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Milk fermented with *Lactobacillus casei* NCDC19 improves high fat and sucrose diet alters gene expression in obese mice

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ABSTRACT

Dietary incorporation of milk fermented with indigenous probiotic *Lactobacillus casei* NCDC19 (Lc19) was investigated in mice fed a high-fat and sucrose diet (HFS diet). Epididymal fat mass and adipocyte size were significantly reduced in the group fed HFS diet supplemented with Lc19 compared with the group fed HFS diet only. However, body weight differences were not statistically significant. Fasting blood glucose, serum triglyceride, insulin and homeostatic model assessment of insulin resistance score were found to be significantly decreased in the Lc19 treatment group; liver triglyceride levels remained unaffected. Epididymal fat adiponectin and liver carnitine palmitoyl-transferase 1, peroxisome proliferator-activated receptor α, forkhead box protein A2 and peroxisome proliferator-activated receptor gamma coactivator 1β were significantly up-regulated in the Lc19 group. The influence of Lc19 on the expression of genes related to energy expenditure suggests a possible role of *L. casei* in gut physiology modulation, which might be affecting the host metabolism.

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1. Introduction

Probiotics are defined as live microorganisms that confer health-benefits upon the host when administered in an adequate amount (FAO/WHO, 2002). They are an important class of functional foods that have attracted consumers' attention because of their potential effectiveness in prevention/treatment of hypercholesterolaemia (Shin et al., 2010), certain cancers (Kumar et al., 2010) and immune diseases (Borchers, Selmi, Meyers, Keen, & Gershwin, 2009). In addition, certain reports have also demonstrated the beneficial effects of some bacterial strains on obesity (Kang et al., 2013; Park et al., 2013; Pothuraju, Sharma, Chagalamarri, Kavadi, & Jangra, 2015; Rather et al., 2014) and insulin resistance (Ma, Hua, & Li, 2008; Okubo, Takemura, Yoshida, & Sonoyama, 2013).

Different probiotics have different capacities to modulate metabolic phenotypes. *Lactobacillus rhamnosus* PL60 and *Lactobacillus plantarum* PL62 have been reported to exert beneficial effects on diet-induced obesity through production of CLA (Lee et al., 2006, 2007). VSL#3 (mixture of viable lyophilised bifidobacteria, lactobacilli and *Streptococcus thermophilus*) was shown to attenuate diet-induced obesity, hepatic steatosis and insulin resistance by increasing hepatic natural killer T cells and reducing inflammatory signalling (Ma et al., 2008). *Lactobacillus gasseri* SBT2055 reduced adipocyte size by inhibiting dietary fat absorption (Hamad et al., 2009) and anti-obesity effects through improvement of inflammatory state in adipose tissue (Miyoshi, Ogawa, Higurashi, & Kadooka, 2014). *L. gasseri* BNR17 has also been reported to attenuate fat accumulation in body by increasing energy expenditure through up-regulating the expression of genes involved in fatty acid β-oxidation (Kang et al., 2013). *Lactobacillus casei* NCDC19 exerts anti-obesity effects by up-regulating the expression of adiponectin in epididymal fat, and bifidobacterial counts in the colon (Rather et al., 2014). However, the exact mechanism of action of probiotics has not been completely elucidated as yet.

Moreover, beneficial effects of probiotics have been reported to be strain dependent (Fak & Backhed, 2012; Qiao et al., 2015; Yin, Yu, Fu, Liu, & Lu, 2010). There are also reports of probiotics indicating no effects on body weight gain (Arora et al., 2012), and also in some cases probiotics have been reported to cause weight gain in rodents (Andersson et al., 2010; Yin et al., 2010). Evidently, the contradictory reports suggest that more studies are necessary to delineate the effects of probiotics belonging to different species/strains.

L. casei NCDC19 is an indigenous strain that has been reported to exhibit potential probiotic attributes (Kapila, Vibha, & Sinha, 2006)

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and has not been fully explored especially in relation to adiposity, insulin resistance and related gene expression. Fermented milk is an important vehicle for delivery of probiotics. Various studies are available on animals and humans involving the administration of probiotic through fermented milk (Hamad et al., 2009; Kadooka, 2010; 2013; Sato et al., 2008). Therefore, the present investigation was carried out to evaluate the effects of dietary supplementation of milk fermented with probiotic *L. casei* NCDC19 (Lc19) on adiposity, insulin resistance and expression of genes related to lipid metabolism in C57BL/6 mice.

2. Materials and methods

2.1. Preparation of *Lactobacillus casei* NCDC19 fermented milk

L. casei NCDC19 was obtained from the National Collection of Dairy Cultures, Dairy Microbiology Division, National Dairy Research Institute, Karnal, India. Before preparation of probiotic fermented milk, the culture was activated by sub-culturing three times in skim milk. Thereafter, fermented milk was prepared by inoculating (1%, v/v) the sterile skim milk with activated culture, and incubation at 37 °C for 20–22 h. Fermented milk was prepared on alternate days and stored at 4 °C. Viable counts in fermented milk were determined by pour plating method as per formula:

cfu g^{-1} = (no. of colonies × dilution factor)/volume of sample plated.

