deionised water was freely available throughout the feeding periods.

On the day of surgery, all rats were anesthetised with Nembutal before permanent cannulation of the thoracic duct according to a method modified by our laboratory[19]. Briefly, a cannula (Silicon tube SH, internal diameter 0·5 mm and outside diameter 1·0 mm; Kaneka Medics Co., Tokyo, Japan) filled with heparinised saline was inserted into the thoracic duct and secured within the abdominal cavity. After surgery, the rats were returned to their cages and provided with the experimental diets twice per d. On the third postoperative day, the rats were attached to a long PE-cannula (internal diameter 0·58 mm and outside diameter 0·97 mm; Becton-Dickinson Co., Cockeysville, MD, USA) to collect lymph samples. The end of the cannula was 5–10 cm below the bottom of the cage to provide sufficient underside pressure to allow the lymph to enter the cannula. The lymph was collected for 30 min as the blank and the rats were then given free access to the experimental diet for 30 min. After provision of the experimental diet, lymph was collected every hour for 7 h in separate tubes. The rats freely consumed deionised water during lymph collection.

During the experiment, the growth parameters were monitored. At the end of the lymph collection period, the rats were killed by withdrawing blood from the abdominal aorta under diethyl ether anaesthesia. The obtained lymph samples were analysed for total cholesterol, TAG and phospholipids.

Methods of analysis

Analyses of lipids, glucose and adipocytokines. The concentrations of serum TAG, total cholesterol, HDL-cholesterol, phospholipids and glucose were measured using enzyme assay kits (Triglyceride E test, Phospholipids C test and Glucose C test from Wako Pure Chemicals, Osaka, Japan and Determiner TC555 from Kyowa Medix, Tokyo, Japan). Serum adiponectin and leptin concentrations were measured using ELISA kits (Mouse/rat adiponectin ELISA kit from Otsuka Pharmaceutical, Tokyo, Japan and rat leptin ELISA from Yanaihara Institute, Shizuoka, Japan). Liver lipids were extracted by the method of Folch *et al.*[20] and the concentration of TAG, total cholesterol and phospholipids was measured[10].

Measurement of lipoprotein lipase activity in adipose tissue. In accordance with the method of Perona & Ruiz-Gutierrez[21], subcutaneous adipose tissue (0·5–1 g) was homogenised with 40 ml cooled acetone (−20°C), filtered through Whatman no. 2 filter paper, and washed with 20 ml cooled acetone, 40 ml acetone at room temperature and 40 ml diethyl ether. After drying, the remaining powder was weighed and stored at −80°C until use. Ammonium buffer (1 ml; 25 mM; pH 8·1)

containing 1 unit/ml of sodium heparin was added to 5 mg of the powder. The mixture was incubated on ice for 60 min and subsequently centrifuged at 5000 **g** for 10 min at 4°C. The supernatant fraction was employed as a lipoprotein lipase (LPL) source. One sample was used for the activity measurement and another sample for protein quantification according to the method of Lowry *et al.* [22]. Dibutyryl fluorescein (Sigma, St Louis, MO, USA) was prepared as a substrate for the reaction with LPL by dissolving 1 mg in 5 ml ethylene glycol monomethyl ether. Of this solution, 1 ml was dissolved in 20 ml of 0·1 M-phosphate buffer (pH 8). Of the enzyme extract, 200 μl were incubated (37°C for 20 min) with 1500 μl dibutyryl fluorescein solution, 150 μl ethylene glycol monomethyl ether and 1250 μl phosphate buffer. LPL activity was quantified as the difference between the fluorescence emitted by the released fluorescein when 1 M-NaCl was added to the incubation medium and the level emitted in the absence of NaCl. Fluorescence was measured in a fluorescence spectrophotometer (Shimadzu Dual beam difference spectrofluorophotometer RF-520; Seisakusho Ltd, Kyoto, Japan) with excitation at 490 nm and emission at 530 nm.

Measurement of adipocyte cell size. Adipocyte cell size in the mesenteric and subcutaneous adipose tissue was measured as described previously[10]. In short, adipose tissues rinsed with saline solution were fixed in 10 % neutral formalin-buffered solution, embedded in paraffin, cut into 10 μm sections, and stained with haematoxylin. Cell sizes were measured using National Institutes of Health (Bethesda, MD, USA) image software (100 cells per rat).

Analysis of faecal fatty acids and sterols. Faeces were lyophilised and faecal fatty acids were analysed by the method of Van de Kamer *et al.* [23] as modified by Jeejeebhoy *et al.* [24]. The apparent excretion rate of dietary fat per d was calculated as follows: $100 \times$ (amount of faecal fatty acids excreted)/(amount of daily fat intake).

