Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Nutritional Biochemistry 17 (2006) 419–426

Journal of Nutritional Biochemistry

Altered mRNA expression of hepatic lipogenic enzyme and PPARα in rats fed dietary levan from *Zymomonas mobilis*

Soon Ah Kang[a,1], Kyunghee Hong[b,1], Ki-Hyo Jang[c], Yun-Young Kim[d], Ryowon Choue[d], Yoongho Lim[a,*]

[a]*Department of Molecular Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, South Korea*
[b]*Department of Endocrinology, Asan Institute for Life Science, Ulsan 680-749, South Korea*
[c]*Department of Food and Nutrition, Samcheok National University, Gangwon 245-711, South Korea*
[d]*Department of Medical Nutrition, Graduate School of East–West Medical Science, Kyung Hee University, Seoul 130-701, South Korea*

Abstract

Levan or high molecular β-2,6-linked fructose polymer is produced extracellularly from sucrose-based substrates by bacterial levansucrase. In the present study, to investigate the effect of levan feeding on serum leptin, hepatic lipogenic enzyme and peroxisome proliferation-activated receptor (PPAR) α expression in high-fat diet-induced obese rats, 4-week-old Sprague-Dawley male rats were fed high-fat diet (beef tallow, 40% of calories as fat), and, 6 weeks later, the rats were fed 0%, 1%, 5% or 10% levan-supplemented diets for 4 weeks. Serum leptin and insulin level were dose dependently reduced in levan-supplemented diet-fed rats. The mRNA expressions of hepatic fatty acid synthase and acetyl CoA carboxylase, which are the key enzymes in fatty acid synthesis, were down-regulated by dietary levan. However, dietary levan did not affect the gene expression of hepatic malic enzyme, phosphatidate phosphohydrolase and HMG CoA reductase. Also, the lipogenic enzyme gene expression in the white adipose tissue (WAT) was not affected by the diet treatments. However, hepatic PPARα mRNA expression was dose dependently up-regulated by dietary levan, whereas PPARγ in the WAT was not changed. The results suggest that the in vivo hypolipidemic effect of dietary levan, including anti-obesity and lipid-lowering, may result from the inhibition of lipogenesis and stimulation of lipolysis, accompanied with regulation of hepatic lipogenic enzyme and PPARα gene expression.

Keywords: High-fat diet; Levan; Bacterial levansucrase; PPARα; Acetyl CoA carboxylase

1. Introduction

Disturbances in lipid metabolism are strongly associated with the development of insulin resistance-related diseases of the metabolic syndrome, particularly obesity and type 2 diabetes. The etiology of obesity is uncertain; however, positive energy balance over time is believed to be the primary cause [1]. It is probable that there are multiple causes of obesity including dietary fat and genetic factors. High-fat diet may promote obesity through increased calorie intake and possibly through diminished efficiency in the metabolism of fat [2].

Lipid accumulation by adipose tissue depends on plasma lipid that is derived from hepatic lipogenesis and absorbed from the diet [3]. Therefore, hepatic lipogenesis and the export of lipid are crucial steps linked to adipose tissue lipid accretion. The decrease in serum triglyceride in animals has been shown to result from reduction of very low density lipoprotein–triglyceride secretion and inhibition of hepatic lipogenesis through the reduction of activity and gene expression of lipogenic enzymes [4,5]. Additionally, it has been reported that, with the development of high-fat diet-induced insulin resistance, which is characterized by hyperinsulinemia, lipogenesis is elevated in the liver, which further exacerbates the accumulation of excess visceral fat, increasing serum free fatty acid levels [6].

Peroxisome proliferation-activated receptors (PPARs) are transcription factors that have important effects on lipid homeostasis via regulation of the expression of genes involved in lipid metabolism. PPARα is predominantly expressed in the liver and regulates the transcription of genes involved in hepatic fatty acid uptake and oxidation [7], and agonists of PPARα are shown to be anti-hyperlipidemic agents [8,9]. PPARγ is expressed mainly in white

* Corresponding author. Tel.: +82 2 450 3760; fax: +82 2 453 3761.
E-mail address: yoongho@konkuk.ac.kr (Y. Lim).
[1] The first two authors contributed equally to this study.

0955-2863/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2005.08.012

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, Bethlehem, PA, USA); 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).
[c] AIN-76 Vitamin mix (Dyets).

adipose tissue (WAT) where its activation stimulates the expression of genes involved in fatty acid uptake and storage. Recently, the insulin-sensitizing effects of PPARγ agonists have been used clinically for the treatment of type 2 diabetes [10,11]. Consequently, it is generally believed that the ability of PPAR agonists to up-regulate lipid metabolism in WAT and liver is central to their insulin-sensitizing effects [12].

Leptin, the product of the ob gene, is a hormone secreted by adipocyte [13]. By acting as a satiety factor and increasing energy expenditure, leptin, which is regulated by neuropeptide Y (NPY) in the hypothalamus, plays a major role in body weight homeostasis [3]. When the NPY level is decreased, leptin increases sympathetic activation, thermogenesis and metabolic rate [14,15], thereby increasing the energy expenditure and adapting thermogenesis in brown adipose tissue [16,17].

