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Anti-Obesity and Hypolipidemic Effects of Dietary Levan in High Fat Diet-Induced Obese Rats

KANG, SOON AH[1], KYUNGHEE HONG[2], KI-HYO JANG[3], SOHYE KIM[4], KYUNG HEE LEE[5], BYUNG-IL CHANG[6], CHUL-HO KIM[7], AND RYOWON CHOUE[8]*

[1]*Department of Molecular Biotechnology, Bio/Molecular Informatics Center, Konkuk Univesity, Seoul 143-701, Korea*
[2]*Asan Institute for Life Science,* [3]*Department of Food & Nutrition, Samcheok National University, Gangwon*
[4]*Health Medical Center, Seoul National University Bundang Hospital, Bundang, Korea*
[5]*Department of Food Management, Kyung Hee University, Seoul 130-701, Korea*
[6]*RealBio Tech Co. Ltd., Taejon 305-333, Korea*
[7]*Biotechnology Research Division, KRIBB, 52 Oun-dong, Yusong, Taejon 305-333, Korea*
[8]*Department of Medical Nutrition, Graduate School of East-West Medical Science, Kyung Hee University, Seoul 130-701, Korea*

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Abstract We found previously that dietary high fat caused obesity, and levan supplementation to the regular diet reduced adiposity and serum lipids. In the present study, we examined the effects of levan [high-molecular-mass β-(2,6)-linked fructose polymer] supplement on the development of obesity and lipid metabolism in rats fed with high-fat diet. Thus, to determine whether the dietary levan may have the anti-obesity and hypolipidemic effects, 4-wk-old Sprague Dawley male rats were fed with high-fat diet for 6 wk to induce obesity, and subsequently fed with 0, 1, 5, or 10% levan supplemented high-fat diets (w/w) for another 4 wk. For the comparison, a normal control group was fed with AIN-76A diet. Supplementation with levan resulted in a significant reduction of high-fat-induced body weight gain, white fat (i.e., epididymal, visceral, and peritoneal fat) development, adipocyte hypertrophy, and the development of hyperinsulinemia and hyperlipidemia in a dose-dependent manner. Serum triglyceride and free fatty acid levels were greatly reduced by levan supplementation. Serum total cholesterol level was reduced, whereas the HDL cholesterol level was increased by dietary levan. The expression of uncoupling protein (UCP) was increased by dietary high fat, and was further induced by levan supplementation. The mRNA level of UCP1, 2, and 3 in brown adipose tissue (BAT) and UCP3 in skeletal muscle was upregulated in rats fed with dietary levan. In conclusion, upregulated UCP mRNA expression may contribute to suppression of development of obesity through increased energy expenditure. The present results suggest that levan supplementation to the diet is beneficial in suppressing diet-induced obesity and hyperlipidemia.

Key words: Obesity, hypolipidemic effects, levan, UCP, high-fat diet, rat

*Corresponding author
Phone: 82-2-961-0769; Fax: 82-2-965-8904;
E-mail: sakang@khu.ac.kr

Obesity is the most prevalent nutritional disorder that results from imbalance between energy intake and expenditure [25]. It is often associated with hyperlipidemia, type 2 diabetes mellitus, hypertension, and increased risk of coronary heart disease. The optimal treatment for obesity would be the one that both suppresses food intake and increases energy expenditure. Food intake is regulated by leptin and other neurotransmitters, affecting appetite [36], and diet modulation can affect energy expenditure. An important component of energy expenditure is adaptive thermogenesis, which is based on the functionality of uncoupling protein (UCP). UCP is a member of the inner mitochondrial membrane transporters, which induces heat production by uncoupling respiration from ATP synthesis. It has been reported that the expression of UCP is related to energy expenditure and it plays a role in the development of obesity [9].

Interestingly, nondigestible carbohydrates such as fructan has been shown to exert systemic effects by modifying energy and lipid metabolism. Indeed, consumption of nondigestible oligosaccharides suppresses weight gain and fat accumulation [31], and reduces serum triglyceride and cholesterol concentrations [21]. The mechanism of these effects on serum lipids remains incompletely elucidated. Fructan stimulates the growth of bifidobacteria [6] and

improves the intestinal microflora production of short-chain fatty acids, which are expected to have physiologic roles in human health [35]. Animal studies demonstrated that short-chain fatty acids produced by bacterial fermentation and absorbed into the portal blood flow may have an inhibitory effect on hepatic fatty acid and cholesterol synthesis [8, 27].