Faecal neutral sterols were derivatised to trimethylsilyl ethers, and faecal acidic sterols were methylated and derivatised to trimethylsilyl ethers. The products were analysed by gas–liquid chromatography (GC-17A Shimadzu; Seisakusho Ltd, Kyoto, Japan) using 5α-cholestane (Nacalai Tesque, Kyoto, Japan) and 23-nordeoxycholic acid (Steraloids, Inc., Wilton, NH, USA) as internal standards for neutral sterols and bile acids, respectively[25].

Analysis of lymph lipids. Lymph lipid levels were measured using commercially available enzyme assay kits (Triglyceride E test and Phospholipids C test from Wako Pure Chemicals, Osaka, Japan; Determiner TC555 from Kyowa Medix, Tokyo, Japan) as described previously[10].

Statistical analysis

The data were expressed as mean values with their standard errors. The effects of diet (SM *v.* LGSP) and genotype (lean *v.* obese) were analysed by two-way ANOVA. Data for which the variances were unequal were logarithmically transformed before ANOVA or *post hoc* analysis. When the effect of diet or its interaction with genotype in ANOVA resulted in a significant *F* test, Student's *t* test was used for comparisons between the two groups of each genotype. However, the cell size for adipocytes of each size (Figs. 1 and 2) was statistically analysed only by Student's *t* test between the two groups of

each genotype because of the large difference (more than three times) in the average adipocyte size between the genotypes (Table 1). In the second experiment, Student's *t* test was used for analysing data. Differences were considered statistically significant for $P \leq 0.05$. The analysis was carried out with Excel 2002 (Microsoft Corp., Redmond, WA, USA).

Results

Experiment 1

Body and organ weights as well as several metabolic and morphometric parameters were determined in all rat groups (Table 1). The obese Zucker rats had a higher average food intake and body-weight gain compared with the lean rats ($P < 0.001$). Food intake per d was similar between LGSP- and SM-fed groups in each rat strain. Body-weight gain was not affected by diet. The LGSP diet resulted in an increased relative liver weight in both lean and obese rats compared with the SM diet ($P < 0.001$).

The weight of individual adipose tissues and the total weight were greater in the obese rats than in the lean rats ($P < 0.001$). The LGSP diet resulted in reduced total, mesenteric and subcutaneous adipose tissue masses ($P < 0.05$), whereas no significant effect was noticed on other white adipose tissues. In lean rats the mesenteric fat mass was significantly reduced by the LGSP diet (22 %; $P < 0.05$) more prominently than in obese rats. The size of mesenteric and subcutaneous adipocytes was greater in the obese rats compared with the lean rats ($P < 0.001$). While there was no effect of diet on the average size of subcutaneous adipocytes, there was a significant effect of diet ($P < 0.05$) and genotype × diet interaction ($P < 0.001$) on the average size of mesenteric adipocytes. Student's *t* test showed that the LGSP diet was associated with a reduction in average adipocyte size in mesenteric adipose tissue in lean rats (28 %; $P < 0.001$) but not in obese rats.

Likewise, the numbers of smaller adipocytes increased while the numbers of large adipocytes decreased in the mesenteric and subcutaneous tissues of lean and obese rats, respectively, when they were fed the LGSP-containing diet (Figs. 1 and 2). On the contrary, there was no significant effect of the LGSP diet on the profile of the distribution of adipocyte size in mesenteric adipose tissue of obese rats or from subcutaneous adipose tissue of lean rats (Figs. 1 and 2).

LPL activity in the subcutaneous adipose tissue was greater in the obese rats compared with the lean rats ($P < 0.001$). There was a significant effect ($P < 0.05$) of genotype × diet interaction on the LPL activity in the subcutaneous adipose tissue of Zucker rats (Table 1). This significant effect was attributed to a tendency towards decreased LPL activity ($P = 0.07$) in obese rats fed the LGSP diet.

