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Bacterial β-Glucan Exhibits Potent Hypoglycemic Activity via Decrease of Serum Lipids and Adiposity, and Increase of UCP mRNA Expression

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Abstract This study was undertaken to evaluate the effect of bacteria-derived β-glucan fiber on serum lipids, adiposity and uncoupling protein (UCP) expression in rats. In order to induce obesity, Sprague-Dawley weanling male rats were allowed free access to AIN-76A diet until 4 weeks of age, and fed high-fat diet (beef tallow, 40% of calories as fat) for 6 weeks until 10 weeks of age. Rats were then fed with 0% (high-fat control group), 1%, or 5% bacterial β-glucan supplemented high-fat diets (w/w) for another 6 weeks. For comparison, normal control group was fed with AIN-76 diet (11.7% fat). Supplementation with bacterial β-glucan resulted in a significant reduction of high-fat-induced white fat (i.e., visceral and peritoneal fat) development, adipocyte hypertrophy, and development of hyperinsulinemia and hyperleptinemia. Serum triglyceride, total cholesterol, and free fatty acid levels were greatly reduced, but, HDL-cholesterol concentrations were increased by bacterial β-glucan supplementation. Serum leptin level was lower in the β-glucan groups than in the high-fat group. The expression of UCPs (UCP1, UCP2, and UCP3) in brown adipose tissue (BAT) were significantly increased by 5% bacterial β-glucan-containing diet. This study suggests that the anti-obesity effect of 5% bacterial β-glucan is attributed to upregulation of UCPs and inefficient energy utilization.

Key words: Obesity, hypolipidemic effects, β-glucan, UCP, leptin

β-Glucan is an insoluble microbial exopolymer and composed almost exclusively of β-(1,3)-glucosidic linkages [17]. β-Glucan has an approximately 450 average degree of polymerization (DP) with a number of structural variants. β-Glucans are present in a variety of living systems, including fungi, yeasts, algae, bacteria, and higher plants. However, only bacteria belonging to the *Alcaligenes* and *Agrobacterium* species have currently been reported to produce the linear β-(1,3)-glucan type of homopolymer [17]. β-Glucan is useful as a gelling material to improve the textural quality, water-holding capacity, and thermal stability of various foods. The foods employing these functions include soybean curd (tofu), sweet bean paste jelly, boiled fish paste, noodles, sausages, jellies, and jams [5]. In addition, β-glucan is well known to have immune-stimulatory effects and may act as a prebiotic to change the intestinal microflora, thereby offering beneficial effects [12, 25].

Previous work in our laboratory showed that the effects of a high-fat diet (40% of calories as fat) caused obesity in rats, with increased body weight and fat accumulation [8]. Recently, exobiopolymers produced from submerged mycelial culture of *Ganoderma lucidum*, levan, and mushrooms have shown the hypolipidemic effects [9, 10, 11, 28, 29]. The β-glucan from oat or yeast has been shown to improve lipid profiles. On the basis of these earlier studies, the present study was undertaken to test the hypothesis that β-glucan is able to decrease adiposity and post-prandial lipremia in obese rats induced by high-fat diet, thus examining whether supplementation of β-glucan has anti-obesity and hypolipidemic effect. Furthermore, we examined the effect of *Agrobacterium* species-derived β-glucan on blood lipids in hypercholesterolemic obese rats to confirm whether the fiber from a bacterial source works similarly.

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Uncoupling proteins (UCPs; UCP 1, 2, 3) are mitochondrial inner membrane proteins that uncouple the respiratory chain from oxidative phosphorylation and generate heat instead of ATP, thereby increasing energy expenditure [7]. Uncoupling is especially prominent in thermogenic brown adipose tissue (BAT), which expresses a tissue-specific UCP1. The recent discovery of UCP1 homologs such as UCP2 and UCP3 raised the possibility that innate proton leak and metabolic rate are regulated by UCPs. UCP1 is expressed exclusively in BAT, while UCP2 is expressed widely, and UCP3 is expressed abundantly in skeletal muscle in humans, and in BAT and skeletal muscle in rodents. The expression of UCP genes is presently of considerable interest because of their putative relation to the defense against obesity. The gene expression of UCP was investigated in order to find potential effect of bacterial β-glucan in energy intake and expenditure.