In contrast to the extensive studies on the effects of β-(2,1) fructan, such as oligofructose and inulin, few investigation of levan which belongs to a class of fructan composed of β-(2,6)-linked fructose have been carried out. Although oligofructose and inulin are available as natural oligomers and polymers of the sugar fructose found in many plants and microbial products, it has not been possible to obtain sufficient quantities of β-(2,6) fructan levan from natural sources. Microbial levans are produced from bacteria, yeasts, and fungi [10, 18-20, 34]. The production and utilization of levan at an industrial level are not well developed, and only a few papers have reported on the production of levan using fermentation techniques [17, 32]. We used synthesized high molecular weight (ca. 6,000,000) levan from sucrose by using bacterial levansucrase (sucrose-6-fructosyltransferase, EC 2.4.1.10) which was isolated from *Zymomonas mobilis* [34]. Levan is soluble in water and not hydrolyzed by human digestive enzymes, and the fermentability and the bifidogenic effect of levan have been confirmed *in vivo* [12]. Also, levan can be used as a prebiotic for stimulating the growth of lactic acid-producing bacteria in an animal model [11].

Previous work in our laboratory showed that dietary high-fat diet (40% of calories as fat) caused obesity in rats with increased body weight and fat accumulation [13], and that the oral administration of 2% levan reduced the adiposity and serum lipids [14]. Recently, exobiopolymers produced from submerged mycelial culture of *Ganoderma lucidum* and mushrooms have been shown to have hypolipidemic effect [43, 44]. On the basis of these earlier studies, the present study was undertaken to test the hypothesis that nondigestible carbohydrates, namely levan, are able to decrease adiposity and post-prandial lipemia in obese rats induced by high-fat diet, thus clarifying whether supplementation of levan has anti-obesity and hypolipidemic effects. The gene expression of UCP was investigated in order to find a potential effect of levan on energy intake and expenditure.

MATERIALS AND METHODS

Animals and Diets

Three-weeks-old Sprague-Dawley male rats were purchased from Central Experimental Animals (Samtaco, Seoul Korea) and housed individually. After adaptation for 1 week, rats were weighed, randomly assigned, and fed normal or high-fat diet. Six weeks later, high-fat fed rats were randomly assigned to four groups and allowed to one of the four diets; high fat with 0, 1, 5, or 10% (wt/wt) levan diets for 4 weeks. The composition of experimental diets is shown in Table 1. Water and food were *ad libitum*. The food intake and body weight were weighed twice a week and food efficiency ratio (FER) was calculated.

Table 1. Composition of experimental diets (g/kg diet).[a]

	N	HF	HF-L1	HF-L5	HF-L10
Casein	200	200	200	200	200
DL-Methionine	3	3	3	3	3
Corn starch	150	150	140	100	50
Sucrose	500	345	345	345	345
Cellulose	50	50	50	50	50
Corn oil	50	-	-	-	-
Beef tallow	-	205	205	205	205
Mineral mixture[b]	35	35	35	35	35
Vitamin mixture[c]	10	10	10	10	10
Choline bitartrate	2	2	2	2	2
Levan	-	-	10	50	100
Fat % (Calories)	11.7	40.0	40.0	40.0	40.0

[a]N; Normal diet, AIN-76A diet #100000. HF; high-fat diet, AIN-76 diet #100496 (Dyets Inc., Bethlehem, PA, U.S.A.). HF-L1; high fat with 1% levan. HF-L5; high fat with 5% levan. HF-L10; high fat with 10% levan diet.
[b]AIN-76 Mineral mix; Dyets Inc., Bethlehem, PA, U.S.A.
[c]AIN-76 Vitamin mix; Dyets Inc., Bethlehem, PA, U.S.A.

Sample Collection

After 4 weeks of feeding normal, high fat, or levan supplemented high-fat diets, blood was collected from the portal vein under anesthesia with diethyl ether, and serum was separated by centrifugation (3,000 ×*g*, for 15 min at 4°C). After collecting blood samples, soleus muscle, interscapular brown adipose tissue (BAT), epididymal fat pad, visceral fat, and peritoneal fat pad were immediately excised, weighed, and frozen in liquid N_2. All serum and tissue samples were stored at -70°C until analysis.

Abdominal Fat Distribution

Abdominal fat distribution of rats was examined one day before sacrifice, using MRI (Magnetic Resonance Imaging System).

Adipocyte Size Determination

Adipose tissue samples (0.5 g) were taken from visceral fat depots and adipocytes were isolated using collagenase [24]. Adipose tissue was immediately washed in 145 mmol/l NaCl-buffer containing 3% BSA, cut into small pieces, and added to 1 ml of NaCl-buffer containing 1.5 mg collagenase (Sigma Chemical, St. Louis, MO, U.S.A.), and the mixture was incubated in a shaking water bath at

80 cycles/min for 1 h at 37°C. After incubation, the cells was filtered through 450 μm nylon mesh, and adipocytes were allowed to float for 3 min. The adipocytes were washed twice with 3 ml of NaCl-buffer containing 5 mM glucose and 3% BSA. Between each washing, the adipocytes were centrifuged at 470 ×*g* for 1 min. Then, the cells were resuspended in 1-2 ml of NaCl-buffer with glucose and BSA. The adipocytes were evaluated by a microscope, using a calibrated grid, and the mean diameter of 30 cells from each cells preparation was calculated.