Fermented skim milk (Lc19) contained viable counts in the range of 8.5–9.0 log cfu g^{-1}.

2.2. Experimental animals and diets

All animal experiments were conducted at the small animal house, National Dairy Research Institute, India, in accordance with the guidelines of Institutional Animal Ethics Committee. Eighteen C57BL/6 male mice (10–12 weeks old) were purchased from Indian Institute of Integrative Medicine, Jammu, India. Mice were kept in ventilated plastic cages with soft husk bedding at controlled room temperature (22 ± 2 °C). The animals were acclimatised for 2 weeks during which they were fed on normal chow ad libitum and had free access to water. After the acclimatisation period animals were randomised according to their body weights, divided into 3 groups (n = 6 animals per group; 2 mice per cage), and assigned on experimental diets as follows: (i) HFS group, received high-fat and sucrose diet (HFS diet) ad libitum; (ii) HFS-SM group, received skim milk along with HFS diet; (iii) HFS-FM group, received Lc19 along with HFS diet. The composition of HFS diet is shown in Table 1. Skim milk comprised (g 100 mL^{-1}): protein, 3.6, carbohydrate 4.9, and fat, 0.3; energy content was 35 kcal 100 mL^{-1}.

Skim milk/Lc19 was provided around 9:30 a.m. and removed around 4:30 p.m. During this period, HFS diet and water was removed so that the animals consumed the skim milk/Lc19. HFS diet and water was presented to the animals after removal of skim milk/Lc19, and fed till next morning. This feeding schedule was followed for 18 weeks. Body weight was taken weekly, and feed intake was recorded daily. After 18 weeks, mice were fasted overnight, and blood glucose level was determined by puncturing the tail vein and using a glucometer (ACCU-CHECK® active, Roche Diagnostics, Germany). Overnight fasted mice were sacrificed by cervical dislocation under diethyl ether anaesthesia. Weight of visceral organs (spleen, liver and kidney) and epididymal white adipose tissue was measured. For relative gene expression analysis, tissues (liver, epididymal fat and distal ileum) were stored in RNA*later*® (Sigma–Aldrich, Saint Louis, Missouri, USA) at −20 °C until used. To quantitate liver lipids, liver tissues were washed with 1× phosphate buffered saline (PBS) and stored at −20 °C in PBS until analysed.

Table 1
Composition of experimental high-fat and sucrose diet.[a]

Ingredient	Content (%)
Casein	19.5
L-Methionine	0.3
Sucrose	28.78
Corn starch	15.0
Milk fat	21.0
Soybean oil	–
Cellulose	10.52
Mineral mixture (AIN-76)	3.5
Vitamin mixture (Teklad)	1.0
$CaCO_3$	0.4
Ethoxyquin	0.004
Fat (% kcal)	42.63
Total energy (kcal 100 g^{-1} diet)	443.3

[a] Casein was a product of M/s Modern Dairies, Karnal (India); cow milk fat (ghee) prepared by experimental dairy, NDRI, Karnal (India).

2.3. Analysis of blood parameters

At the end of study, blood obtained by cardiac puncture was transferred into non-heparinized tubes. Tubes were kept undisturbed at room temperature for 30 min, centrifuged at 2000 ×*g* for 15 min at 4 °C, and serum was collected. Serum total cholesterol (TC), HDL-C and triglyceride (TG) levels were determined using commercial kits (Span Diagnostics Pvt. Ltd., Surat, India). LDL-C concentration was calculated by using Friedewald's equation (Friedewald, Levy, & Fredrickson, 1972), and VLDL-C calculated by dividing triglycerides value by 5. Serum insulin level was measured by using ultrasensitive mouse insulin ELISA kit (Crystal Chem Inc., Elk Grove Village, IL, USA). Serum PYY was measured by using Human/Mouse/Rat enzyme immunoassay kit (RayBiotech Inc., Norcross, GA, USA). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the following equation (Haffner, Miettinen, & Stern, 1997):

HOMA-IR = fasting insulin (μU mL^{-1}) × fasting glucose (mmol L^{-1})/22.5.

2.4. Analysis of liver lipids

Analysis of liver lipids was done following the method described by Morton et al. (2005). Briefly, liver tissue was homogenised in isopropanol (1:10), and then shaken for 45 min. The supernatant obtained by centrifugation at 3000 ×*g* for 10 min was used for analysis with a commercial kit (Span diagnostics Pvt. Ltd.).