The concentration of serum leptin and adiponectin was greater in the obese rats compared with the lean rats ($P < 0.001$). In addition, there was a significant effect ($P < 0.05$) of the LGSP diet on serum leptin concentrations (Table 1). The leptin levels in the serum of lean rats were decreased (36 %; $P < 0.05$) by the LGSP diet. In contrast, there was no effect of the LGSP diet on the serum levels of both glucose and adiponectin in Zucker rats (Table 1).
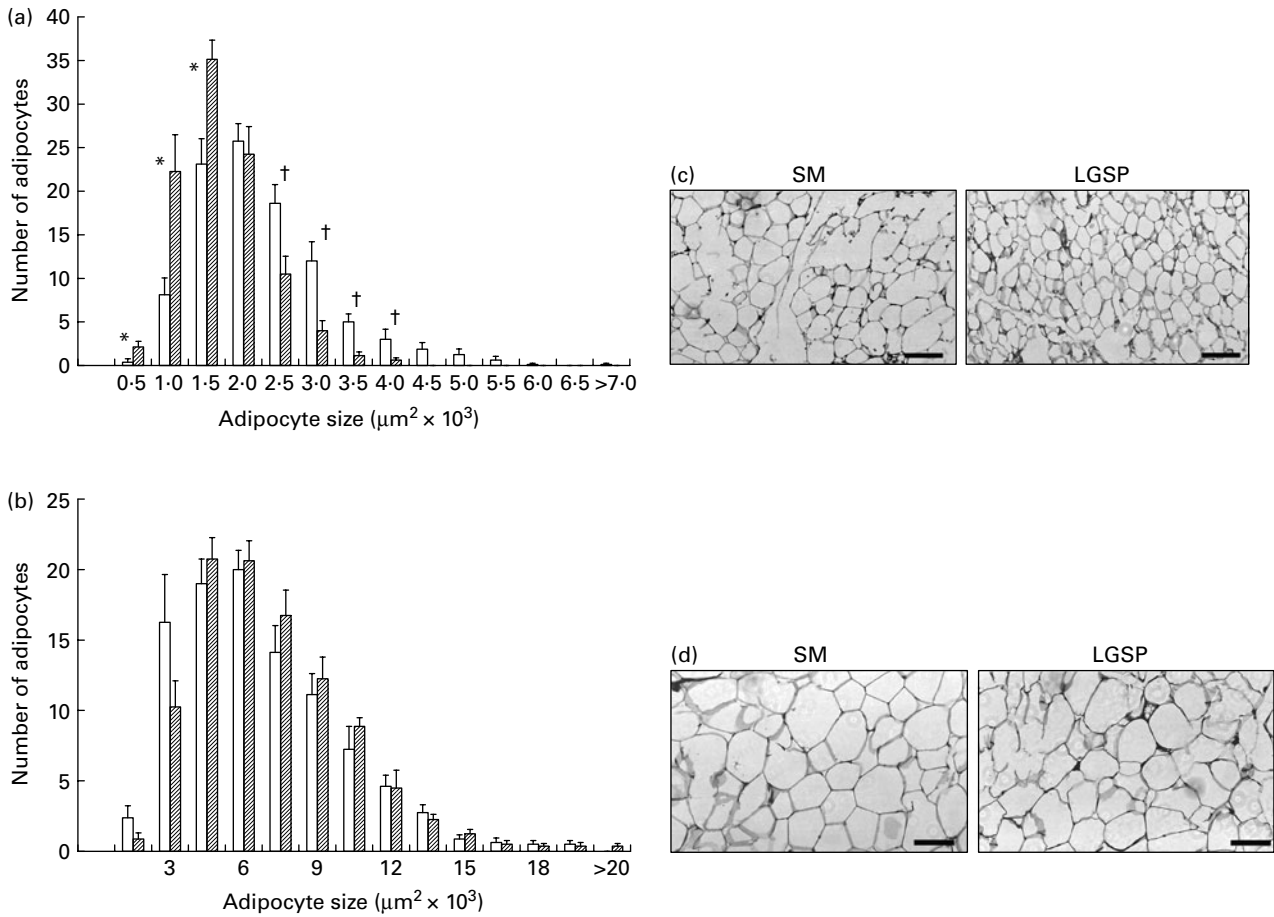
Fig. 1. The effect of dietary skimmed milk (SM) and *Lactobacillus gasseri*-fermented milk product (LGSP) on cell size in mesenteric adipose tissue. Profile of the distribution of cell size of adipocytes from lean (a) and obese (b) Zucker rats (SM, □; LGSP,▨). Values are means for eight rats per group, with their standard errors represented by vertical bars. * Mean value for small adipocytes was significantly higher in the LGSP group than in the SM group ($P<0.05$). † Mean value for large adipocytes was significantly lower in the LGSP group than in the SM group ($P<0.05$). Adipocytes in paraffin sections (scale bar: 100 μm) of lean (c) and obese (d) Zucker rats.