Blood Analyses

Serum cholesterol, HDL-cholesterol, triglyceride (TG), and free fatty acid were measured using commercial kits (Sigma Chemical, St. Louis, MO, U.S.A.).

Quantitative RT-PCR for Gene Expression Analyses

Total RNA from BAT, epididymal fat, and soleus muscle was extracted with the Trizol reagent (Gibco). The yield and quality of the extracted RNA were assessed by the 260/280 nm optical density ratio and by electrophoresis on 1% agarose gels under denaturating conditions. Reverse transcription (RT) reactions consisted of 2 μg of total RNA denatured for 10 min at 72°C for cDNA synthesis. The final composition of the reaction mixture was as follow: M-MLV (Promega) 200 units, dNTP (each 2.5 mM) mix 2 μl, RNasin (Promega) 40 units, oligo (dT) primer (Invitrogen) 1 μl. Reverse transcriptase reaction was then performed in a final volume of 25 μl for 60 min at 42°C and stopped after 30 min at 75°C. UCP primers were: UCP1 sense 5'-TAC CCA CAT CAG GCA ACA G-3', antisense 5'-TCA TTG CAC AGC TGG GTA C-3' (product size 842 bp), UCP2 sense 5'-ACA GCA GCC TGT ATT GCA G-3', antisense 5'-TTG TAG GCT TCG ACA GTG C-3' (product size 428 bp), UCP3 sense 5'-ACC ATG GTT GGA CTT CAG C-3', antisense 5'-AGT TCC CAG CGT ATC CAT G-3' (product size 450 bp).

Polymerase chain reaction (PCR) was performed in 25 μl final volume containing Taq polymerase (Takara) 0.125 μl, 10×PCR buffer 2.5 μl, dNTP (each 2.5 mM) mix 2 μl, 10 pmol each of the gene specific primers, and 10 pmol each of the primers and β-actin. The PCR cycle was 94°C for 30 s, 58°C or 60°C for 60 s, and 72°C for 90 s, repeated for 27, 32, and 30 cycles for UCP1, UCP2, and UCP3, respectively. A final elongation step was 10 min at 72°C. The PCR products (10 μl) were resolved in 1.5% agarose gel, and the DNA was visualized by ethidium bromide using an U.V. transilluminator and then photographed. The level of gene expression was determined as the ratio of integrated peak area for each individual gene PCR product relative to that of the coamplified α-actin internal standard. Values are presented as mean±SE of 4 individual determinations.

Statistical Analyses

Results are expressed as means±SE. The significance of difference between the normal and the high-fat diet groups before levan supplementation was determined by Student's t-test. ANOVA and Duncan's multiple range test were used to determine the significance of differences after 4 weeks of levan supplementation. Statistical analyses were carried out with the SAS program (SAS 8.0, SAS institute, Cary, NC, U.S.A.) and statistical significance of difference was defined at $P<0.05$.

RESULTS

Food Intake, Body Weight, Weight Gain, and FER After Levan Supplementation

Food intake was lower in the high-fat diet fed rats, compared to normal diet fed rats, and higher in the levan supplemented diet fed rats than the high-fat diet fed rats. Body weight gain was markedly lower in the rats fed diets containing 5% and 10% levan, compared to the rats fed with high-fat diet alone. At the end of the study, body weight gain was 38% lower in the rats fed 10 % levan diet, compared to the high-fat diet fed rats, and 35% lower compared to the normal diet fed rats. Thus, food efficiency ratio (FER) was significantly lowered by dietary levan in a dose-dependent manner. FER of the high-fat diet fed rats was higher than the normal diet fed rats, and lowered by 15%, 34%, and 43% in 1%, 5%, and 10% levan supplemented diet fed rats, respectively (Table 2).

Table 2. Daily food intake, weight gain, and food efficiency ratio in rats fed with experimental diets for 4 wk.

	N	HF	HF-L1	HF-L5	HF-L10
Food intake (g/day)	28.99 ± 0.49^{a}	$21.98\pm0.56^{d}_{z}$	$27.20\pm0.52^{b}_{x}$	$24.24\pm0.60^{c}_{y}$	$23.95\pm0.49^{c}_{y}$
Weight gain (g/day)	2.48 ± 0.21^{ab}	$2.58\pm0.29^{a}_{x}$	$2.77\pm0.17^{a}_{x}$	$1.94\pm0.16^{bc}_{y}$	$1.61\pm0.21^{c}_{y}$
FER	0.087 ± 0.007^{c}	$0.121\pm0.013^{a}_{x}$	$0.103\pm0.006^{ab}_{xy}$	$0.080\pm0.007^{bc}_{yz}$	$0.069\pm0.007^{c}_{yz}$

Each value is the mean±S.E. for 9 rats.

Statistical analysis was performed using one-way ANOVA followed by Duncan's Multiple Range test. Values with different superscript letters are significantly different from each other at $p<0.05$. a, b, c, d; significance between N, HF, HF-L1, HF-L5, and HF-L10. x, y, z; significance between HF, HF-L1, HF-L5, and HF-L10.