Levan is found in plants and in the bioproducts of microorganisms [18,19]. Recently, an enzymatic process to efficiently produce levan has been developed using a levansucrase (sucrose-6-fructosyltransferase, EC2.4.1.10) isolated from *Zymomonas mobilis* [20]. Fructose polymers such as levan are fermented by gut microflora, which in turn improves the intestinal microflora and produces short-chain fatty acids [21]. In animal studies, short-chain fatty acids produced by bacterial fermentation and absorbed into the portal blood flow showed an inhibitory effect on hepatic fatty acid and cholesterol synthesis [22,23].

Recent studies demonstrated that supplementation of levan was able to decrease adiposity and postprandial lipidemia in high-fat diet-induced obese rats through enhancement of the gene expression of uncoupling protein (UCP) [24,25]. In the present study, in order to more specifically identify the mechanisms of hypolipidemic action at the molecular level, we analyzed mRNA expression of genes involved in lipogenesis and lipid metabolism in the liver and WAT.

2. Materials and methods

2.1. Experimental animals and sampling procedures

Experiments were performed on 3-week-old Sprague-Dawley male rats purchased from Central Experimental Animals (Samtaco, Seoul, South Korea) and housed individually. Rats were housed in a temperature-controlled environment with a 12:12-h light/dark cycle. After adaptation for 1 week, rats were weighed, randomly assigned and fed normal (11.7% fat of total dietary calories) or high-fat diet (40% fat of total dietary calories). Six weeks later, high-fat diet-fed rats were randomly grouped into four groups and assigned 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 [24]. Water and food were consumed ad libitum. The food intake and body weight were weighed twice a week, and energy intake was calculated, based on the caloric value of 1.5 kcal/g of levan, similar to all the other carbohydrates that are fermented in

Table 2
Primer sequences used in the quantitative RT-PCR for FAS, ACC, ME, PAP, HMG CoA reductase, HSL, PPARα and PPARγ

Gene		Primer sequence	Product size (bp)
FAS	Forward	5′ -GGA AAG CTG AAG GAT CTG TC-3′	435
	Reverse	5′ -AGG TGT TAG GCT TTA GCA GC-3′	
ACC	Forward	5′ -CGG CTT GCA CCT AGT AAA AC-3′	565
	Reverse	5′ -AAT CCA CTC GAA GAC CAC TG-3′	
ME	Forward	5′ -ACC CGC ATC TCA ACA AGG AC-3′	574
	Reverse	5′ -CCG CAG CCC AAT ATA CAA GG-3′	
PAP	Forward	5′ -GGT CCT CGA TGT GAT TTG CG-3′	412
	Reverse	5′ -TGT AGC CAT CGC TGC AGT TG-3′	
HMG CoA reductase	Forward	5′ -CGA TTA GGT CCC CAA AAC GG-3′	549
	Reverse	5′ -CCA GGA TTG TCT TTG CAC GC-3′	
HSL	Forward	5′ -CCA GGA AAG AGG GCA AAA GC-3′	434
	Reverse	5′ -AGC GAC TGT GTC ATT GTG CG-3′	
PPARα	Forward	5′ -TTC GTG GAG TCC TGG AAC TG-3′	702
	Reverse	5′ -TGT CGT ACG CCA GCT TTA GC-3′	
PPARγ	Forward	5′ -CCC ACC AAC TTC GGA ATC AG-3′	481
	Reverse	5′ -GCA AGG CAC TTC TGA AAC CG-3′	

Table 3
Energy intake, weight gain and EER in rats fed experimental diets for 4 weeks*

	N	HF	HF-L1	HF-L5	HF-L10
Energy intake (kcal/day)	116.62 ± 1.91^{b}	$101.68\pm2.58^{c}_{y}$	$125.12\pm2.40^{a}_{x}$	$109.09\pm2.72^{bc}_{y}$	$104.76\pm3.84^{bc}_{y}$
Weight gain (g/day)	2.48 ± 0.21^{ab}	$2.58\pm0.29^{a}_{x}$	$2.77\pm0.17^{a}_{x}$	$1.94\pm0.16^{bc}_{y}$	$1.61\pm0.21^{c}_{y}$
EER**(g/kcal)	0.021 ± 0.003^{c}	$0.025\pm0.007^{a}_{x}$	$0.022\pm0.005^{ab}_{xy}$	$0.018\pm0.007^{bc}_{xy}$	$0.015\pm0.003^{c}_{y}$

* Each value is mean±S.E. for nine 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 $P<.05$. a–d: Significance between N, HF, HF-L1, HF-L5 and HF-L10. x–z: Significance between HF, HF-L1, HF-L5 and HF-L10.