The concentrations of serum lipids were higher in the obese rats relative to the lean rats (Table 1). Moreover, rats fed the LGSP diet displayed a significant reduction in the levels of total and HDL-cholesterol in their serum ($P<0.05$), whereas there was no significant effect of diet on the remaining serum lipids (Table 1). The concentration of liver TAG was remarkably greater in the obese rats compared with the lean rats ($P<0.001$). Genotype significantly affected the liver cholesterol ($P<0.05$) and phospholipid ($P<0.01$) levels. In addition, the LGSP diet reduced the level of hepatic phospholipids and cholesterol ($P<0.05$), with no effect on the level of hepatic TAG (Table 1).

Faecal weight was greater in the obese rats compared with the lean rats (Table 2) ($P<0.001$). Faecal weight was significantly greater ($P<0.05$) in the LGSP group than in the SM group. The amount of faecal fatty acids was greater in the obese rats compared with the lean rats ($P<0.001$), but there was no significant difference between the strains in terms of the apparent excretion rate. The LGSP diet resulted in a significant ($P<0.01$) increase in the amount of faecal NEFA and the apparent excretion of NEFA in the faeces of both Zucker rat strains (Table 2). The faecal excretion of cholesterol ($P<0.01$), coprostanol ($P<0.05$) and total neutral

sterol ($P<0.001$) (Table 2) was influenced by the strain. The LGSP diet resulted in a significant ($P<0.05$) increase in faecal cholesterol (Table 2). There was a significant effect ($P<0.05$) of genotype × diet interaction on total neutral sterols in the obese rats. The amount of faecal total bile acid and individual components (lithocholic, deoxycholic, chenodeoxycholic, hyodeoxycholic, ursodeoxycholic and β-muricholic acid) was greater in the lean rats compared with the obese rats ($P<0.001$) (Table 2). In addition, the LGSP diet significantly increased the deoxycholic acid ($P<0.05$) and decreased β-muricholic acid ($P<0.01$) levels in the obese rats. There was a significant effect ($P<0.01$) of genotype × diet interaction on the excretion of ursodeoxycholic acid. There was no significant effect of the LGSP diet on the excretion of total acidic sterols.