FER; Food efficiency ratio=body weight gain (g/day)/food intake (g/day).

N: normal diet, HF: high fat diet, HF-L1: high fat with 1% levan diet, HF-L5: high fat with 5% levan diet, HF-L10: high fat with 10% levan diet group.

Adipose Tissue Mass and Adipocyte Size

BAT mass was higher in the rats fed 1% and 5% levan containing diet, and lower in the 10% levan diet fed rats than that of the rats fed high-fat diet alone. Compared with the normal diet, the epididymal and visceral fat masses in the rats fed 10% levan supplemented high-fat diet were nearly identical. Moreover, the peritoneal fat mass of 5% (1.40±0.11 g/100 g B.W.) and 10% (1.52±0.15 g/100 g B.W.) levan supplement to diet was lower than that of the normal diet group (2.06±0.15 g/100 g B.W.). Supplementation with levan significantly suppressed the relative white adipose tissue mass in a dose-dependent manner; i.e., epididymal, visceral, and peritoneal fat accumulation, in comparison with that in the high-fat diet group. Epididymal, visceral, and peritoenal fat mass was remarkably lower than the high-fat diet fed rats by 45%, 57%, and 44%, respectively (Fig. 1).

MRI, which visualizes the fat accumulation in the intra-abdomen, revealed great fat in the visceral and subcutaneous areas in the high-fat diet fed rats, and the body fat accumulation was lowered by levan supplementation in a dose-dependent manner (Fig. 2).

The adipocyte size was measured from collagenase treated visceral fat pad (Fig. 3). The cell size was bigger (187.2±16.8 μm) in the high-fat diet fed rats than the normal diet fed rats (107.3±11.4 μm), and became smaller dose dependently by levan supplement, which is consistent with the fat mass. The size of adipocytes in the rats fed 5% (114.3±3.3 μm) and 10% (93.4±11.4 μm) levan-containing diet was nearly identical with that of the normal diet fed rats.

Serum Triglyceride, Total Cholesterol, HDL Cholesterol, and Free Fatty Acid

The levan supplementation in the high-fat diet markedly lowered the serum triglyceride concentration, as compared to the rats fed the high-fat diet alone. The triglyceride level in the 10% levan diet fed rats was significantly lower than the high-fat fed rats and normal diet fed rats by 48% and 32%, respectively ($P<0.05$) (Table 3). Total cholesterol level was higher in the rats fed high-fat diet than the normal diet fed rats. This increase in total cholesterol was suppressed by 5% or 10% levan supplementation to the