2.5. Histological examination of epididymal fat tissue

Epididymal fat tissue was fixed in 10% (v/v) formalin and sent to a local pathology lab for further processing. Samples were embedded in paraffin, sectioned (4 μm), and mounted on glass slides. Three replicates were taken for each sample. Haematoxylin-eosin staining was performed using the standard technique and examined the morphology (three fields) under 200× magnification (Olympus BX51 fitted with Olympus DP71 camera). Images were captured and cell size and number were measured using Image J software (National Institutes of Health, Bethesda, MD, USA).

2.6. Relative mRNA expression analysis

Total RNA was isolated using TRI Reagent® (Sigma, Saint Louis, Missouri, USA). Five hundred nanograms of total RNA was reverse transcribed into cDNA using a RevertAid first strand cDNA synthesis

Table 2
Primer sequences used for real-time quantitative PCR.[a]

S. No.	Target gene	Primer sequence
1	Foxa2 F	5′-GCG GCC AGC GAG TTA AAG TAT-3′
	Foxa2 R	5′-TCA TTC CAG CGC CCA CAT A-3′
2	Pgc1β F	5′-TTG TAG AGT GCC AGG TGC TG-3′
	Pgc1β R	5′-GAT GAG GGA AGG GAC TCC TC-3′
3	Cpt1 F	5′-CCA GGC TAC AGT GGG ACA TT-3′
	Cpt1 R	5′-GAA CTT GCC CAT GTC CTT GT-3′
4	Pparα F	5′-CAG TGC CCT GAA CAT CGA GTG T-3′
	Pparα R	5′-TTC GCC GAA AGA AGC CCT T-3′
5	Glut4 F	5′-GAT TCT GCT GCC CTT CTG TC-3′
	Glut4 R	5′-ATT GGA CGC TCT CTC TCC AA-3′
6	Glut2 F	5′-GTC CAG AAA GCC CCA GAT ACC-3′
	Glut2 R	5′-GTG ACA TCC TCA GTT CCT CTT AG-3′
7	Fiaf F	5′-TAG AGT CCC TGA AGG CCA GA-3′
	Fiaf R	5′-AAT GAG CTG GGT CAT CTT GG-3′
8	Adiponectin F	5′-TGT TGG AAT GAC AGG AGC TG-3′
	Adiponectin R	5′-CGA ATG GGT ACA TTG GGA AC-3′
9	Leptin F	5′-TGA CAC CAA AAC CCT CAT CA-3′
	Leptin R	5′-TCA TTG GCT ATC TGC AGC AC-3′
10	Pparγ F	5′-CTG GCC TCC CTG ATG AAT AA-3′
	Pparγ R	5′-GGC GGT CTC CAC TGA GAA TA-3′
11	Srebp1c F	5′-GGC ACT AAG TGC CCT CAA CCT-3′
	Srebp1c R	5′-GCC ACA TAG ATC TCT GCC AGT GT-3′
12	β-actin F	5′-TGT TAC CAA CTG GGA CGA CA-3′
	β-actin R	5′-GGG GTG TTG AAG GTC TCA AA-3′

[a] Abbreviations are: FOXA2, forkhead box protein A2; PGC1β, peroxisome proliferator-activated receptor gamma coactivator 1β; CPT1, carnitine palmitoyltransferase 1; PPARα, peroxisome proliferator-activated receptor α; GLUT4, glucose transporter 4; GLUT2, glucose transporter2; FIAF, fasting induced adipose factor; PPARγ, peroxisome proliferator-activated receptor γ; SREBP-1c, sterol regulatory element-binding protein-1c.

kit (Thermo Scientific, Waltham, MA, USA). Relative mRNA expression of genes was determined using a Maxima SYBR Green qPCR Master Mix (2×) kit (Thermo Scientific, Waltham, MA, USA) and a Roche Light Cycler 480 system. The gene specific primers used are shown in Table 2 β-Actin was used as an internal control to normalise the mRNA levels of all genes. Melting peaks for all the reactions were analysed for the presence of primer dimers or secondary structures (if any). Samples were run in duplicate in a 96-well reaction plate, and real time PCR data was analysed using the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001).

2.7. Statistical analysis

All data are expressed as mean ± SEM. Analysis of variance (ANOVA) followed by post hoc Tukey's multiple comparison tests was used to compare different experimental groups (GraphPad Software, San Diego, CA, USA). Intake of skim milk/Lc19 was analysed by an unpaired t-test to compare the two groups, i.e., HFS-SM and HFS-FM; *P*-values < 0.05 were considered statistically significant.