** EER=body weight gain (g/day)/energy intake (kcal/day).

the colon. After 4 weeks of feeding the normal, high-fat or levan-supplemented high-fat diets, blood was collected from the portal vein under anesthesia with dietyl ether, and serum was separated by centrifugation (3000×*g*, for 15 min at 4°C). After collecting blood samples, liver and epididymal fat pad were excised immediately, weighed and frozen in liquid nitrogen. All serum and tissue samples were stored at −70°C until analysis. All experimental procedures were conducted strictly in accordance with the ethical guidelines of Konkuk University.

2.2. Blood analyses

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using commercial kits (Sigma, St. Louis, MO). Serum leptin and insulin were measured by radioimmunoassay using the Linco leptin assay kit and insulin assay kit (Linco Research Immunoassay, St. Louis, MO), respectively.

2.3. Quantitative RT-PCR for gene expression of hepatic and adipocytic enzyme analyses

Total RNA was extracted from the liver and epididymal fat pad with the Trizol reagent (Invitrogen) for analysis of gene expression of liver and adipose tissue. The yield and quality of the extracted RNA were assessed by 260/280 nm optical density ratio and by electrophoresis on 1.5% agarose gels under denaturing conditions. Reverse transcription (RT) reaction mixture, containing 2 μg of total RNA, was denatured for 10 min at 72°C. Reverse transcriptase reaction in a final volume of 25 μl was then performed for 60 min at 42°C and stopped after 30 min at 75°C. The final composition of the reaction mixture was as follows: M-MLV (Promega, Madison, WI) 200 U, dNTP mix (each 2.5 mM) 2 μl, RNasin (Promega) 40 U, oligo(dT) primer (Invitrogen, Carlsbad, CA) 1 μl.

Polymerase chain reaction (PCR) was performed in a total volume of 25 μl, containing Taq polymerase (Takara, Shiga, Japan) 0.125 μl, 10× PCR buffer 2.5 μl, dNTP (2.5 mM each) mix 2 μl, 10 pmol each of the gene-specific primers and 10 pmol each of the primers and β-actin (see Table 2 for primer sequence). Thermal cycling parameters were as follows: 94°C for 30 s, 58–62°C for 60 s and 72°C for 60 s, and repeated for 27–35 cycles with an elongation step of 10 min at 72°C.

The PCR products (7 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. of four individual determinations.

2.4. Statistical analyses

Results are expressed as means±S.E. The significance of difference between the normal and the high-fat diet group 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 (ver. 8.0 for Windows), and statistical significance of difference was defined as $P<.05$.

3. Results

3.1. Energy intake and energy efficiency ratio after levan supplementation

As shown in Table 3, energy intake in the high-fat diet-fed rats (101.68±2.58 kcal/day) was lower than in the normal diet-fed rats (116.62±1.91 kcal/day), but lower than in the 1% levan-supplemented diet-fed rats (125.12±2.40 kcal/day). Body weight gain was 2.48±0.21, 2.58±0.29, 2.77±0.17, 1.94±0.16 and 1.61±0.21 g/day in normal diet (N), high-fat diet (HF), high-fat diet with 1% levan (HF-L1), high-fat diet with 5% levan (HF-L5) and

Table 4
Liver weight, serum aspartate aminotransferase (AST) and alanine aminotrasferase (ALT) in rats fed experimental diets for 4 weeks[a]

	N	HF	HF-L1	HF-L5	HF-L10
Liver weight (g/100 g BW)	2.86±0.07	2.86±0.07	2.93±0.03	2.93±0.09	2.87±0.12
Serum AST (IU/L)	95.14±14.59	108.40±4.62	116.30±21.98	90.22±3.23	94.89±3.02
Serum ALT (IU/L)	43.57±4.45	42.10±2.82	43.90±6.61	39.78±1.52	41.89±2.58

[a] Each value is mean±S.E. for nine rats. Statistical analysis was performed using one-way ANOVA, followed by Duncan's multiple range test ($P<.05$).

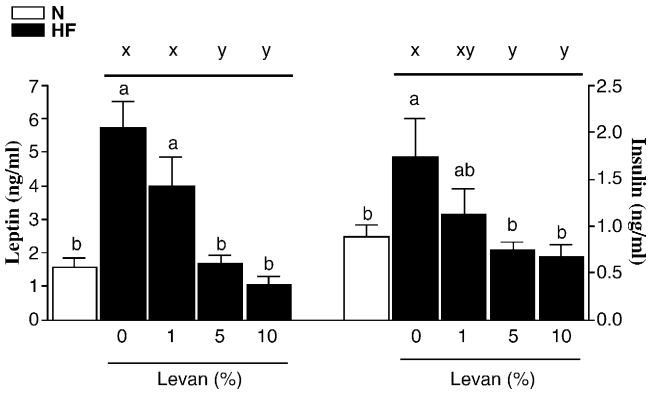

Fig. 1. Effect of dietary levan on serum leptin and insulin levels in rats fed HF diet for 4 weeks. Values are mean±S.E. (n=9). Different letters indicate significant difference (P<.05) by Duncan's multiple range test. a–c: Significance between N, HF and levan-supplemented HF groups. x–z: Significance between HF and levan-supplemented HF groups.

high-fat diet with 10% levan (HF-L10), respectively. Body weight gain was lower in the rats fed 5% or 10% levan-supplemented high-fat diet than in those fed high-fat or normal diet alone. Thus, the energy efficiency ratio (EER) was significantly lowered dose-dependently by dietary levan. The EER of the high-fat diet-fed rats was higher than that of normal diet-fed rats; however, it was lowered by 12%, 28% and 40% in 1%, 5% and 10% levan-supplemented diet-fed rats, respectively (Table 3).