MATERIALS AND METHODS

Materials and Media Preparation

Bacterial β-glucan produced by fermentation with a mutant strain of *Agrobacterium* species [13] was obtained from DMJ Biotech Corp. (Daejeon, Korea). The estimated molecular size of bacterial β-glucan was about 3×10^5 dalton. Purity of β-glucan was above 99.5%, judging by HPLC analysis [13]. The purified β-glucan was found to consist exclusively of glucose, based on the analysis of monosaccharide. The infrared (IR) spectroscopy showed an absorption band at 890 cm^{-1}, indicating that no α-configuration existed, since there was no characteristic absorption at 840 cm^{-1}. From the NMR spectrum, β-glucan had linear (1→3)-linkages.

Animals and Diets

Three-week-old Sprague-Dawley male rats were purchased from Central Experimental Animals (Samtaco, Seoul, Korea) and housed individually. After adaptation for one week, rats were weighed, randomly assigned, and fed normal or high-fat diet. Six weeks later, the high-fat fed rats were randomly assigned to 6 groups (n=8) by body weight and fed experimental diets (Table 1) containing fiber-free diet (control), high-fat diet, high-fat diet containing 1% bacterial β-glucan, and high-fat diet containing 5% bacterial β-glucan for 6 weeks from 9:00–11:00 A.M. The cages were placed in a room with controlled temperature (22±2°C), relative humidity (50±5%), and 12 h light/dark cycle. The composition of experimental diets is shown in Table 1. Water and food were consumed *ad libitum*. At night on the final day of the experiment, the food was removed for 12–14 h. The food intake and body weight were weighed twice a week from 9:00–11:00 A.M., and the food efficiency ratio (FER) was calculated.

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

	N	HF	HF-BG1	HF-BG5
Casein	200	200	200	200
DL-Methionine	3	3	3	3
Corn starch	150	150	140	100
Sucrose	500	345	345	345
Cellulose	50	50	50	50
Corn oil	50	–	–	–
Beef tallow	-	205	205	205
Mineral mixture[2]	35	35	35	35
Vitamin mixture[3]	10	10	10	10
Choline bitartrate	2	2	2	2
β-Glucan	–	–	10	50
Fat % (calories)	11.7	40.0	40.0	40.0

[1]N, Normal diet, AIN-76A diet #100000; HF, high fat diet, AIN-76 diet #100496 (Dyets Inc., Bethlehem, PA, U.S.A.); HF-BG1, high fat with 1% bacterial β-glucan. HF-BG5, high fat with 5% bacterial β-glucan.
[2]AIN-76 Mineral mix; Dyets Inc., Bethlehem, PA, U.S.A.
[3]AIN-76 Vitamin mix; Dyets Inc., Bethlehem, PA, U.S.A.

Sample Collection

After 6 weeks of feeding the normal, high-fat, or β-glucan-supplemented high-fat diets, food was withheld for 12 h before the rats were sacrificed. Twelve-h fasting blood was drawn from the heart under anesthetization with pentobarbitol and diethyl ether, and serum was separated by centrifugation (3,000 ×*g*, for 15 min at 4°C) for lipid, leptin, and insulin analyses. After collecting blood samples, interscapular 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.

Adipocyte Size Determination

Adipose tissue samples (0.5 g) were taken from visceral fat depots, and adipocytes were isolated using collagenase [16]: adipose tissue was immediately washed in 145 mM NaCl-buffer containing 3% BSA, cut into small pieces, and added to 1 ml of NaCl-buffer containing 1.5 mg of collagenase (Sigma Chemical, St. Louis, MO, U.S.A.), and incubated in a shaking water bath at 80 cycles/min for 1 h at 37°C. After incubation, the cells were 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, 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 mean diameter of 30 cells from each 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.). Serum leptin and insulin levels were analyzed by RadioImmunoAssay (RIA) using Linco leptin assay kit (Linco Research Immunoassay, St. Louis, MO, U.S.A.) and insulin standards (Linco Research Immunoassay), respectively.