3. Results

3.1. Body and epididymal fat weights

The mean body weight of animals in the HFS-FM group was found to be lower than that of the HFS as well as the HFS-SM groups. However, the differences in body weight were not statistically significant (Fig. 1A). Administration of Lc19 was found to be significantly effective ($P < 0.05$) in resisting the epididymal fat mass accumulation compared with the HFS group (Fig. 1B). No effect on visceral organs (liver, kidney and spleen) was observed among different groups. No statistically significant difference in cumulative feed intake, skim milk versus probiotic fermented milk intake,

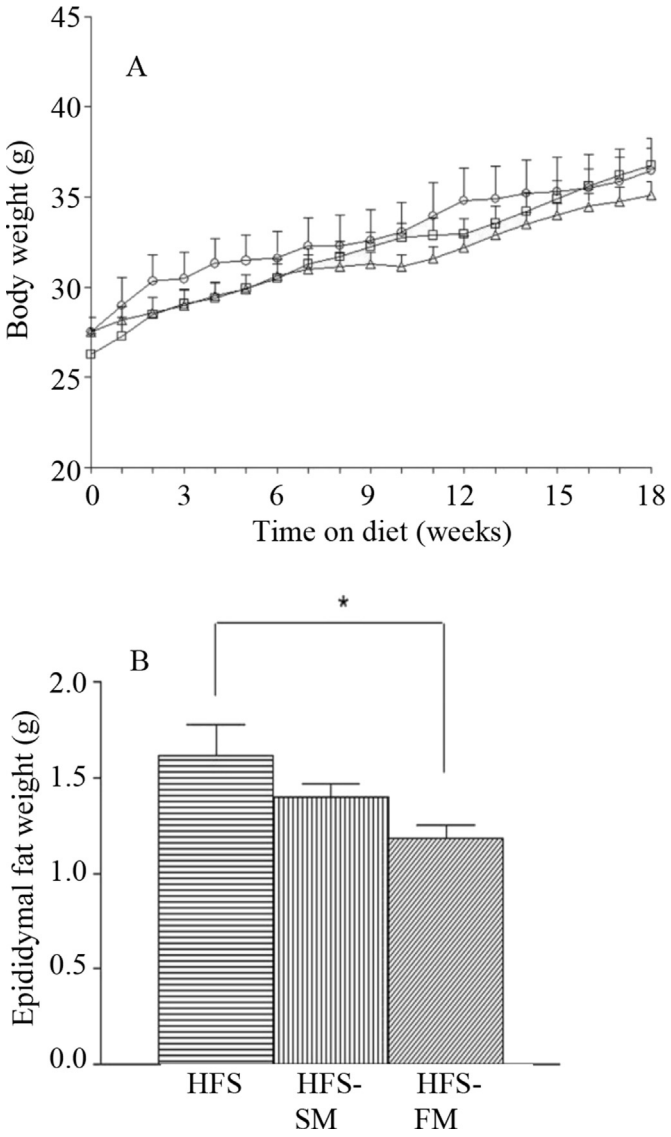

Fig. 1. Effect of Lc19 on HFS diet-induced adiposity in mice: effect on (A) body weight (○, HFS □, HFS-SM △, HFS-FM) and (B) epididymal white adipose tissue weight gain (values are the mean ± SEM (n = 6 per group); *$P < 0.05$ versus HFS group).

and energy intake among different experimental groups was observed (Fig. 2).

3.2. Adipocyte size and number

Adipocyte size was significantly smaller ($P < 0.01$) in the HFS-FM group (2138 ± 151.6 μm^2) compared with the HFS group (3237 ± 205.9 μm^2) after 18 weeks. Although the size of adipocytes was smaller in the HFS-SM group, it was not found to be statistically significant when compared with the HFS group (Fig. 3). Adipocyte number per spot among different experimental groups did not significantly differ.

3.3. Serum and liver lipid profile

The serum and liver lipid profile was determined in the different experimental groups to evaluate the effect of dietary supplementation of Lc19, and the data are presented in Table 3. Serum TG and