3.2. Liver weight, serum AST and ALT after levan supplementation

The range of relative liver weight was 2.86–2.93 g/100 g body weight, and serum AST and ALT levels were 94.89–95.14 and 39.78–43.90 IU/L, respectively (Table 4). However, there was no significant difference between the groups in relative liver weight, and serum AST and ALT levels.

3.3. Serum leptin and insulin

Serum leptin and insulin levels are presented in Fig. 1. In accordance with the previous study, serum leptin and insulin levels in the rats fed high-fat diet were significantly higher than in the normal diet-fed rats, suggesting the development of hyperleptinemia and hyperinsulinemia. In contrast to the high-fat diet-fed rats (5.74±0.77 ng/ml), those that received 5% (1.67±0.26 ng/ml) and 10% (1.04±0.26 ng/ml) levan supplementation showed significant suppression of elevated serum leptin concentrations by 71% and 82%, respectively (Fig. 1). Furthermore, serum insulin level was markedly lower in the rats fed 5% (0.75±0.08 ng/ml) and 10% (0.67±0.13 ng/ml) levan-supplemented high-fat diet than in the rats fed high-fat diet alone (1.74±0.40 ng/ml). The suppressing effect of dietary levan on serum leptin and insulin was dose-dependent. It should be noted in Fig. 1 that 5% and 10% levan supplementation restored serum leptin (1.58±0.26 ng/ml) and insulin (0.67±0.13 ng/ml) levels to normal.

3.4. mRNA expression of lipogenic enzymes and PPAR in liver and WAT

The effects of dietary levan supplementation on mRNA levels of genes involved in lipid metabolism in the liver and WAT (epididymal fat pad) were examined by quantitative RT-PCR: the mRNA expressions of three key lipogenic enzymes [fatty acid synthetase (FAS), acetyl CoA carboxylase (ACC) and malic enzyme (ME)]; phosphatidate phosphohydrolase (PAP), a key enzyme controlling fatty acid esterification; β-hydroxy-β-methylglutaryl-CoA (HMG CoA reductase), the key enzyme in cholesterol synthesis; and hormone-sensitive lipase (HSL). Furthermore, the mRNA expression of the transcriptional controlling factor in lipid metabolism, PPARs, was measured, such as PPARα in liver and PPARγ in WAT (Table 4; Figs. 2 and 3).

Hepatic mRNA levels of FAS were significantly lower in HF-L1, HF-L5 and HF-L10 rats than in those fed high-fat diet alone by 61%, 54% and 61%, respectively, and hepatic ACC mRNA level was lower in HF-L1, HF-L5 and HF-L10 rats than in those fed high-fat diet alone by 38%, 20% and 33%, respectively. There was no significant difference in the mRNA levels of ME, PAP and HMG CoA reductase in the liver between the experimental groups.

The mRNA expression of PPARα in the liver was higher in the HF-L1, HF-L5 and HF-L10 rats than in the rats fed high-fat diet alone by 3%, 21% and 44%, respectively, which was dose-dependent (Fig. 2). In WAT, however, the mRNA levels of lipogenic enzyme, HSL and PPARγ were not statistically different among the experimental groups (Fig. 3).

4. Discussion

In the present study, we observed a reduction in the mRNA expression of FAS, ACC and PPARα in the liver of obese rats with levan diet supplementation, concomitant with a decrease in leptin and insulin levels, which were significantly correlated with a decrease in free fatty acid level. On the other hand, although the rats lost fat mass, the change in the mRNA levels of lipogenic enzyme, HSL and PPARγ expression in WAT was not associated with the degree of fat mass loss.

There is increasing evidence that serum leptin level is correlated with percentages of body fat and adiposity [26]. In addition, obese humans and rats are insensitive to endogenous leptin production, leptin resistance [27] and increase in leptin resistance by high-fat diet-induced obesity [28]. Our recent studies showed that levan supplementation in high-fat diet reduced the adiposity and serum lipid by increasing UCP gene expression [24,25]. In the present result, in consistency with the above reports, the levan supplementation was found to suppress body fat development and adipocyte hypertrophy, resulting in decreased leptin production and secretion. Since there is a strong link between leptin and insulin in the