Quantitative RT-PCR for Gene Expression Analyses

Total RNA from BAT was extracted with the Trizol reagent (Gibco-BRL, Bethesda, MD, U.S.A.). The yield and quality of the extracted RNA were assessed by the 260/280 nm optical density ratio and by electrophoresis under denaturating conditions on 1% agarose gels. Reverse transcription (RT) reaction mixture containing 2 μg of total RNA was denatured for 10 min at 72°C for cDNA synthesis. Reverse transcriptase reaction was then performed in 25 μl final volume for 60 min at 42°C and stopped by 30 min at 75°C. The final composition of the reaction mixture was as follows: M-MLV (Promega WI, U.S.A.) 200 units, dNTP (each 2.5 mM) mix 2 μl, RNasin (Promega) 40 units, oligo(dT) primer (Invitrogen Corporation, Carlsbad, CA, U.S.A.) 1 μl. 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 containing *Taq* polymerase (Takara Co., Japan) 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 a 1.5% agarose gel, and the DNA was visualized by ethidium bromide, using a UV 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 β-actin internal standard. Values are presented as mean±S.E.M. of 4 individual determinations.

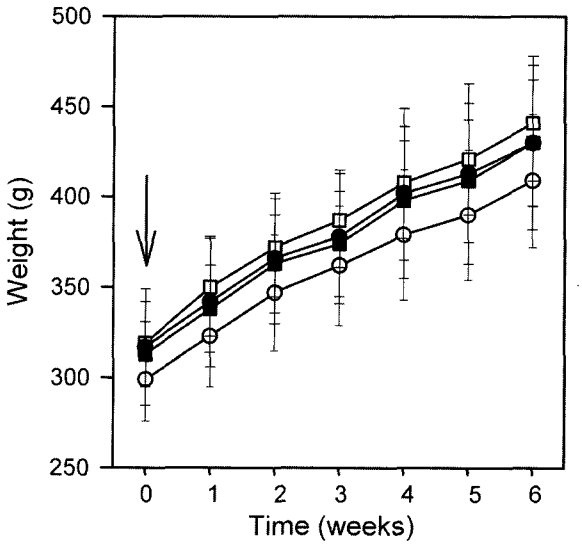

Fig. 1. Body weight change of animals during experiment.
Experimental diets (see Table 1 for details) were fed to the rat (indicated by arrow sign). Symbols: open circle, normal diet; open square, high fat diet; closed circle, high fat with 1% bacterial β-glucan; closed square, high fat with 5% bacterial β-glucan.

Statistical Analyses

Results are expressed as means±standard error mean (S.E.M.). Duncan's multiple range test was used to determine the significance of differences after 6 weeks of β-glucan 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 a $P<0.05$.

RESULTS

Food Intake, Body Weight, and Food Efficiency Ratio

Food intake was lower in high-fat diet fed rats (18.31± 1.40 g/day) compared to normal diet fed rats (23.75± 2.24 g/day), but weight gain and FER were markedly higher in the high-fat diet fed rats (2.91±0.42 g/day and 0.16± 0.02) compared to the normal diet fed rats (2.61±0.45 g/day and 0.11±0.02). β-Glucan diet had no influence on food intake. Body weight gradually increased with time, but it

Table 2. Daily food intake, weight gain, and food efficiency ratio in rats fed with experimental diets for 6 weeks.

	N	HF	HF-BG1	HF-BG5
Food intake (g/day)	23.75±2.24[a]	18.31±1.40[b]	18.56±1.27[b]	18.48±1.98[b]
Weight gain (g/day)	2.61±0.45	2.91±0.42	2.77±0.66	2.79±0.57
FER	0.110±0.018[b]	0.159±0.023[a]	0.149±0.036[ab]	0.151±0.033[ab]

Each value is the mean±S.E.M. for 8 rats.
[ab]Values within a column with different superscript letters are significantly different from each other at $p<0.05$ Duncan's Multiple Range test. FER, Food efficiency ratio=body weight gain (g/day)/food intake (g/day).
N, normal diet; HF, high-fat diet; HF-BG1, high fat with 1% bacterial β-glucan; HF-BG5, high fat with 5% bacterial β-glucan.