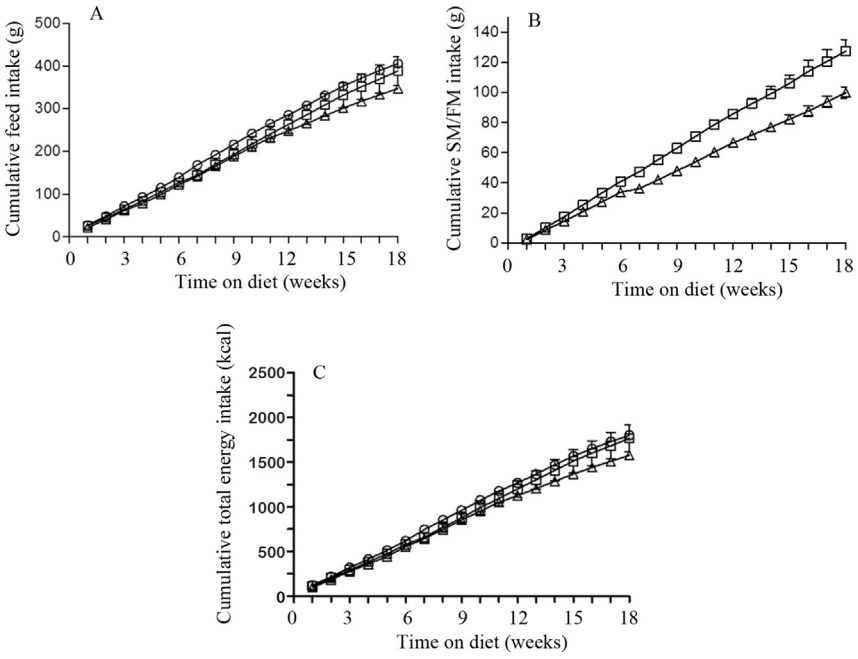

Fig. 2. Effect of Lc19 on energy intake among different experimental groups (○, HFS □, HFS-SM △, HFS-FM): (A) cumulative feed (solid) intake; (B) cumulative skim milk/Lc19 intake; (C) cumulative total energy (solid + SM/Lc19) intake. Values are the mean ± SEM (n = 6 per group).

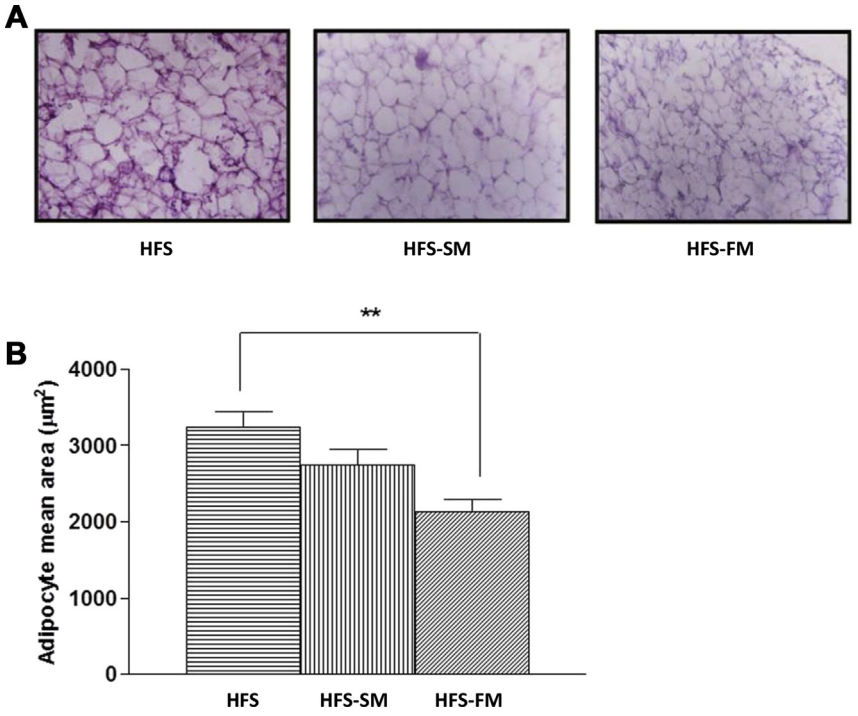

Fig. 3. Effect of Lc19 on adipocyte hypertrophy in mice fed HFS diet for 18 weeks: (A) photomicrographs of haematoxylin and eosin staining of adipocytes, 200× magnification; (B) adipocyte mean area (μm^2). Values are expressed as mean ± SEM (n = 3 per group); $^{**}P < 0.01$ versus HFS group.

VLDL levels were lowered significantly ($P < 0.01$) in the HFS-FM group compared with the HFS group. No significant effect on serum TC, HDL-C, and LDL-C was found with dietary supplementation of Lc19. Also, hepatic TC and TG contents were not altered significantly on feeding Lc19.

3.4. Blood glucose, serum insulin and HOMA-IR score

Dietary supplementation of Lc19 was found to be significantly effective in resisting an increase in blood glucose ($P < 0.01$) and insulin levels ($P < 0.01$) due to HFS diet feeding (Table 3). The

Table 3
Effect of Lc19 on blood parameters and hepatic lipids.[a]