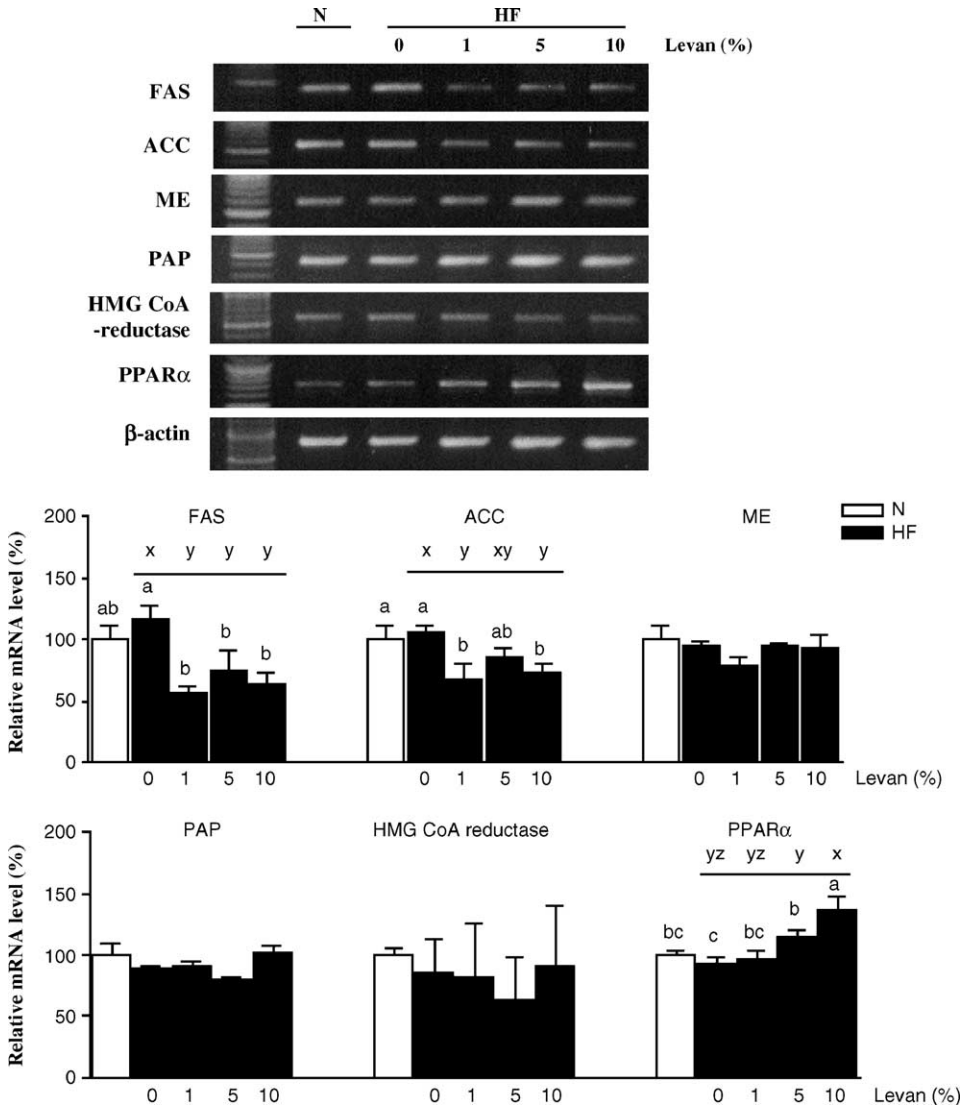

Fig. 2. mRNA expressions of hepatic enzymes and PPARα in rats fed experimental diets for 4 weeks. Quantitative RT-PCR was used for the mRNA determination. Levels of mRNA were calculated as percentage values of the N diet group. Values are mean±S.E. ($n=4$). Different letters indicate significant difference ($P<.05$) by Duncan's multiple range test. a–c: Significance between N, HF and levan-supplemented HF groups. x–z: Significance between HF and levan-supplemented HF groups.

long-term regulation of energy homeostasis [29], it is quite possible that dietary levan modifies the kinetics of absorption of dietary carbohydrates, leading to modifications of both serum glucose and insulin, analogous to fermentable dietary fibers such as pectin.

Postprandial insulin was significantly lower and in hepatic lipogenesis was strongly inhibited in high-fat diet-fed rats, when fructan was added to the diet [30]. Consistent with the report, 1–10% of levan supplementation markedly suppressed the hyperinsulinemia induced by high-fat diet (Fig. 1), thereby suppressing hepatic lipogenesis and excess body fat development accompanied by decreased serum free fatty acid.

In order to understand the putative atherogenic role of hyperlipidemia in humans, it is essential to elucidate the mechanism involved in levan-treated rats after a fat load. However, the mechanism by which such nondigestible carbohydrate as levan modulates systemic lipid metabolism remains unknown. One of the mechanisms might be related to the fermentation in the ceco-colon. Yamamoto et al. [31] reported that the levan in their study was not fermented; however, the levan used in the present study was fermented by bifidobacteria in the colon, and short-chain fatty acids were produced [32,33]. It should be noted that short-chain fatty acids such as acetate and propionate are end products of bacterial fermentation of fructans, which reach the liver through the portal vein, and that acetate is a lipogenic substrate, and propionate is an effective inhibitor of lipid synthesis in isolated hepatocytes [34].