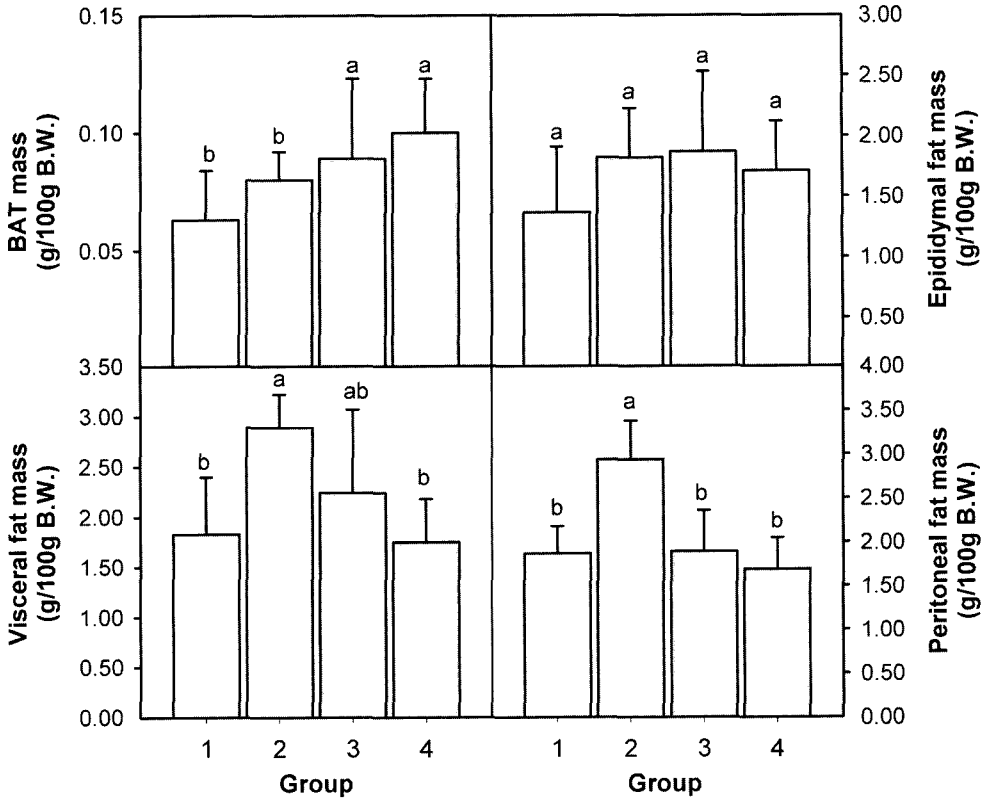

Fig. 2. Adipose tissue mass (BAT, epididymal fat, visceral fat, peritoneal fat) in rats fed with experimental diets for 6 weeks.
Levels of tissue mass were calculated as a weight per unit body weight. Values are mean±S.E.M. n=8. [ab]Different superscript letters are significantly different from each other at $p<0.05$ by Duncan's Multiple Range test. 1, Normal diet; 2, high-fat diet; 3, high fat with 1% bacterial β-glucan; 4, high fat with 5% bacterial β-glucan.

was lower in β-glucan fed rats than in high-fat diet fed rats, resulting in 4% lower body weight gain in 5% bacterial β-glucan fed rats than in high-fat diet fed rats (Fig. 1 and Table 2). FER of high-fat diet fed rats was higher than normal diet fed rats, and lowered by 6% in 1% bacterial β-glucan and by 5% in 5% bacterial β-glucan supplemented diet fed rats, respectively (Table 2).

Adipose Tissue Mass and Adipocyte Size

The masses of various adipose tissues (interscapular brown adipose tissue, epididymal fat, visceral fat, and peritoneal fat) are shown in terms of weight per 100 g of body weight (Fig. 2). BAT mass ranged between 0.06–0.10 g/100 g of body weight and was higher in rats fed with 5% bacterial β-glucan. The β-glucan diet significantly suppressed the relative white adipose tissue mass, such as visceral and peritoneal fat accumulation, in comparison with that in the high-fat diet group. Similar results were obtained from epididymal fat mass without reaching a statistically significant level. For 5% bacterial β-glucan group, visceral and peritoneal fat masses were remarkably lower than those of high-fat diet fed rats, by 40% and 42%, respectively (Fig. 2).