Parameters	HFS	HFS-SM	HFS-FM
Blood			
TC (mg dL^{-1})	144.9 ± 12.21	136.0 ± 9.41	114.8 ± 12.62
HDL-C (mg dL^{-1})	41.53 ± 4.30	39.43 ± 4.51	50.72 ± 4.16
LDL-C (mg dL^{-1})	68.0 ± 10.79	66.82 ± 15.39	40.29 ± 9.58
VLDL-C (mg dL^{-1})	35.33 ± 1.77	29.81 ± 2.35	23.81 ± 1.81**
TG (mg dL^{-1})	176.7 ± 8.84	149.0 ± 11.76	119.1 ± 9.07**
Blood glucose (mg dL^{-1})	199.7 ± 12.32	171.2 ± 8.74	145.5 ± 6.72**
Insulin (ng mL^{-1})	1.52 ± 0.06	1.26 ± 0.10	1.08 ± 0.07**
HOMA-IR score	19.44 ± 2.14	13.82 ± 1.50	9.68 ± 0.87**
PYY (pg mL^{-1})	74.35 ± 34.81	97.25 ± 39.56	148.3 ± 32.77
Hepatic lipids			
TC (mg g^{-1} tissue)	6.35 ± 0.25	6.53 ± 0.43	5.90 ± 0.58
TG (mg g^{-1} tissue)	28.81 ± 2.57	26.71 ± 2.15	21.15 ± 1.98

[a] Values are means ± SEM (n = 4–5); a double asterisk indicates $p < 0.01$ compared with HFS group. Abbreviations are: TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; VLDL-C, very low density lipoprotein cholesterol; TG, triglycerides; HOMA-IR, homeostasis model assessment of insulin resistance; PYY, peptide tyrosine tyrosine.

HOMA-IR score in the different experimental groups revealed a trend similar to that observed in the case of blood glucose and serum insulin levels. Supplementation with Lc19 reduced the increase in HOMA-IR score significantly ($P < 0.01$ versus the HFS group) (Table 3). These results suggested the effectiveness of Lc19 treatment in amelioration of insulin resistance in mice fed HFS diet. Although an increase in serum PYY level (anorexic gut peptide) was visible in the HFS-FM group compared with the HFS group, the difference was not statistically significant.

3.5. Relative mRNA expression

Relative mRNA expression of adiponectin in epididymal white adipose tissue was found to be up-regulated significantly ($P < 0.01$) in the HFS-FM group compared with the HFS group. Feeding of Lc19 exhibited the tendency to resist the increase in leptin and PPARγ expression due to HFS diet feeding, but not to a significant extent. Expression of glut4 in the HFS-FM group did not differ significantly compared with the HFS group (Fig. 4).

Whereas genes related to β-oxidation in the liver, i.e., cpt1, pparα, foxa2 and pgc1β, were up-regulated significantly in the HFS-FM group, expression of srebp1c showed a tendency to be lower in the HFS-FM group compared with the HFS group (Fig. 5). Although mRNA expression of fiaf and glut2 in the distal ileum were not affected significantly by Lc19 feeding, there was a visible trend of higher expression of fiaf in the HFS-FM group compared with the HFS group (Fig. 6).

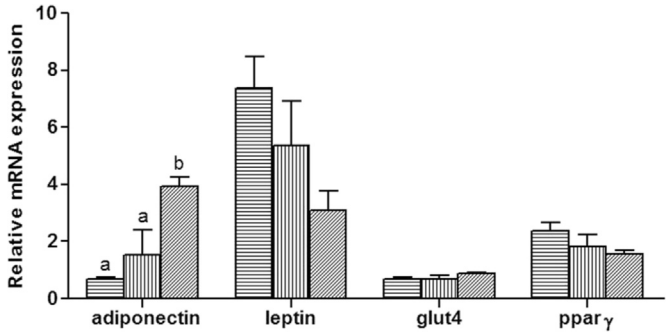

Fig. 4. Effect of Lc19 on relative gene expression in epididymal white adipose tissue of mice fed HFS diet for 18 weeks (▤, HFS; ▥, HFS-SM; ▨, HFS-FM. Values are the mean ± SEM (n = 3 per group); values with different superscript letters differ significantly ($P < 0.05$).

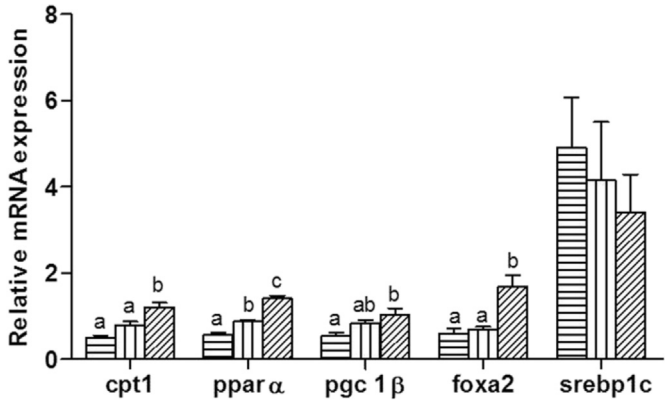

Fig. 5. Effect of Lc19 on relative gene expression in liver tissue of mice fed HFS diet for 18 weeks (▤, HFS; ▥, HFS-SM; ▨, HFS-FM). Values are the mean ± SEM (n = 3 per group); values with different superscript letters differ significantly ($P < 0.05$).