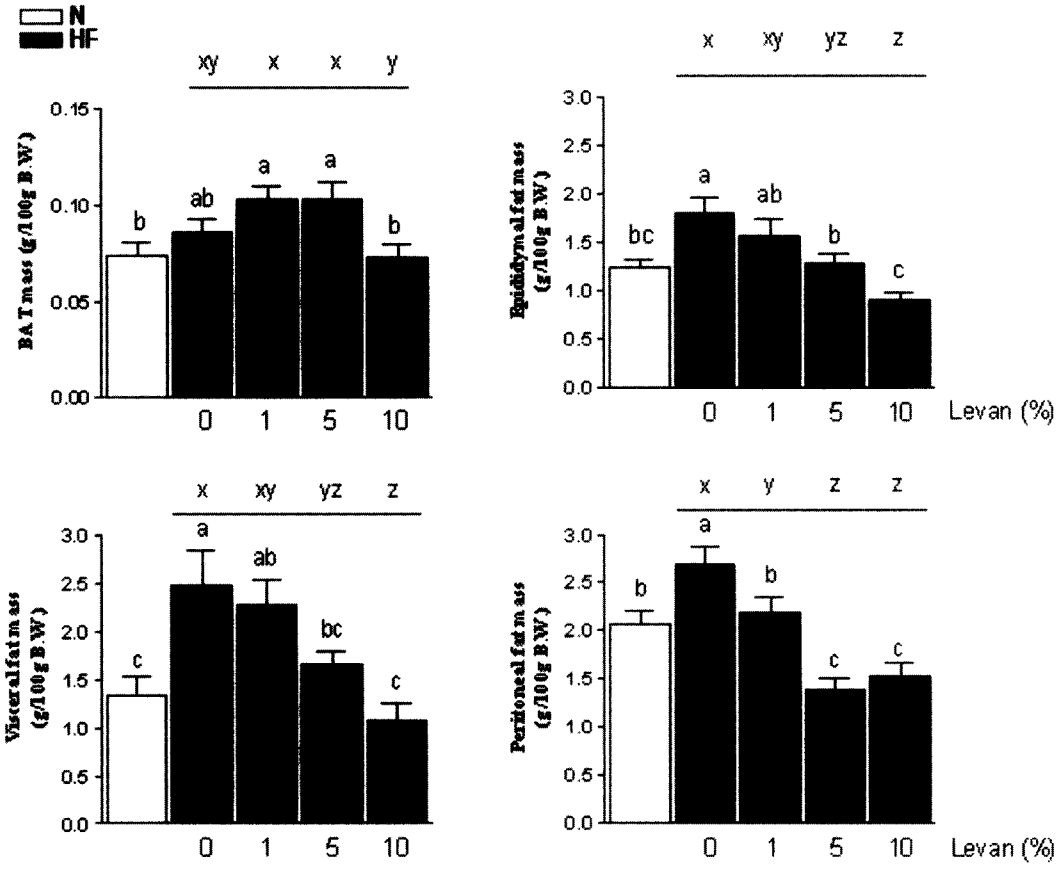

Fig. 1. Adipose tissue mass (BAT, epididymal fat, visceral fat, peritoneal fat) in rats fed with experimental diets for 4 wk.
Levels of tissue mass were calculated as a weight per unit body weight. Values are mean±SE n=9. Different letters indicate significant difference ($p<0.05$) by Duncan's Multiple Range Test. a, b, c; significance between N, HF, and levan supplemented HF groups. x, y, z; significance between HF and levan supplemented HF groups. N; normal diet. HF; high-fat diet group.

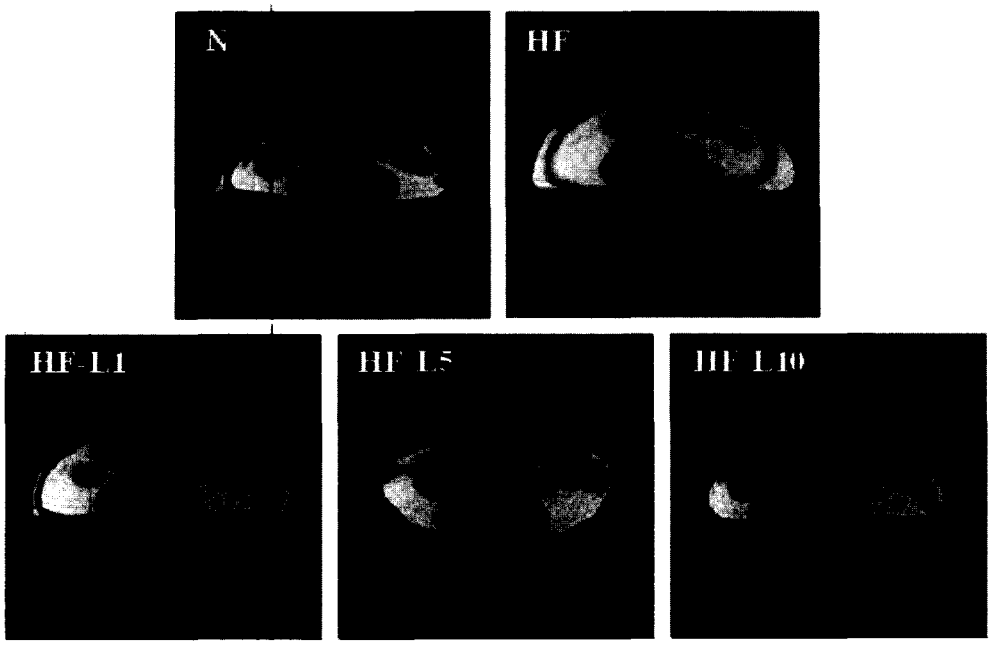

Fig. 2. Magnetic resonance images of abdominal fat distribution in rats fed with experimental diets for 4 wk.
The white area is the fat deposition in images. N; normal diet. HF; high-fat diet. HF-L1; high-fat with 1% levan diet. HF-L5; high fat with 5% levan diet. HF-L10; high-fat with 10% levan diet group.

high-fat diet and lowered to the value of the normal diet fed rats. On the other hand, HDL cholesterol level in the levan fed rats was significantly higher than in the high-fat or normal diet fed rats ($P<0.05$). The ratio of HDL/total cholesterol (HTR) in the rats fed levan-containing high-fat diet was significantly higher than in the high-fat diet or normal diet fed rats. Serum free fatty acid level in the high-fat fed rats was higher than the normal diet fed rats. The levan supplementation to the high-fat diet resulted in significant reduction in the serum free fatty acid level: 21%, 35%, and 29% reduction in 1%, 5%, and 10% levan supplemented rats, compared to the rats fed with high-fat diet alone.