The adipocyte size was measured from collagenase-treated visceral fat pad (Fig. 3). The cell size was greater (209.0±41.4 μm) in high-fat diet fed rats than normal diet fed rats (105.9±27.7 μm), and lowered by β-glucan supplement, which is consistent with the fat mass (Fig. 4): For instance, the adipocyte size in rats fed with 1% bacterial β-glucan was 118.4±20.5 μm, and with 5% bacterial β-glucan was 100.9±12.9 μm. Bacterial β-glucan-containing

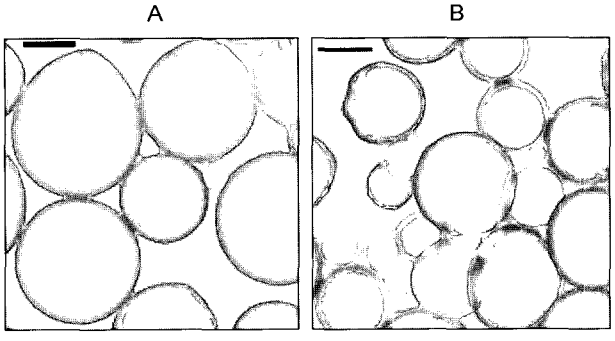

Fig. 3. Photomicrographs of adipocyte obtained from collagenase-treated visceral fat tissue.
A, High-fat diet; B, high fat with 5% bacterial β-glucan. Bars, 100 μm.

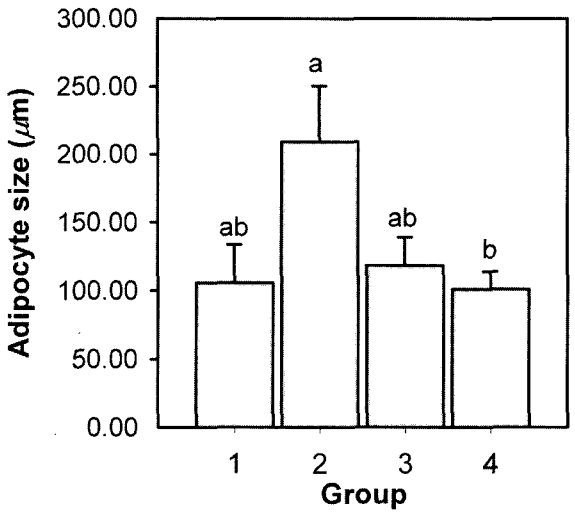

Fig. 4. The adipocyte size in rats fed with experimental diets for 6 weeks. Adipocyte was isolated from visceral fat pad by collagenase treatment.
Values are mean±S.E.M. n=8. [ab]Different superscript letters are significantly different from each other at $p<0.05$ Duncan's Multiple Range test. 1, normal diet; 2, high-fat diet; 3, high fat with 1% bacterial β-glucan; 4, high fat with 5% bacterial β-glucan.

diet was nearly identical to that of rats fed with the normal diet.

Serum Lipid Profiles, Leptin, and Insulin

The serum triglyceride was greater (129.9±40.3 mg/dl) in high-fat diet fed rats than normal diet fed rats (99.3± 35.2 mg/dl). The bacterial β-glucan supplementation in the high-fat diet significantly lowered the serum triglyceride concentration by 59%, as compared to rats fed with high fat diet alone (Table 3). Total cholesterol level in rats fed with high-fat diet was suppressed by 25%, when 5% bacterial β-glucan was supplemented to high-fat diet. HDL cholesterol level was lower in 5% bacterial β-glucan diet fed rats ($P<0.05$), compared to high-fat fed rats. The ratio of HDL/total cholesterol (HTR) was significantly higher in rats fed with 5% bacterial β-glucan-containing high-fat diet (1.00±0.16) than in high-fat diet (0.67±0.12) or normal diet fed rats (0.88±0.21). Serum free fatty acid level was slightly higher in high-fat fed rats, compared to normal diet fed rats. The β-glucan supplementation to high-fat diet caused significant reduction in the serum free fatty acid level by 41% and 48% in 1% and 5% bacterial β-glucan-supplemented rats, respectively, compared to rats fed high-fat diet alone. The β-glucan diet decreased the leptin level, whilst no difference was found in the insulin level.