Experiment 2

Food consumption by the rats within the feeding period did not differ between the two groups (11·3 (SE 0·3) and 10·5 (SE 0·9) g/d for the SM and LGSP diets, respectively). Lymph flow also did not differ between the two groups (data not shown). The maximum transport rates (after 4 h
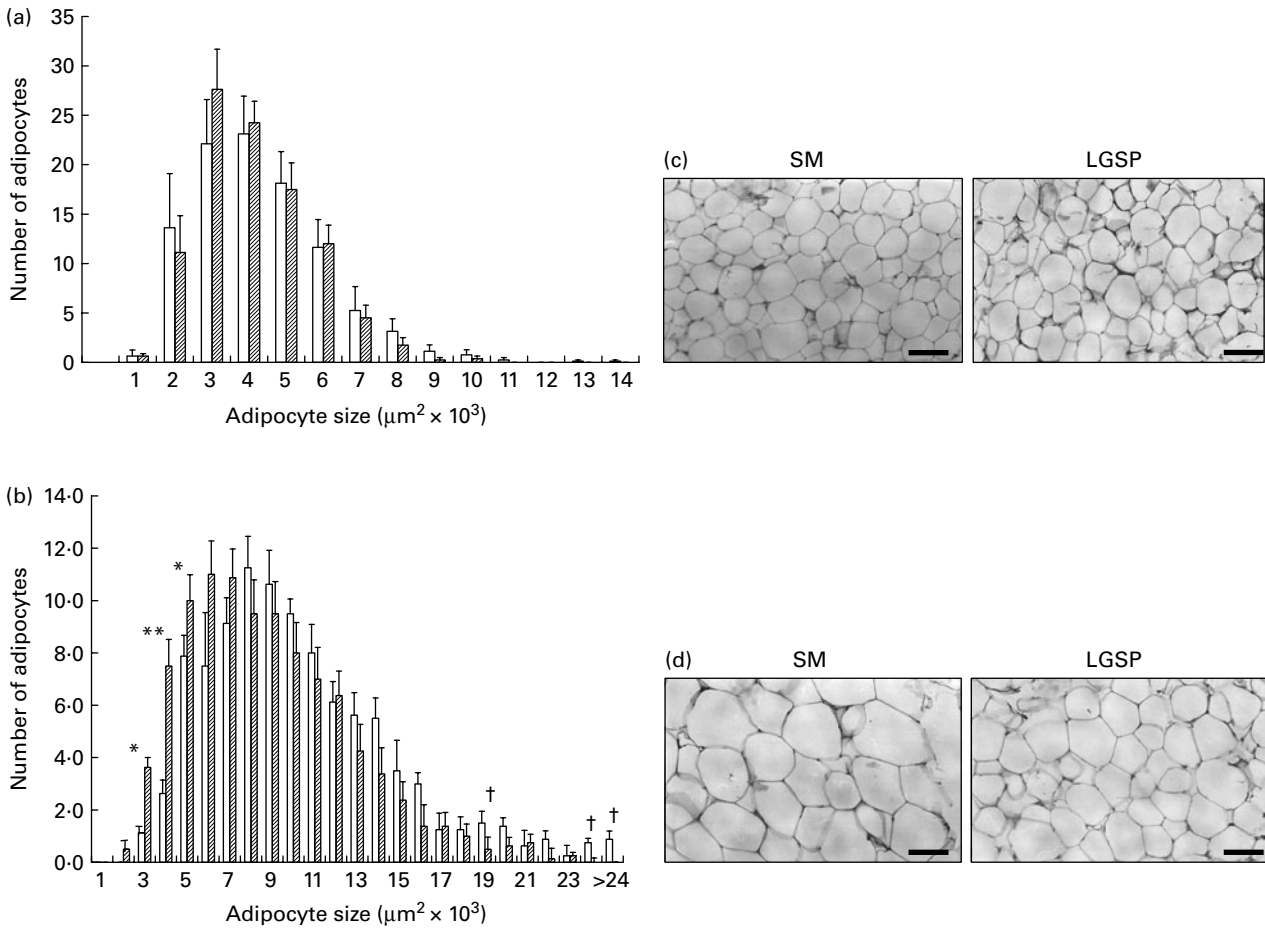
Fig. 2. The effect of dietary skimmed milk (SM) and *Lactobacillus gasseri*-fermented milk product (LGSP) on cell size in subcutaneous adipose tissue. Profile of the distribution of cell size of adipocytes from lean (a) and obese (b) Zucker rats (SM, □; LGSP, ▨). Values are means for eight rats per group, with their standard errors represented by vertical bars. * Mean value for small adipocytes was significantly higher in the LGSP group than in the SM group ($P<0.05$). † Mean value for large adipocytes was significantly lower in the LGSP group than in the SM group ($P<0.05$). Adipocytes in paraffin sections (scale bar: 100 μm) of lean (c) and obese (d) Zucker rats.

lymph collection) of both TAG and phospholipids were lower in the LGSP group than in the SM group (Fig. 3). The maximum transport rate of cholesterol tended to be lower in the LGSP group than in the SM group ($P=0.06$).

Discussion

In Zucker rats fed a diet containing milk fermented by *L. gasseri* SBT2055 (LGSP) there was a reduction in the fat mass, adipocyte size and leptin levels and an increase in the faecal fatty acid content. Moreover, there was a significant effect of the LGSP diet on the maximum absorption of lymphatic lipids in Sprague–Dawley rats. This is the first report showing that a fermented milk product containing *L. gasseri* SBT2055 affects adipocyte size in Zucker rats and lymphatic lipid absorption in Sprague–Dawley rats. These findings suggest a close relationship between adipocyte size and fatty acid absorption in Zucker rats.

In the present study, the size and mass of mesenteric adipose tissue were significantly reduced (28 and 23 %, respectively) by feeding the LGSP diet to lean Zucker rats, but there was almost no effect on the mesenteric adipose tissue of obese Zucker rats. In contrast, the LGSP diet resulted in

a decreased mass of subcutaneous adipose tissue in Zucker rats compared with the SM diet. In particular, the number of smaller adipocytes was increased in the subcutaneous adipose tissue of obese rats fed on the LGSP diet. These results indicate that the LGSP diet led to changes in mesenteric and subcutaneous adipose tissues. The effect of diet observed in the lean Zucker rats is very similar to that observed in our previous study in Sprague–Dawley rats[10]. This similarity may be because the growth of the adipose tissue in lean Zucker rats is quite similar to that reported for Sprague–Dawley rats[26].