Fig. 6. Effect of Lc19 on relative gene expression in distal ileum of mice fed HFS diet for 18 weeks (▤, HFS; ▥, HFS-SM; ▨, HFS-FM. Values are the mean ± SEM (n = 3 per group); values with different superscript letters differ significantly ($P < 0.05$).

4. Discussion

In the present study, dietary supplementation with Lc19 seemed to resist the gain in body weight caused by HFS diet feeding, but not to a significant level. Contrary to this, Rather et al. (2014) observed reduced gain in body weight to a significant extent from the 7th week onwards as a result of dietary supplementation probiotic dahi containing *L. casei* NCDC19 and dahi culture NCDC167 in high fat diet fed mice. This discrepancy in findings might be ascribed to the different type of fat (lard) in the high fat diet and the age of mice used in their study. In the present investigation, the HFS diet contained 21% milk fat (cow ghee), and the fermented milk contained the probiotic organism only. Similar to our study, no effect on body weight has been reported by other workers also (Arora et al., 2012; Fak & Backhed, 2012; Qiao et al., 2015; Salaj et al., 2013; Takemura, Ozawa, Kimura, Watanabe, & Sonoyama, 2010).

Our results indicate a possible protective effect of Lc19 feeding in terms of reduction in epididymal fat mass deposition and adipocyte size. The mechanisms of how probiotics might reduce the fat mass accumulation are not clearly understood. Obesity is considered to be linked with low-grade inflammation of visceral adipose tissue (Hotamisligil, 2006). Ding et al. (2010) have reported that inflammation in small intestine, which is closely connected to visceral adipose tissue via blood vessels, occurs in early phase of

obesity development. Miyoshi et al. (2014) observed that *L. gasseri* SBT2055 significantly prevented body weight gain, fat accumulation and pro-inflammatory gene expression in adipose tissue. It is possible that the resistance to fat accumulation by administration of Lc19 observed in the present study might be through an anti-inflammatory action. Hamad et al. (2009) suggested that suppression of adipocyte hypertrophy by *L. gasseri* SBT2055 (LGSP) might be due to the reduction in energy input (intestinal absorption of lipids). They showed that the apparent absorption of dietary fat, based on faecal NEFA excretion levels, was lower when Zucker rats were fed on normal diet containing LGSP fermented milk powder and this might explain the reduction of both fat mass and adipocyte size in the adipose tissue. Larger adipocytes are known to produce more pro-inflammatory cytokines (IL6 and TNFα) and free fatty acids in the circulation. These molecules are known to initiate the inflammatory signalling pathway in the cell that leads to insulin resistance in peripheral tissues. From our results, it could be implied that Lc19 might reduce the levels of pro-inflammatory cytokines through decreasing the size of adipocytes and improvement of insulin sensitivity.

Feeding of Lc19 was also effective in providing protection against development of hyperglycemia and hyperinsulinemia caused by high fat and sucrose diet. The improvement in insulin resistance index (HOMA-IR) due to treatment with Lc19 could be correlated with their anti-adiposity effects. The available literature related to both rodents and humans indicates that insulin sensitivity improves as visceral fat reduces (Fantuzzi & Mazzone, 2007). Modulation of gut microbiota could be another mechanism for improvement of insulin sensitivity. There are many reports showing the positive relationship between bifidobacterial counts in the intestine and improvement of high fat diet-induced metabolic syndrome (Cani et al., 2007; Chen, Wang, Li, & Wang, 2011). The increase in the bifidobacterial counts in caecal content by *L. casei* NCDC19 has been reported previously (Rather et al., 2014).

Supplementation of Lc19 with HFS diet seemed to be effective in amelioration of dyslipidaemia. Administration of Lc19 resulted in significant reduction of serum TG and VLDL-C levels as compared with the HFS group. However, serum LDL-C, TC, HDL-C, and hepatic TG and TC were not affected. Lee et al. (2006) administered *L. rhamnosus* PL60 cells to C57BL/6 mice fed a high fat diet and, similar to our findings, observed no effect on serum HDL-C, LDL-C and TC. Rather et al. (2014) observed significantly reduced plasma TC and LDL-C in C57BL/6 mice fed high fat diet (60% kcal from lard) supplemented with probiotic dahi for 8 weeks. Although, the probiotic culture was *L. casei* NCDC19, it was administered in the form of probiotic dahi containing a mixed dahi culture (*Lactococcus lactis* ssp. *lactis*, *Lc. lactis* ssp. *cremoris*, *Lc. lactis* ssp. *lactis* biovar. *diacetylactis*). Other researchers have also reported amelioration of TG levels without any effect on other lipid parameters on administration of probiotics (Huang, Korivi, Tsai, Yang, & Tsai, 2013; Lee, Paek, Lee, Park, & Lee, 2007; Salaj et al., 2013). Harisa, Taha, Khalil, and Salem (2009) thought that hypotriglyceridaemic effect of probiotics might be due to initiation of lipase activity, decreasing intestinal absorption of lipids or increased lipid catabolism and/or antioxidant activity. Up-regulation of LDL receptor mRNA expression on supplementation of *L. rhamnosus* GG (Kumar et al., 2013) and *Lactobacillus acidophilus* ATCC 43121 (Park, Kim, Shin, Kim, & Whang, 2007) has also been reported. Regulation of serum cholesterol by probiotic strains has also been linked with bile salt hydrolase produced by certain probiotic organisms. Deconjugated bile salts are less reabsorbed in the intestinal lumen resulting in the excretion of free bile acids in faeces. Assimilation of cholesterol by probiotic organisms has also been suggested to be one of the mechanisms (Liong & Shah, 2005; Pereira & Gibson, 2002).