Expression of UCP mRNA in BAT

To examine whether dietary bacterial β-glucan would change the expression of UCPs mRNA levels, we measured the expression of UCP 1, 2, and 3 in BAT, which is responsible for the thermogenesis activity, as shown in Fig. 5. BAT mRNA levels of UCP 1, 2, and 3 in 5% bacterial β-glucan-containing high-fat fed rats were 20%, 51%, and 69% higher than in high-fat diet fed rats, respectively. UCP1, 2, and 3 mRNA expressions in BAT were upregulated by high-fat diet, and induced more strongely by 5% bacterial β-glucan supplementation (Fig. 5). In contrast, BAT mRNA levels of UCP 1, 2, and 3 in 1% bacterial β-glucan-containing high-fat fed rats were 22%, 49%, and 52% lower than in the high-fat diet fed rats, respectively.

DISCUSSION

The majority of previous studies on the anti-obesity and hypolipidemic effects of dietary β-glucan have been concentrated on plant-derived β-glucan. The US Food and Drug Administration published a final report on the relation between soluble fiber from whole oats and plasma cholesterol concentrations [6]. It concluded that the soluble β-glucan is the primary component responsible for the hypocholesterolemic properties of oat bran. The present study investigated whether the addition of dietary bacterial

Table 3. Serum triglyceride, total cholesterol, HDL cholesterol, HTR, free fatty acid, leptin, and insulin level in rats fed with experimental diets for 6 weeks.

	N	HF	HF-BG1	HF-BG5
Triglyceride (mg/dl)	99.28±35.17[b]	129.85±40.27[a]	73.57±21.50[bc]	53.12±11.07[c]
Total cholesterol (mg/dl)	88.85±20.92[a]	83.85±9.66[a]	65.14±12.01[b]	63.12±9.41[b]
HDL cholesterol (mg/dl)	75.42±9.44[a]	55.42±4.54[c]	56.14±6.54[bc]	62.75±11.65[b]
HTR	0.88±0.21[a]	0.67±0.12[b]	0.88±0.17[a]	0.99±0.16[a]
Free fatty acid (UEq/l)	822.28±182.29[a]	862.01±229.71[a]	510.71±103.89[b]	447.37±72.94[b]
Leptin (ng/ml)	3.57±0.72[b]	5.61±2.29[a]	3.20±1.80[b]	2.69±0.83[b]
Insulin (ng/ml)	1.15±0.37	1.72±0.91	1.37±0.61	1.22±0.46

Each value is the mean±S.E.M. for 8 rats.
[a,b,c]Values within a column with different superscript letters are significantly different from each other at $p<0.05$ Duncan's Multiple Range test. HTR=HDL cholesterol/Total cholesterol ratio.
N, normal diet; HF, high-fat diet; HF-BG1, high fat with 1% bacterial β-glucan; HF-BG5, high fat with 5% bacterial β-glucan.