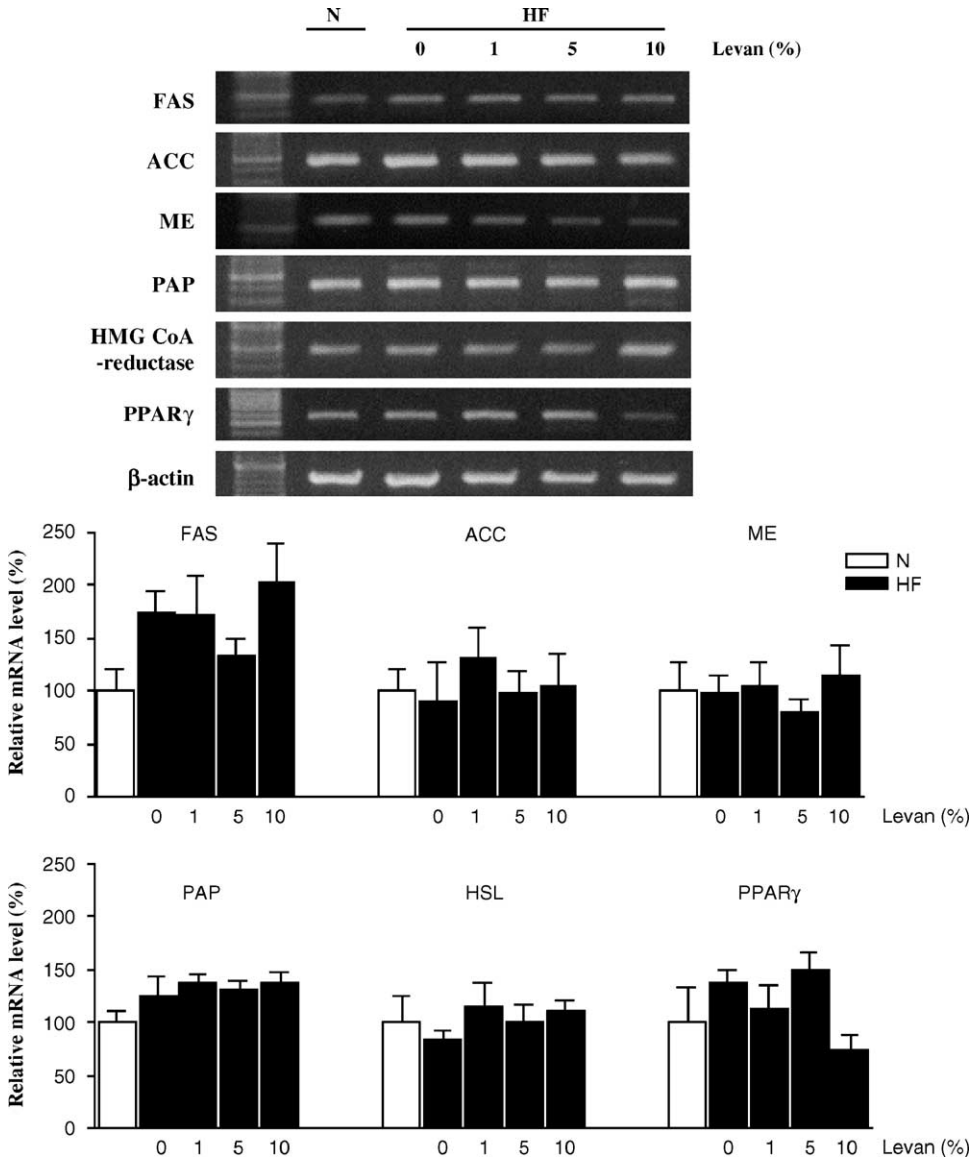

Fig. 3. mRNA expressions of various enzymes and PPARγ of WAT (epididymal fat pad) in rats fed experimental diets for 4 weeks. Quantitative RT-PCR was used for the mRNA determination. Levels of mRNA were calculated as percentage of the values of the N diet group. Values are mean±S.E. (n=4). Different letters indicate significant difference (P<.05) by Duncan's multiple range test.

Considering these findings, the hypotriglyceridaemic effect of levan seems likely to be due to the decrease of hepatic synthesis of triglycerides. Indeed, triglyceride synthesis and secretion from acetate were significantly reduced in hepatocytes isolated [4] from oligofructosesaccharide (OFS)-fed rats, and the inulin supplementation reduced hepatic lipogenesis and plasma triglyceride concentrations in humans [35]. In this study, the expression of lipogenic enzymes, FAS and ACC, was found to be low in the liver of levan-fed rats compared to rats fed high-fat diet without levan, in accordance with the robust decrease of FAS mRNA observed in the liver of OFS-fed Wistar rats [36]. In this study, the expression of the enzyme, which drives the esterification pathway (PAP, which converts phosphatidate to diacylglycerol, the common intermediate for phospholipid and triglyceride biosynthesis), was not changed by the levan treatment. These results suggest that levan decreased the hepatic triglyceride synthesis through fatty acid synthesis, but not esterification pathway. However, Delzenne and Kok [36] reported that the activity of all lipogenic enzymes, including FAS, ACC, carnitine palmitoyltransferase, ME, glucose 6-phophate dehydrogenase and PAP, was reduced in OFS-fed rats. In obese Zucker rats, when 10% OFS was added to the diet, the only significant change was a lower activity of ME, whereas FAS and PAP activity and FAS mRNA were not different [14].