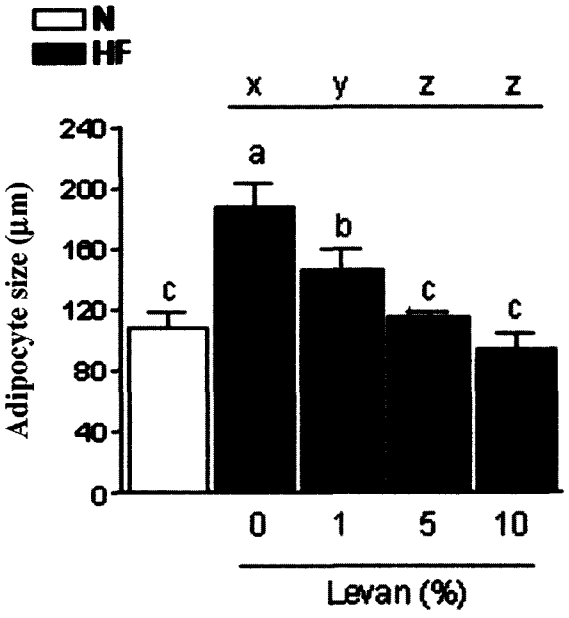

Fig. 3. The level of adipocyte size in rats fed with experimental diets for 4 wk. Adipocyte was isolated by collagenase treatment from visceral fat pad.
Each value is mean±SE for 9 rats. Different letters indicate significant difference ($p<0.05$) by Duncan's Multiple Range Test (B). a, b, c; significance between N, HF, and levan supplemented HF groups. x, y, z; significance between HF and levan supplemented HF groups. N; normal diet. HF; high-fat diet group.

Expression of UCP mRNA in BAT, Skeletal Muscle, and WAT

The effects of dietary levan on mRNA levels of UCPs, which may influence metabolic efficiency, were examined. BAT mRNA levels of UCP 1, 2, and 3 in 10% levan-containing high-fat fed rats were 121%, 42%, and 22% higher than the high-fat diet fed rats, respectively. BAT UCP2 mRNA expression was higher in HF-L1, HF-L5, and HF-L10 compared to HF group alone by 32, 67, and 52%, respectively. UCP1, 2, and 3 mRNA expressions in BAT were upregulated by the high-fat diet and further induced by levan supplementation (Fig. 4A). UCP2 mRNA expressions in skeletal muscle and WAT were upregulated by the high-fat diet by 25%, but were not affected by levan supplementation (Figs. 4B, 4C). In contrast to the UCP2 gene, UCP3 gene expression in skeletal muscle exhibited a pattern similar to the UCP3 gene in BAT. Skeletal muscle UCP3 mRNA expression was also affected by the

Table 3. Serum triglyceride, total cholesterol, HDL cholesterol, HTR, and free fatty acid levels in rats fed with experimental diets for 4 wk.[1]

	N[2]	HF	HF-L1	HF-L5	HF-L10
Triglyceride (mg/dl)	63.00 ± 6.59^{ab}	$82.50\pm8.65^{a}_{x}$	$75.40\pm8.45^{a}_{x}$	$61.33\pm6.97^{ab}_{xy}$	$42.66\pm6.59^{b}_{y}$
Total cholesterol (mg/dl)	60.71 ± 3.62^{c}	$73.10\pm4.18^{ab}_{xy}$	$74.00\pm2.18^{a}_{x}$	$64.44\pm4.40^{abc}_{xy}$	$61.55\pm3.62^{bc}_{y}$
HDL cholesterol (mg/dl)	45.57 ± 2.80^{ab}	$44.60\pm2.81^{b}_{y}$	$53.80\pm1.65^{a}_{x}$	$54.33\pm3.89^{a}_{x}$	$53.77\pm2.80^{a}_{x}$
HTR[3]	0.77 ± 0.06^{abc}	$0.62\pm0.05^{c}_{z}$	$0.73\pm0.01^{bc}_{yz}$	$0.85\pm0.05^{ab}_{xy}$	$0.90\pm0.07^{a}_{x}$
Free fatty acid (UEq/l)	678.9 ± 56.1^{bc}	$853.8\pm75.5^{a}_{x}$	$762.2\pm43.3^{ab}_{x}$	$557.8\pm46.3^{c}_{y}$	$610.4\pm29.2^{bc}_{y}$

[1]Each value is the mean±SE for 9 rats.

Statistical analysis was performed using one-way ANOVA followed by Duncan's Multiple Range test. Values with different superscript letters are significantly different from each other at $p<0.05$. a, b, c; significance between N, HF, HF-L1, HF-L5, and HF-L10. x, y, z; significance between HF, HF-L1, HF-L5, and HF-L10.

[2]N; normal diet. HF; high-fat diet. HF-L1; high-fat with 1% levan diet. HF-L5; high-fat with 5% levan diet. HF-L10; high-fat with 10% levan diet group.

[3]HTR=HDL cholesterol/Total cholesterol ratio.