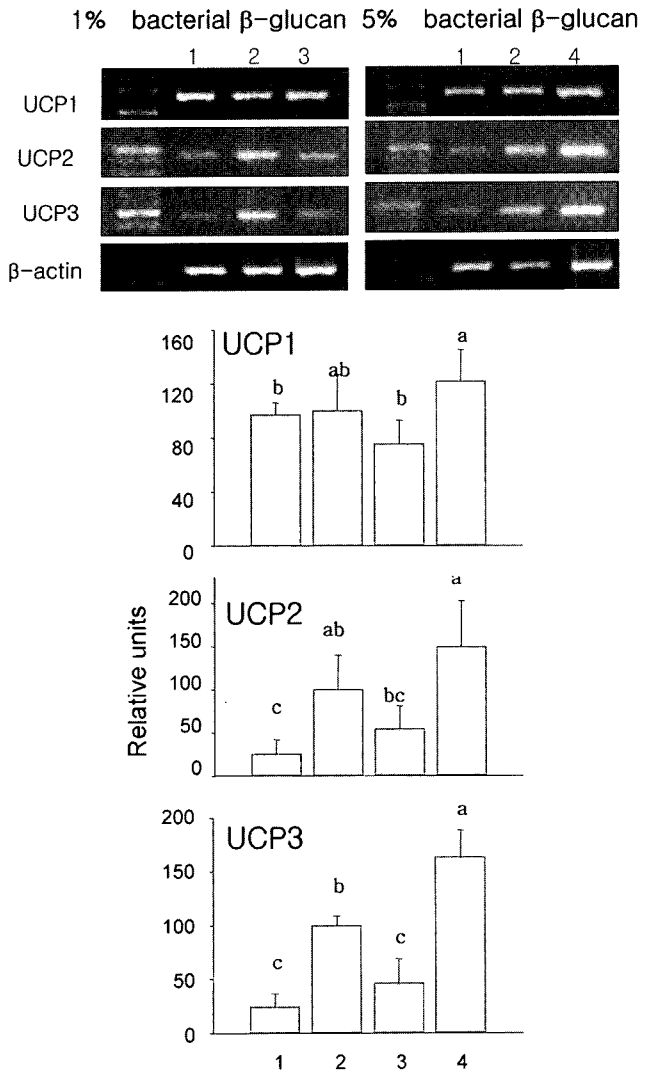

Fig. 5. Changes in UCP mRNA expression in BAT. mRNA levels of UCP1, 2, and 3 in BAT in rats fed with control or β-glucan-supplemented diets for 6 weeks.

Quantitative RT-PCR was used for the mRNA determination. Levels of mRNA were calculated as a percentage of the values of the high-fat diet group. Values are mean±S.E.M. n=8. [a,b,c]Different superscript letters are significantly different from each other at $p<0.05$ Duncan's Multiple Range test. 1, Normal diet; 2, high-fat diet; 3, high fat with 1% bacterial β-glucan; 4, high fat with 5% bacterial β-glucan.

β-glucan could have potential regulatory effects on the adiposity, lipid metabolism, and gene expression of UCP. It is well known that the abdominal fat accumulation could be a main risk factor for the numerous health complications, including cardiovascular diseases. Therefore, the suppressive effect of bacterial β-glucan on visceral or peritoneal fat development suggests the potential anti-obesity effect of dietary β-glucan.

The higher BAT mass in rats fed with high fat compared to normal diet fed rats agrees with our previous report [8] and the BAT hypertrophy induced by long-term overeating of highly palatable cafeteria diet, which generally contains high-fat content [19]. Furthermore, adaptive thermogenesis takes place in BAT, therefore, a higher BAT mass in rats fed with 5% bacterial β-glucan may be due to increased thermogenesis and energy expenditure [21]. Although statistically not significant, 1% bacterial β-glucan supplementation to high-fat diet tended to reduce the adipocyte size. However, the adipocyte size was significantly reduced by 5% bacterial β-glucan supplementation to high-fat diet, nearly identical with those of normal diet fed rats. In human studies, the levels of leptin mRNA were higher in obese women than in non-obese women [14]. In animal model, the plasma leptin levels increased when *ob/ob* mice were treated with a high-fat diet, implying that leptin gene expression might control body energy balance. These studies showed a similar regulatory pattern for leptin gene expression to what we found in the present study. Supplementation of β-glucan might have decreased the leptin production, and this might attribute to decreased amounts of body fat.

