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<0.001) and serum leptin concentration (36%; P<0.005) 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 extensively1,2. 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 properties3, enhancement of intestinal functions4,5, hypocholesterolaemic effect5 and inhibitory action against undesirable bacteria6.

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 faeces7. This strain has the ability to reach and establish in the intestine in both man and mice8. Therefore, this strain was chosen as an excellent probiotic strain due to its suitability as a starter for preparing fermented milk9.

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 rats10. 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 lipids11. Furthermore, L. gasseri strains have been reported to bind micellar lipids, thereby raising a possibility of inhibiting the absorption of fatty acids and cholesterol12.

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 deficiency13 and overeating14,15. 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.

Abbreviations: LGSP, Lactobacillus gasseri-fermented milk product; LPL, lipoprotein lipase; SM, skimmed milk.
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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) as described previously \(^{16}\). 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 \(^{15}\)) 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 x 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 (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 anaesthetised 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 0.2 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 (Triacylglyceride 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 Yamaibara 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 measure-
ment and another sample for protein quantification according
to the method of Lowry et al.\(^{22}\). Dibutylryl 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
dibutylryl fluorescein solution, 150 µl ethylene glycol mono-
methyl ether and 1250 µl phosphate buffer. LPL activity was
quantified as the difference between the fluorescence emitted
by the released fluorescein when 1M-NaCl was added to the
incubation medium and the level emitted in the absence of
NaCl. Fluorescence was measured in a fluorescence spectropho-
tometer (Shimadzu Dual beam difference spectrofluorophot-
ometer 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-buf-
fered 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 × (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 derivat-
ised to trimethylsilyl ethers. The products were analysed by
gas–liquid chromatography (GC-17A Shimadzu; Seisakusho
Ltd, Kyoto, Japan) using 5a-cholestanate (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 trans-
formed 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 sig-
nificant for *P*≤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 mesen-
teric and subcutaneous tissues of lean and obese rats, respecti-
vely, 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 subcu-
taneous 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). 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 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<0.001), 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
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 leptin.

![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.](https://www.cambridge.org/core/terms).
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)

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th></th>
<th>Obese</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SM</td>
<td>LGSP</td>
<td>SM</td>
<td>LGSP</td>
</tr>
<tr>
<td>Genotype</td>
<td>P (ANOVA)</td>
<td></td>
<td>Genotype</td>
<td>P (ANOVA)</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>15.0 0.3</td>
<td>14.7 0.1</td>
<td>21.4 0.2</td>
<td>21.3 0.1</td>
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<tr>
<td>Body-weight gain (g)</td>
<td>123 3</td>
<td>115 2</td>
<td>184 3</td>
<td>184 4</td>
</tr>
<tr>
<td>Relative liver weight (g/100 g body weight)</td>
<td>3.26 0.02</td>
<td>3.53** 0.02</td>
<td>3.61 0.06</td>
<td>3.97** 0.07</td>
</tr>
<tr>
<td>Mesenteric fat mass (g)</td>
<td>1.83 0.14</td>
<td>1.41* 0.12</td>
<td>7.73 0.26</td>
<td>7.54 0.12</td>
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<td>Periportal fat mass (g)</td>
<td>0.61 0.07</td>
<td>0.49 0.05</td>
<td>2.25 0.11</td>
<td>2.37 0.10</td>
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<td>Retroperitoneal fat mass (g)</td>
<td>2.23 0.19</td>
<td>1.82 0.18</td>
<td>7.97 0.25</td>
<td>7.56 0.09</td>
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<td>Epididymal fat mass (g)</td>
<td>2.39 0.09</td>
<td>2.22 0.15</td>
<td>8.75 0.21</td>
<td>8.49 0.17</td>
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<td>Subcutaneous fat mass (g)</td>
<td>8.24 0.76</td>
<td>6.75 0.53</td>
<td>30.97 1.27</td>
<td>33.66 1.21</td>
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<td>Total fat mass (g)</td>
<td>15.30 1.19</td>
<td>12.69 0.95</td>
<td>62.76 1.35</td>
<td>59.63 1.52</td>
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<tr>
<td>Mesenteric adipocyte size (μm² x 10³)</td>
<td>2.01 0.11</td>
<td>1.44*** 0.08</td>
<td>6.02 0.37</td>
<td>6.38 0.21</td>
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<td>Subcutaneous adipocyte size (μm² x 10³)</td>
<td>3.83 0.43</td>
<td>3.65 0.21</td>
<td>9.83 0.43</td>
<td>8.30 0.21</td>
</tr>
<tr>
<td>Lipoprotein lipase activity in subcutaneous adipose tissue (nmol/min per mg protein)</td>
<td>0.224 0.030</td>
<td>0.338 0.067</td>
<td>0.727 0.122</td>
<td>0.449 0.076</td>
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<tr>
<td>Serum leptin (ng/l)</td>
<td>11.0 1.0</td>
<td>7.0* 0.5</td>
<td>149.5 5.0</td>
<td>129.6 13.2</td>
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<tr>
<td>Serum adiponectin (μg/ml)</td>
<td>8.3 0.5</td>
<td>7.8 0.5</td>
<td>13.0 0.4</td>
<td>12.7 0.4</td>
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<tr>
<td>Serum glucose (mg/l)</td>
<td>1870 80</td>
<td>1810 60</td>
<td>1810 50</td>
<td>1780 50</td>
</tr>
<tr>
<td>Serum lipids (mg/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAG</td>
<td>1440 110</td>
<td>1260 150</td>
<td>2990 160</td>
<td>3410 240</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>1570 50</td>
<td>1570 40</td>
<td>3130 60</td>
<td>3240 60</td>
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<td>Total cholesterol</td>
<td>795 28</td>
<td>726 15</td>
<td>1600 40</td>
<td>1480 30</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>563 16</td>
<td>493* 11</td>
<td>994 32</td>
<td>946 41</td>
</tr>
<tr>
<td>Liver lipids (mg/g)</td>
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<tr>
<td>TAG</td>
<td>8.9 0.9</td>
<td>8.2 0.6</td>
<td>76.3 16.5</td>
<td>78.6 12.0</td>
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<tr>
<td>Phospholipids</td>
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<td>30.9 0.6</td>
<td>30.1 0.7</td>
<td>28.4 0.7</td>
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<tr>
<td>Cholesterol</td>
<td>2.2 0.0</td>
<td>2.1 0.0</td>
<td>2.2 0.1</td>
<td>1.9* 0.1</td>
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</table>