The present study showed that the PPARα mRNA expression was dose-dependently up-regulated by levan supplementation. Since the fatty acid oxidation in the liver is regulated by the PPARα [37], one possible mechanism in

the triglyceride-lowering effects of levan may be multiple inductions of intra- and extracellular fatty acid catabolism and utilization pathways (e.g., induction of fatty acid β-oxidation and triglyceride hydrolysis), by up-regulation of PPARα gene expression. The induction of lipid catabolism potently suppressed body fat development.

We conclusively demonstrated that levan supplementation was beneficial for the suppression of high-fat diet-induced obesity, accompanied by activation of lipid metabolism and suppression of lipogenesis in the liver. These hypolipidemic effects are probably due to decreased lipogenic enzyme expression, resulting from the action of short-chain fatty acids produced by levan fermentation and reduced insulinemia. The precise molecular mechanism by which levan stimulates lipid metabolism is unclear at present. Further studies are needed to elucidate the detailed interrelationship between levan feeding, energy and lipid metabolism, and neuropeptide-related food intake.

Acknowledgments

This study was supported by the Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea. The authors gratefully acknowledge RealBioTech, Co. Ltd., for generously providing the levan sample.

References

[1] Leonhardt M, Hrupka B, Langhans W. New approaches in the pharmacological treatment of obesity. Eur J Nutr 1999;38:1–13.
[2] Albu J, Allison D, Boozer CN, Heymsfield S, Kissileff H, Kretser A, et al. Obesity solutions: report of a meeting. Nutr Rev 1997;55(5): 150–6.
[3] Griffen HD, Guo K, Windsor K, Butterwith SC. Adipose tissue lipogenesis and fat deposition in leaner broiler chicken. J Nutr 1992;122:363–8.
[4] Kok N, Roberfroid M, Delzenne N. Involvement of lipogenesis in the lower VLDL secretion induced by oligofructose in rats. Br J Nutr 1996;76:881–90.
[5] Delzenne N, Ferre P, Beylot M, Daubioul C, Declercq B, Diraison F, et al. Study of the regulation by nutrients of the expression genes involved in lipogenesis and obesity in humans and animals. Nutr Metab Cardiovasc Dis 2001;11(4 Suppl):118s–21s.
[6] Commins SP, Watson PM, Padgett MA, Dudley A, Argyropoulos TW, Gettys TW. Induction of uncoupling protein expression in brown and white adipose tissue by leptin. Endocrinology 1999; 140(1):292–300.
[7] Escher P, Wahli W. Peroxisome proliferators-activated receptors: insight into multiple cellular functions. Mutat Res 2000;448:121–38.
[8] Guerre-Millo M, Gervois P, Raspe E, Madsen L, Poulain P, Derudas B, et al. Peroxisome proliferators-activated receptor alpha activators improve insulin sensitivity and reduce adiposity. J Biol Chem 2000; 275:16638–42.
[9] Avogaro A, Miola M, Favaro A, Gottardo L, Pacini G, Manzato E, et al. Gemfibrozil improves insulin sensitivity and flow-mediated vasodilation in type 2 diabetic. Eur J Clin Invest 2001;31:603–9.
[10] Hegarty BD, Furler SM, Oakes ND, Kraegen EW, Cooney GJ. PPAR activation induces tissue specific effects on fatty acid uptake and metabolism in vivo—a study using the novel PPAR agonist tesaglitazar. Endocrinology 2004;10:1210–22.
[11] Bailey CJ. New pharmacological agents for diabetes. Curr Diab Rep 2001;1:119–26.
[12] Moller DE. New drug targets for type 2 diabetes and the metabolic syndrome. Nature 2001;414:821–7.
[13] Schwartz MW, Woods SC, Porte Jr D, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature 2000;404:661–71.
[14] Daubioul CA, Taper HS, Wispelaere LD, Delzenne NM. Dietary oligo-fructose lessens hepatic steatosis, but does not prevent hypertriglyceridemia in obese Zucker rats. J Nutr 2000;130:1314–9.
[15] Mortensen A, Poulsen M, Frandsen H. Effect of a long-chain fructan Raftline HP on blood lipids and spontaneous atherosclerosis in low density receptor knockout mice. Nutr Res 2002;22:473–80.