The effects of oat β-glucan on blood lipids have been studied in hyperlipidemic or obese humans and animals; however, the results on hypolipidemic effects in human studies are controversial. In 1997, the US Food and Drug Administration passed a unique ruling that allowed oat bran at a dosage of 3 g of β-glucan/day to be registered as the first cholesterol-reducing food [6]. However, it was found by Lovegrove *et al.* [20] that a low dosage of β-glucan (3 g/day) did not significantly reduce total cholesterol or LDL cholesterol in volunteers. Based on this observation, they concluded that large reductions in total cholesterol were associated with high doses of β-glucan. In this experiment, the high-fat diet raised the serum triglyceride and cholesterol concentrations, and bacterial β-glucan supplementation to the high-fat diet had both triglyceride-lowering and cholesterol-lowering effect in rats, which is in agreement with a significant reduction in serum lipids in rats fed with yeast β-glucan [22]. We could not find any other paper describing such effect of bacterial β-glucan. It is interesting to note that yeast-derived β-glucan increases HDL-cholesterol concentrations, whereas oat-derived β-glucan decreases them [20, 22]. In the present study, we observed that ingestion of bacterial β-glucan increased HDL cholesterol. The ability of bacterial β-glucan to improve cholesterol metabolism, similar to soluble fiber, might have several explanations. Firstly, β-glucan might increase the viscosity of the digesta and increase the thickness of the unstirred layer in the small intestine. Therefore, it might be expected to inhibit uptake of sterol and lipids [3, 24]. Secondly, it is the substrate for fermentation by the lumen bacteria in the cecum and colon. One of the important beneficial properties of lumen bacteria is the formation of short-chain fatty acids (SCFAs), including propionate. Pripionate has been demonstratred to lower

cholesterol synthesis both in isolated rat hepatocytes *in vitro* [27] and in humans [26]. The pH values for the colon samples from the rats in normal diet were 6.72±0.24, 6.32±0.29 in high-fat diet, 6.03±0.20 in high-fat diet containing 1% bacterial β-glucan diet, and 5.90±0.21 in high-fat diet containing 5% bacterial β-glucan. The acidic colonic pH, resulting from ingestion of the β-glucan diet, was probably caused by greater production of total SCFAs. β-Glucan could also decrease the serum cholesterol level by reducing hepatic cholesterol synthesis by short-chain fatty acids, including propionate [25]. Thirdly, the hypocholesterolemic effect of β-glucan might also be due to alterations in hormone secretions [3] and modifications of lipoprotein metabolism [15]. It has previously been suggested that the hypocholesterolemic effect of β-glucan in animals reflects reduced activity of the liver enzyme, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase [1].

The suppressive effects of dietary bacterial β-glucan on body fat development and adipocyte hypertrophy were accompanied by the increase in the mRNA levels of the UCP that consume energy via thermogenesis. It is likely that increased expression of UCPs would increase energy expenditure, thereby contributing to the suppression of body fat accumulation [4]. UCPs are regulated by various nutritional perturbations in a tissue-selective manner. Thus, diets that are capable of activating or increasing expression of the UCP have recently been investigated as an interesting therapeutic candidate for obesity [2]. However, the influence of β-glucan on UCP expression has rarely been studied. Obesity induced by high-fat diet increased expression of UCP in our previous reports [4] and other studies [18, 23]. Consistent with these results, we found in the present study that the UCP mRNA in BAT was increased by dietary high fat. In addition, 5% bacterial β-glucan supplementation to the high-fat diet upregulated the expression of UCP in BAT. Our data on UCP expression in BAT suggest that the thermogenic effect of the high-fat diets was enhanced by 5% bacterial β-glucan supplementation. BAT mRNA levels of UCP 1, 2, and 3 in 1% bacterial β-glucan-containing high-fat fed rats were lower than those of high-fat diet fed rats. The current data demonstrate that the concentration of β-glucan might affect the anti-obesity by changing both adipocyte size and UCPs expression. Since the effect of low concentration β-glucan supplementation on hypoglycemic activity was not determined in the present study, different β-glucan concentrations, ranging from 0.1% to 5%, should be further investigated.

In summary, our results demonstrate that bacterial β-glucan supplementation resulted in a significant reduction of high-fat-induced white fat (i.e., visceral and peritoneal fat) development, adipocyte hypertrophy, and development of hyperinsulinemia and hyperleptinemia. Serum triglyceride, total cholesterol, and free fatty acid levels were greatly reduced, but HDL-cholesterol concentration was increased by bacterial β-glucan supplementation. We postulate that upregulation of UCPs and inefficient energy utilization are responsible for the anti-obesity effect of 5% bacterial β-glucan.

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