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).
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 (mg/ml) than leptin (ng/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 accounted for by a reduction in energy input (intestinal absorption of lipids). 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 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

### 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)

<table>
<thead>
<tr>
<th>Genotype Diet</th>
<th>SM Mean SE</th>
<th>LGSP Mean SE</th>
<th>SM Mean SE</th>
<th>LGSP Mean SE</th>
<th>Genotype Diet</th>
<th>Genotype × diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P (ANOVA)</td>
<td></td>
</tr>
<tr>
<td>Neutral steroids (mg/rat per d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>2.57 ± 0.18</td>
<td>2.55 ± 0.04</td>
<td>1.86 ± 0.09</td>
<td>2.38 ± 0.16</td>
<td>0.01 ± 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Coprostanol</td>
<td>2.07 ± 0.17</td>
<td>2.30 ± 0.19</td>
<td>1.66 ± 0.14</td>
<td>1.95 ± 0.30</td>
<td>0.05 ± 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>4.64 ± 0.18</td>
<td>4.85 ± 0.19</td>
<td>3.53 ± 0.13</td>
<td>4.33 ± 0.25</td>
<td>0.001 ± 0.05</td>
<td>0.05 ± 0.05</td>
</tr>
<tr>
<td>Cholic acid</td>
<td>0.88 ± 0.11</td>
<td>0.76 ± 0.07</td>
<td>0.69 ± 0.05</td>
<td>0.64 ± 0.11</td>
<td>NS ± NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ursodeoxycholic acid</td>
<td>0.18 ± 0.03</td>
<td>0.17 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.001 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Total</td>
<td>0.04 ± 0.08</td>
<td>0.31 ± 0.04</td>
<td>0.08 ± 0.01</td>
<td>0.04 ± 0.04</td>
<td>0.001 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Total</td>
<td>2.21 ± 0.12</td>
<td>2.23 ± 0.15</td>
<td>2.63 ± 0.17</td>
<td>2.47 ± 0.14</td>
<td>0.001 ± 0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

**NS** not detected.

* Mean value was significantly different from that of the rats fed the SM diet (P<0.05; Student’s t test).
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 and ursodeoxycholic acids and to lower the excretion of ursodeoxycholic 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).

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.

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