Similarly, there was a prominent reduction in the serum levels of leptin in lean rats fed the LGSP diet but not in obese Zucker rats. In fact, the serum level of leptin is correlated with adipose tissue weight or adipocyte size in both rodents[27] and humans[28]. Therefore, the lower serum level of leptin in the LGSP group of lean Zucker rats may reflect the reduction of the fat mass and adipocyte size of their mesenteric adipose tissue. Despite the increase in the number of smaller adipocytes in subcutaneous depots in obese Zucker rats (Fig. 2), no change was noted in their serum leptin levels. This is likely to be due to the lower contribution of subcutaneous adipose tissue to the expression of

Table 1. Effect of skimmed milk (SM) and *Lactobacillus gasseri*-fermented milk product (LGSP) on morphometric and metabolic parameters in lean and obese Zucker rats

(Mean values with their standard errors for eight rats per group)

| | Lean | | | | Obese | | | | P (ANOVA) | | |
| | SM | | LGSP | | SM | | LGSP | | | | |
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Genotype	Diet	Genotype × diet
Food intake (g/d)	15·0	0·3	14·7	0·1	21·4	0·2	21·3	0·1	0·001	NS	NS
Body-weight gain (g)	123	3	115	2	184	3	184	4	0·001	NS	NS
Relative liver weight (g/100 g body weight)	3·26	0·02	3·53**	0·02	3·61	0·06	3·97**	0·07	0·001	0·001	NS
Mesenteric fat mass (g)	1·83	0·14	1·41*	0·12	7·73	0·26	7·54	0·12	0·001	0·05	NS
Perirenal fat mass (g)	0·61	0·07	0·49	0·05	2·25	0·11	2·37	0·10	0·001	NS	NS
Retroperitoneal fat mass (g)	2·23	0·19	1·82	0·18	7·97	0·25	7·56	0·09	0·001	NS	NS
Epididymal fat mass (g)	2·39	0·09	2·22	0·15	8·75	0·21	8·49	0·17	0·001	NS	NS
Subcutaneous fat mass (g)	8·24	0·76	6·75	0·53	36·07	1·27	33·66	1·21	0·001	0·05	NS
Total fat mass (g)	15·30	1·19	12·69	0·95	62·76	1·35	59·63	1·52	0·001	0·05	NS
Mesenteric adipocyte size ($\mu m^2 \times 10^3$)	2·01	0·11	1·44***	0·08	6·02	0·37	6·38	0·21	0·001	0·05	0·001
Subcutaneous adipocyte size ($\mu m^2 \times 10^3$)	3·83	0·43	3·65	0·21	9·83	0·43	8·30	0·21	0·001	NS	NS
Lipoprotein lipase activity in subcutaneous adipose tissue (nmol/min per mg protein)	0·224	0·030	0·338	0·067	0·727	0·122	0·449	0·076	0·001	NS	0·05
Serum leptin (ng/l)	11·0	1·0	7·0*	1·0	149·5	5·0	129·6	13·2	0·001	0·05	NS
Serum adiponectin (μg/ml)	8·3	0·5	7·8	0·5	13·0	0·4	12·7	0·4	0·001	NS	NS
Serum glucose (mg/l)	1870	80	1810	60	1810	50	1780	50	NS	NS	NS
Serum lipids (mg/l)											
TAG	1440	110	1260	150	2990	160	3410	240	0·001	NS	NS
Phospholipids	1570	50	1570	40	3130	60	3240	60	0·001	NS	NS
Total cholesterol	795	28	726	15	1600	40	1480	30	0·001	0·05	NS
HDL-cholesterol	563	16	493*	11	994	32	946	41	0·001	0·05	NS
Liver lipids (mg/g)											
TAG	8·9	0·9	8·2	0·6	76·3	16·5	78·6	12·0	0·001	NS	NS
Phospholipids	31·8	0·4	30·9	0·6	30·1	0·7	28·4	0·7	0·01	0·05	NS
Cholesterol	2·2	0·0	2·1	0·0	2·2	0·1	1·9*	0·1	0·05	0·05	NS

Mean value was significantly different from that of the rats fed the SM diet: * $P<0·05$, ** $P<0·01$, *** $P<0·001$ (Student's *t* test).

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Table 2. Effect of skimmed milk (SM) and *Lactobacillus gasseri*-fermented milk product (LGSP) on faecal steroid composition in lean and obese Zucker rats

(Mean values with their standard errors for eight rats per group)