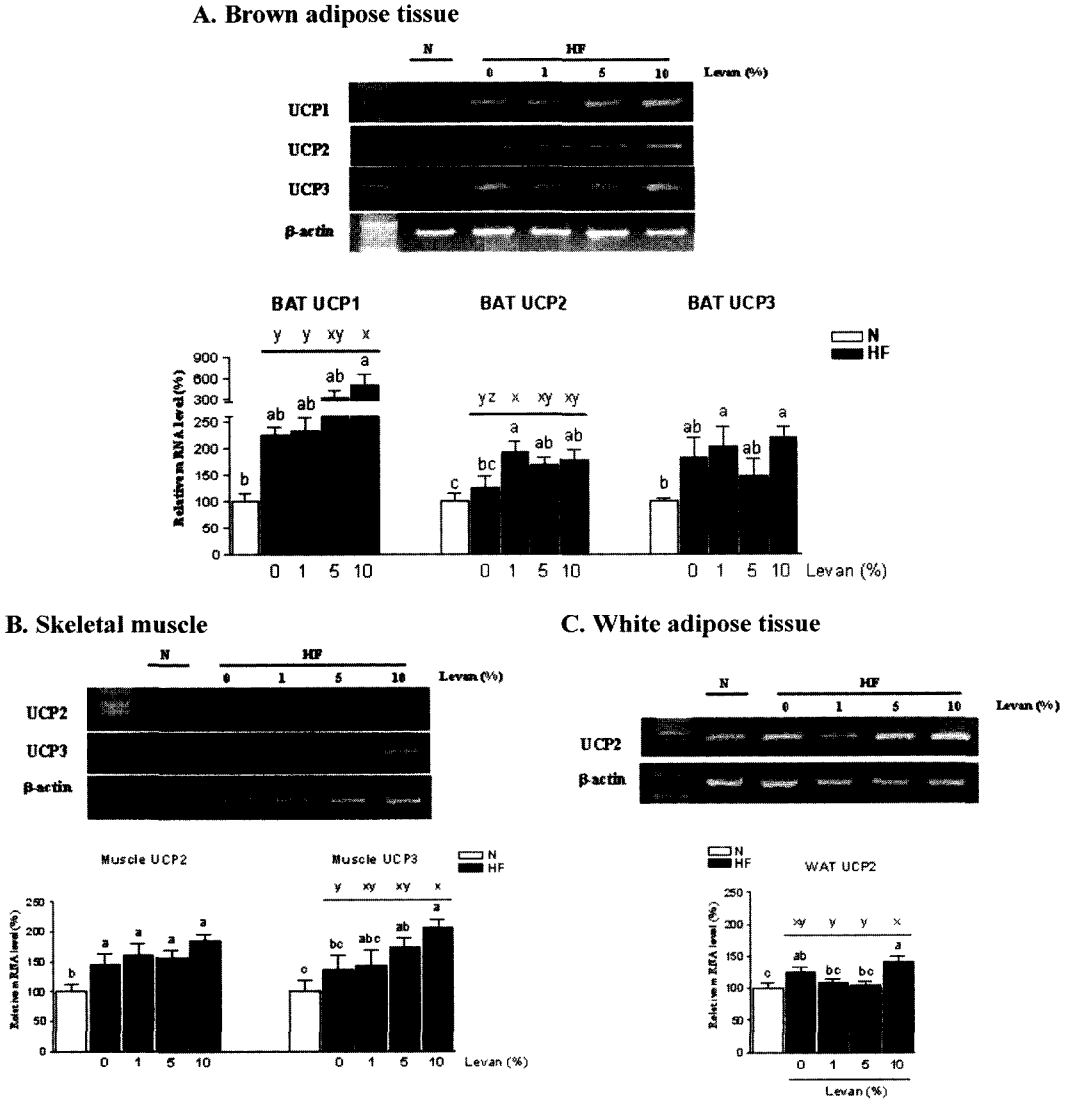

Fig. 4. Changes of UCP mRNA expression in BAT, skeletal muscle, and WAT.

mRNA levels of UCP1, 2, and 3 in BAT (A), mRNA levels of UCP 2 and 3 of skeletal muscle or soleus (B), and mRNA levels of UCP2 of WAT or epididymal fat pad (C) in rats fed with control or levan supplemented diets for 4 wk. Quantitative RT-PCR was used for the mRNA determination. Levels of mRNA were calculated as a percentage of the values of the normal diet group. Each value is mean±SE for 9 rats. Different letters indicate significant difference ($p<0.05$) by Duncan's Multiple Range Test. a, b, c; significance between N, HF, and levan supplemented HF groups. x, y, z; significance between HF and levan supplemented HF groups. N; normal diet. HF; high-fat diet group.

high-fat diet, and induced dose-dependently by levan supplementation. Thus, skeletal muscle UCP3 mRNA levels in the high-fat diet and 1, 5, and 10% levan-containing high-fat diet groups were higher by 35%, 42%, 74%, and 106% than the normal diet group, respectively (Fig. 4B).