Adipose tissue is not simply a storage depot of fat. It is a dynamic organ that is recognised to play an important role in energy homeostasis via some secretory proteins, especially adiponectin and leptin. Adiponectin plays an important role in improvement of insulin sensitivity and fatty acid oxidation, and its level is inversely linked with amount of fat stored in the body, and with serum glucose, insulin, and triglyceride levels (Arita et al., 1999). Leptin level in blood is directly correlated with body fat percentage (Maffei et al., 1995); in obese conditions, normal signalling of leptin gets disturbed, which causes leptin resistance and hyperleptaemia (Myers, Leibel, Seeley, & Schwartz, 2010).

Results of this study indicate significant increase in mRNA expression of adiponectin as a consequence of Lc19 feeding. Lc19 administration was also found to exhibit ameliorative effects in resisting the leptin expression, though not to the statistically significant level, compared with the HFS group. A significantly reduced expression of leptin while enhanced expression of adiponectin in epididymal fat of mice as a consequence of feeding probiotic dahi (containing *L. casei* NCDC19) with high fat diet has also been reported by Rather et al. (2014). Increased adiposity is the consequence of low energy expenditure. Accordingly, we measured the mRNA expression levels of genes related to fatty acid oxidation in the livers of the mice in the different experimental groups. It was demonstrated that expression levels of genes related with regulation of fatty acid oxidation in liver tissue were increased significantly on administration of Lc19. This could be surmised as an important contributory factor for the significant resistance to epididymal fat mass accumulation and adipocyte size in Lc19 fed group.

Wolfrum, Besser, Luca, and Stoffel (2003) reported that the forkhead transcription factor A2 (Foxa2) is phosphorylated under the influence of insulin signalling, resulting in inhibition of its transcriptional activity. Foxa2 plays a central role in maintaining lipid and glucose homeostasis by regulating gene expression of rate-limiting enzymes in response to insulin activation (Wolfrum, Asilmaz, Luca, Friedman, & Stoffel, 2004). Induction of foxa2 activity at low insulin concentrations results in increased mitochondrial and peroxisomal β-oxidation. In the present study, it could also be demonstrated that expression of pparα (a regulator of different genes related with fatty acid oxidation) was enhanced significantly in conjunction with cpt1 expression on administration of Lc19.

Fiaf is a circulating lipoprotein lipase (LPL) inhibitor that controls triglyceride deposition into adipose tissue (Sukonina, Lookene, Olivecrona, & Olivecrona, 2006; Yoshida, Shimizugawa, Ono, & Furukawa, 2002). In this study it was demonstrated that there was a tendency of increased fiaf expression in the distal ileum on administration of Lc19. Aronsson et al. (2010) reported that mice given *Lactobacillus paracasei* ssp. *paracasei* F19 displayed significantly less body fat and higher circulating levels of fiaf, which was inferred as possibly inhibited LPL action through fiaf leading to decreased fat storage. Kondo et al. (2010) also observed significantly up-regulated expression of fiaf in the small intestine of high fat diet-induced obese mice supplemented with *Bifidobacterium breve* B3 and suggested that this may be one of the mechanisms related to the reduction in fat accumulation in adipocytes. In our study also, a link could be demonstrated between increased fiaf expression under the influence of Lc19 and decreased epididymal fat mass storage and adipocyte size. These results suggest that Lc19 changes the expression pattern of different genes in different tissues of mice which leads to increased lipolysis, decreased lipogenesis, and increased response to hormones such as adiponectin, all of these phenomena contribute to decreased adiposity and improved insulin sensitivity.

5. Conclusion

The findings of the present study suggest an important role of Lc19 in regulation of adiposity and resistance to development of diet induced insulin resistance. The influence on the expression of genes related to energy expenditure suggests the possible role of Lc19 in modulation of gut physiology, which might be affecting the host metabolism. The dietary supplementation of skim milk with the HFS diet did not lead to significant difference in different parameters when compared with the group fed HFS diet only. However, further studies are warranted to understand the mechanistic aspects.

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