	Lean				Obese				P (ANOVA)		
	SM		LGSP		SM		LGSP				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Genotype	Diet	Genotype × diet
Faeces dry weight (g/d)	1·55	0·05	1·58	0·03	2·06	0·06	2·27*	0·05	0·001	0·05	NS
Amount of faecal NEFA (mg/d)	26·13	1·17	30·29*	1·50	36·15	2·16	43·41*	2·34	0·001	0·01	NS
Apparent excretion rate of faecal NEFA (%/d)	2·21	0·12	2·63*	0·15	2·10	0·11	2·47*	0·14	NS	0·01	NS
Neutral steroids (mg/rat per d)											
Cholesterol	2·57	0·18	2·55	0·04	1·86	0·09	2·38*	0·16	0·01	0·05	NS
Coprostanol	2·07	0·17	2·30	0·19	1·66	0·14	1·95	0·30	0·05	NS	NS
Total	4·64	0·18	4·85	0·19	3·53	0·13	4·33*	0·25	0·001	0·05	0·05
Acidic steroids (mg/rat per d)											
Lithocholic acid	0·19	0·02	0·21	0·02	0·05	0·01	0·07	0·02	0·001	NS	NS
Deoxycholic acid	0·67	0·08	0·89	0·06	0·15	0·02	0·27*	0·05	0·001	0·05	NS
Chenodeoxycholic acid	0·05	0·01	0·06	0·01	ND		ND		0·001	NS	NS
Cholic acid	0·88	0·11	0·76	0·07	0·69	0·05	0·64	0·11	NS	NS	NS
α-Muricholic acid	0·51	0·13	0·92	0·15	0·02	0·01	0·07	0·04	NS	NS	NS
Hyodeoxycholic acid	0·19	0·10	0·15	0·03	ND		ND		0·001	NS	NS
Ursodeoxycholic acid	0·18	0·03	0·17	0·01	0·05	0·01	0·01	0·01	0·001	0·01	0·01
β-Muricholic acid	0·45	0·08	0·31	0·04	0·08	0·01	0·04*	0·01	0·001	0·01	NS
ω-Muricholic acid	0·90	0·19	0·60	0·11	0·81	0·08	0·76	0·11	NS	NS	NS
Total	4·01	0·29	4·06	0·08	1·85	0·10	1·86	0·10	0·001	NS	NS

ND, not detected.

* Mean value was significantly different from that of the rats fed the SM diet ($P < 0.05$; Student's *t* test).

leptin compared with internal adipose tissues as observed in rodents[29]. In contrast to leptin, there was no significant difference in the concentration of serum adiponectin in both lean and obese Zucker rats. The difference in the response of these adipocytokines to the LGSP diet may be due to the amounts produced, which is then reflected by their serum concentration, and is greater for adiponectin (μg/ml) than leptin (pg/ml)[30]. These results in lean rats are in line with our previous study in Sprague–Dawley rats[10].

The specific mechanism(s) by which the LGSP diet exerts these effects on adipose tissue remains unknown. One mechanism of adipocyte size regulation by the LGSP diet could be the suppression of adipocyte hypertrophy. The suppression of adipocyte hypertrophy by the LGSP diet might be accounted for by a reduction in energy input (intestinal absorption of lipids). Therefore, we determined the effect of the LGSP diet on the faecal NEFA levels of both Zucker rat strains. The present study showed that the apparent absorption of dietary fat, based on the faecal NEFA excretion levels, was lower when the rats of both strains were fed on the LGSP diet. This finding may explain the reduction of both fat mass and adipocyte size in the mesenteric and subcutaneous adipose tissues in Zucker rats and particularly in the lean rats. In agreement with the present results, a reduction in both fat mass and adipose tissue accumulation through an elevation of NEFA excretion was found previously in mice[11]. Likewise, the LGSP diet decreased the maximum lymphatic absorption of TAG (32 %; $P = 0.03$), phospholipids (34 %; $P = 0.02$) and cholesterol (27 %; $P = 0.06$) in Sprague–Dawley rats. This reduction in lymphatic lipid absorption may support the elevation of faecal NEFA excretion that was observed in the LGSP groups. This effect of the LGSP diet might be because it has an ability to bind intestinal lipids. It was found that

L. gasseri SBT2055 has the ability to bind intestinal cholesterol[12]. In addition, there was a significant genotype × diet interaction in the present study, which resulted in increased total faecal neutral sterol excretion in Zucker rats. It is likely that the LGSP diet decreased lymphatic lipids absorption via the binding of lipids and consequently decreased the energy input, which affected adipose tissue hypertrophy. This point requires further research in order to elucidate the mechanism involved.