[16] Zou B, Suwa M, Nakano H, Higaki Y, Ito T, Katsuta S, et al. Decreased serum leptin and muscle oxidative enzyme activity with a dietary loss of intra-abdominal fat in rats. J Nutr Biochem 2004;15: 24–9.
[17] Steinberg GR, Bonen A, Dyck DJ. Fatty acid oxidation and triacylglycerol hydrolysis are enhanced after chronic leptin treatment in rats. Am J Physiol Endocrinol Metab 2002;282:E593–E600.
[18] Jang EK, Jang KH, Koh I, Kim IH, Kang SA, Kim CH, et al. Molecular characterization of levansucrase gene from *Pseudomonas aurantiaca* S-4830 and its expression in *Escherichia coli*. J Microbiol Biotechnol 2002;12:603–9.
[19] Kim HJ, Yang JH, Lee HG, Cha JH. Cloning and sequence analysis of a levansucrase gene from *Rahnella aquatilis* ATCC15552. J Microbiol Biotechnol 2001;11:693–9.
[20] Rhee SK, Song KB, Kim CH, Park BS, Jang EK, Jang KH. Polysaccharides: 1. Levan. In: Vandamme E, De Baets S, Steinbuchel A, editors. Biopolymers, vol. 5. Germany: Wiley-VCH; 2002. p. 351–77.
[21] Rombeau JL, Kripke SA. Metabolic and intestinal effects of short-chain fatty acids. JPEN J Parenter Enteral Nutr 1990;14(Suppl): S181–5.
[22] Delzenne N, Roberfroid MB. Physiological effects of nondigestible oligosaccharides. Lebensm-Wiss Technol 1994;27:1–6.
[23] Lin Y, Vonk RJ, Sloof MJ, Kuipers F, Smit MJ. Differences in propionate-induced inhibition of cholesterol and triacylglycerol synthesis between human and rat hepatocytes in primary culture. Br J Nutr 1995;74:197–207.
[24] Kang SA, Hong KH, Jang KH, Kim SH, Lee KH, Chang BI et al. Anti-obesity and hypolipidemic effects of dietary levan in high fat diet-induced obese rats. J Microbiol Biotechnol 2004; 14:796–804.
[25] Kang SA, Hong KH, Jang KH, Kim SH, Jang EK, Kin CH, et al. Effects of low level of levan feeding on serum lipids, adiposity and UCP expression in rats. J Korean Soc Food Sci Nutr 2002; 31(5):788–95.
[26] Havel PJ, Kasim KS, Mueller W, Johnson PR, Gingerich RL, Stern JS. Relationship of plasma leptin to plasma insulin and adiposity in normal weight and overweight women: effects of dietary fat content and sustained weight loss. J Clin Endocrinol Metab 1996;81(12): 4406–13.
[27] Scarpace PJ, Tümer N. Peripheral and hypothalamic leptin resistance with age-related obesity. Physiol Behav 2001;74:721–7.
[28] Watson PM, Commins SP, Beiler RJ, Hatcher HC, Gettys TW. Differential regulation of leptin expression and functin in a/J vs. C578BL/6J mice during diet-induced obesity. Am J Physiol Endocrinol Metab 2000;279(2):E356–65.
[29] Dagogo JS, Fanelli C, Parmore D, Brother J, Landt M. Plasma leptin and insulin relationship in obese and nonobese humans. Diabetes 1996;45(5):695–8.
[30] Kok NN, Taper HS, Delzenne NM. Oligofructose modulates lipid metabolism alterations induced by a fat-rich diet in rats. J Appl Toxicol 1998;18(1):47–53.
[31] Yamamoto Y, Takahashi Y, Kawano M, Iizuka M, Matsumoto T, Saeki S, et al. In vitro digestibility and fermentability of levan and its hypocholesterolemic effects in rats. J Nutr Biochem 1999;10:13–8.

[32] Jang KH, Kang SA, Cho Y, Kim YY, Lee YJ, Hong KH, et al. The effects of levan and inulin on the growth of lactic acid-producing bacteria and intestinal conditions in rats. Korean J Nutr 2002;35(9): 912–8.

[33] Jang KH, Kang SA, Cho Y, Kim YY, Lee YJ, Hong K, et al. Prebiotics properties of levan in rats. J Microbiol Biotechnol 2003; 13(3):348–53.

[34] Remesy C, Levrat MA, Gamet L, Demigne C. Cecal fermentations in rats fed oligosaccharides (inulin) are modulated by dietary calcium levels. Am J Physiol 1993;264:G855–62.

[35] Letexier D, Diraison F, Beylot M. Addition of inulin to a moderately high-carbohydrate diet reduces hepatic lipogenesis and plasma triacylglycerol concentration in humans. Am J Clin Nutr 2003;77:559–64.

[36] Delzenne NM, Kok NN. Biochemical basis of oligofructose-induced hypolipidemia in animal models. J Nutr 1999;129(7 Suppl): 1467s–70s.

[37] Schoonjans K, Staels B, Auwerx J. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. J Lipid Res 1996;37: 907–25.