DISCUSSION

Numerous studies have shown the possibility of modifying obesity by dietary intervention. Obesity is disturbance often related to lipid metabolism, leading to an increase in serum triglyceride and cholesterol concentrations, which are involved in the development of cardiovascular diseases. The present study showed that dietary levan strongly suppressed the body fat accumulation induced by high-fat diet, which is compatible with the suppressive effect of inulin-type fructan in adiposity. It is well known that the abdominal fat accumulation could be a main risk factor for numerous health complications, including cardiovascular diseases, therefore, the suppressive effect of levan on visceral or peritoneal fat development suggests the potential anti-obesity effect of dietary levan. The higher BAT mass in the rats fed with high fat than the normal diet fed rats is in agreement with our previous study [13] of BAT hypertrophy induced by long-term overeating of a highly palatable cafeteria diet, which generally has high fat content [28]. Furthermore, adaptive thermogenesis takes place in BAT, therefore, higher BAT mass in rats fed 5% and 10% levan may attribute to increased thermogenesis and energy expenditure [30]. In the present study, high-fat feeding induced a significant increase in fat pad weight, which represents adipocyte hypertrophy. Similar to the body fat mass, the size of adipocytes was reduced dose-dependently by levan supplementation to high-fat diet, nearly identical with those of normal diet fed rats. This result confirms the suppressive effect of levan on high-fat diet-induced adiposity.

The effects of inulin-type fructan on blood lipids have been studied in hyperlipidemic or obese humans and animals, however, the results on hypolipidemic effects are controversial. In animals, oligofructose (OFS) supplementation decreased serum triglyceride in high-fat diet fed rats [7, 22], and inulin supplementation resulted in reduction of plasma triglyceride in LDL receptor knockout mice [31] and Syrian hamsters fed with 20% fat and 0.12% cholesterol diet [38]. In this experiment, the high-fat diet raised the serum triglyceride and cholesterol concentrations, and the levan supplementation to the high-fat diet exhibited both triglyceride-lowering and cholesterol-lowering effects in rats, which is in agreement with a significant reduction in serum of lipids in rats fed with inulin-type fructan or soluble fiber [1]. However, to the best of our knowledge, there has been no report on the effect of levan. Cho *et al.* [4] failed to show a hypocholesterolemic effect of levan on rats fed with cholesterol-containing diets, and Yamamoto *et al.* reported that 1% or 5% levan supplementation reduced the serum cholesterol, but not triglyceride [42]. They used smaller molecular weight levan than that of our present study. In this experiment, high molecular weight (ca. 6,000,000) levan was used. The discrepancy in the effects of levan might have resulted from the differences in experimental conditions, and the high molecular weight levan appears to be important for the hypolipidemic effect.

The ability of levan to improve cholesterol metabolism, similar to soluble fiber, might be due to several mechanisms. First, levan might increase the viscosity of the digesta and increase the thickness of the unstirred layer in the small intestine, thereby possibly inhibiting the uptake of cholesterol and bile acids [29, 34]. Indeed, dietary levan was found to increase the fecal excretions of total sterol and lipids [42]. Second, being excellent substrate for fermentation by the microorganisms in the cecum and colon [3], levan could also decrease the serum cholesterol level by reducing hepatic cholesterol synthesis from short-chain fatty acids, including propionate. Propionate has been demonstratred to lower cholesterol synthesis both *in vitro* in isolated rat hepatocytes [41] and *in vivo* in rats [3] and humans [40]. Third, the hypocholesterolemic effect of levan may also be due to alterations in hormone secretions [16] and modifications of lipoprotein metabolism [23]. It has previously been suggested that the hypocholesterolemic effect of fructan in animals reflects reduced secretion of VLDL particles by the liver secondary to inhibition of *de novo* fatty acid synthesis [39].

The suppressive effects of dietary levan on body fat development and adipocyte hypertrophy induced by a high-fat diet must be accompanied by the increased mRNA level of UCP that consumes energy via thermogenesis. It is highly likely that increased expression of UCPs would increase energy expenditure and contribute to the suppression of body fat accumulation [5]. UCPs are regulated by various nutritional perturbations in a tissue-selective manner. Thus, diets which are capable of activating or increasing expression of the UCP have recently been investigated as an interesting therapeutic candidate for obesity [2]. Nevertheless, the influence of dietary fiber or fructan on UCP expression has hardly been studied.

In our previous [13, 15] as well as other studies [26, 35], high-fat feeding was shown to increase expression of UCP. Consistent with these earlier results, we found in the present study that the UCP mRNA in BAT, skeletal muscle, and WAT was increased by dietary high fat. In addition, levan supplementation to the high-fat diet upregulated the expression of UCP in BAT and skeletal muscle, suggesting that the thermogenic effect of the high-fat diets was enhanced by levan supplementation. Skeletal muscle represents

up to 40% of the total body weight and is endowed with substantial mitochondrial capacity. Therefore, the uncoupling of skeletal muscle mitochondria may play an important role in the utilization of fatty acids [45]. In the present study, because of the increased energy expenditure, the increased expression of UCP mRNA in BAT and skeletal muscle might explain the suppression of body fat development in levan supplemented rats, compared with rats fed with high-fat diet alone.

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