Although the amount of faecal NEFA of obese rats was higher than that of lean rats, there was no effect of diet on the mesenteric adipose tissue mass or adipocyte size in obese rats. It is likely that the effect of the LGSP diet on the increasing excretion of faecal NEFA might be cancelled by the large amount of energy intake by obese Zucker rats (daily food intake is about 1·5 times compared with lean rats). Consequently, the LGSP diet might lead to an insignificant effect on the visceral adipose tissue weight of obese Zucker rats. Strikingly, the number of smaller adipocytes in the subcutaneous adipose tissue of obese Zucker rats was significantly increased ($P < 0.05$) by the LGSP diet. Although the reason for this finding is unknown, there was a significant genotype × diet interaction on the LPL activity in subcutaneous adipose tissue of Zucker rats. Therefore, the increased number of smaller adipocytes may be attributed to the tendency towards reduced LPL activity in the obese rats ($P = 0.073$). This reason for this finding is currently unclear and further research is required, as we do not have any data concerning the LPL activity in mesenteric adipose tissue due to the low quantity of tissue available from the present study.

The present study showed an increased faecal excretion of neutral sterols and bile acids in lean rats compared with the obese rats. This finding may account for the increased
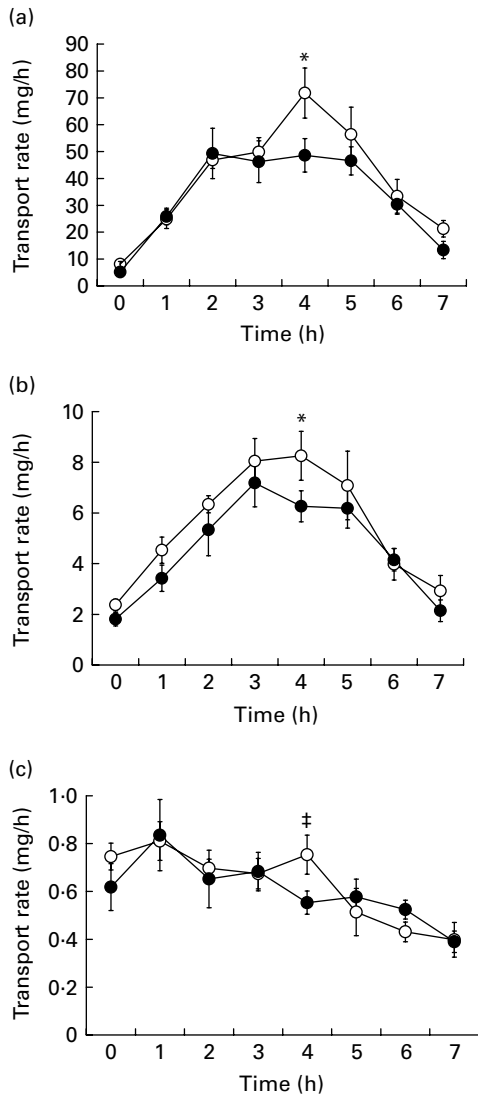
Fig. 3. The effect of dietary skimmed milk (SM) and *Lactobacillus gasseri*-fermented milk product (LGSP) on the transport of TAG (a), phospholipids (b) and cholesterol (c) in Sprague–Dawley rats with thoracic duct cannulation (SM, ○; LGSP, ●). Values are means for six rats per group, with their standard errors represented by vertical bars. * Mean value was significantly lower in the LGSP group than in the SM group ($P<0.05$). ‡ Mean value tended to be lower in the LGSP group than in the SM group ($P=0.06$).

concentration of serum cholesterol in the obese rats compared with the lean rats, possibly due to the decreased excretion of cholesterol. In addition to these strain differences, the diet also affected the faecal bile acid composition. The LGSP diet tended to elevate the excretion of deoxycholic acid and to lower the excretion of ursodeoxycholic and β-muricholic acids in Zucker rats. We have no satisfactory explanation for the effect of *L. gasseri* SBT2055 on the intestinal ecology of our animal models, but this strain might alter the composition and metabolism of the intestinal microflora. Because *L. gasseri* SBT2055 showed an ability to establish in the intestine of both mice and humans[8], we assumed that it can survive and reach the intestine of our animal models. However, the measurement of the *L. gasseri* count in the intestine of our animal models should be required to clarify this effect.

In conclusion, the present results indicate that the fermented milk produced by *L. gasseri* SBT2055 may reduce visceral adipose tissue mass and adipocyte hypertrophy in lean Zucker rat via a reduction in fatty acid absorption, but it may have no effect on rats in which obesity is already established. In addition, we do not know if this effect is due to the viable bacterial cells of *L. gasseri* SBT2055 alone or its milk-fermentation products. Therefore, further experiments are needed to explore the factor responsible of this beneficial effect and to elucidate the mechanism(s) involved.

Acknowledgements

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