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Effects of Long-Term Feeding of Chitosan on Postprandial Lipid Responses and Lipid Metabolism in a High-Sucrose-Diet-Impaired Glucose-Tolerant Rat Model

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ABSTRACT: This study was designed to investigate the effects of long-term feeding of chitosan on postprandial lipid response and lipid metabolism in a high-sucrose (HS)-diet-impaired glucose-tolerant rat model. As the results, HS-diet-fed rats supplemented with 5 and 7% chitosan in diets for 9 weeks had lower postprandial plasma total cholesterol (TC) levels, but 7% chitosan in the diet had higher postprandial plasma triglyceride (TG) and TG-rich lipoprotein TG levels. Supplementation of chitosan significantly decreased the postprandial ratio of apolipoprotein B (apoB)48/apoB100 in TG-rich lipoprotein fractions of HS-diet-fed rats. Long-term supplementation of 5 and 7% chitosan in diets for 16 weeks had lower plasma TC, low-density lipoprotein cholesterol (LDL-C) + very low density lipoprotein cholesterol (VLDL-C), TC/high-density lipoprotein (HDL-C) ratio, leptin, and tumor necrosis factor-α (TNF-α) levels in HS-diet-fed rats. Moreover, it was noticed that the VLDL receptor (VLDLR) protein expression in skeletal muscles of HS-diet-fed rats was significantly decreased, which could be significantly reversed by supplementation of 5 and 7% chitosan. Rats supplemented with 7% chitosan in the diet significantly elevated the lipolysis rate and decreased the accumulation of TG in epididymal fat pads of HS-diet-fed rats. The plasma angiopoietin-like 4 (ANGPTL4) protein expression was not affected in HS-diet-fed rats, but it was significantly increased in 7% chitosan-supplemented HS-diet-fed rats. Taken together, these results indicate that supplementation of chitosan in the diet can improve the impairment of lipid metabolism in a HS-diet-fed rat model, but long-term high-dose chitosan feeding may enhance postprandial plasma TG and TG-rich lipoprotein TG levels in HS-diet-fed rats through an ANGPTL4-regulated pathway.

KEYWORDS: *Chitosan, high-sucrose diet, lipoproteins, angiopoietin-like 4*

INTRODUCTION

Obesity is a growing concern worldwide. The survey of the World Health Organization (WHO) has shown that there are about 1.6 billion overweight adults, of which at least 400 million adults are obese worldwide in 2005, and forecasts about 2.3 billion overweight adults and 700 million adults being obese in 2015.[1] Obesity is known to increase the risk of chronic diseases, such as diabetes, stroke, cancer, chronic liver disease, and cardiovascular disorders. Therefore, obesity is a major public health concern, which may bring significant disability and premature deaths, and needs the appropriate strategies to tackle it.

Many studies have shown that rats fed a high-sucrose (HS) diet can produce hyperlipidemia and insulin resistance.[2−5] Xue et al. have found that a HS diet lead to an increase in plasma triglyceride (TG) (hypertriglyceridemia) in rats by increasing hepatic TG secretion and reducing plasma TG removal.[6] In addition, it has been indicated that a HS diet increases the hepatic very low density lipoprotein (VLDL) secretion rate[7] and increases the TG/protein ratio within VLDL.[8]

Chitosan, a biopolymer of glucosamine derived from chitin, which is chemically similar to cellulose, is not digestible by mammalian digestive enzymes and has been widely employed as a dietary supplement.[9] Chitosan has been shown to reduce liver cholesterol by decreasing cholesterol absorption and increasing bile acid and fat excretions in cholesterol-fed rats.[10] Our previous study has also found that chitosan feeding in hamsters reduces hepatic cholesteryl ester accumulation and lower plasma VLDL cholesterol by enhancing fecal excretion of cholesterol and bile acid.[11] A randomized controlled trial in 250 overweight and obese adults has indicated that chitosan treatment slightly but significantly reduces body weight, circulating total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), and glucose during the 24 week intervention compared to the placebo treatment.[12] Several animal studies have shown that chitosan possesses the antidiabetic potential on type 1 and 2 diabetes.[13−16] Yao et al. have also found that high-molecular-weight (MW) chitosan significantly decreased plasma glucose and TC and increased high-density lipoprotein cholesterol (HDL-C) and fecal cholesterol excretion in streptozotocin-induced diabetic rats.[15] However, further investigation is needed to understand the detail actions and mechanisms of chitosan on postprandial lipid responses and lipid metabolism under an impaired glucose tolerance condition. In the present study, we try to investigate the effects and possible mechanisms of long-term feeding of chitosan on postprandial lipid responses and lipid-related metabolic changes in rats with HS-diet-impaired glucose tolerance.

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MATERIALS AND METHODS

Materials. High-MW chitosan, which was obtained from shrimp shell chitin by alkali fusion, was generously supplied from Taiwan Tanabe Seiyaku Co. (Taipei, Taiwan). Cellulose was purchased from Sigma Chemical Co. (St. Louis, MO). The degree of deacetylation of chitosan was about 90%, and the average MW was about 835 kDa.

Animals and Diets. Male, 7-week-old Sprague−Dawley (SD) rats were purchased from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan). Rats were fed a chow diet (Rodent Laboratory Chow, Ralston Purina, St. Louis, MO) for 2 weeks, and then the animals were randomly divided into two groups: a control group that received standard rodent chow with 5% cellulose and a HS group that received a HS (57.1%) diet with 5% cellulose. After 13 weeks of acclimation, the rats that received a HS diet were further randomly divided into four groups: (1) HS-diet rats with 5% cellulose, (2) HS-diet rats with 3% high-MW chitosan, (3) HS-diet rats with 5% high-MW chitosan, and (4) HS-diet rats with 7% high-MW chitosan. Each group contains 10 animals. The compositions of the experimental diets given to test animals are shown in Table 1. Rats were housed in individual stainless-steel cages in a room kept at 23 ± 1 °C and 60 ± 5% relative humidity with a 12 h light and dark cycle (lighting from 8:00 a.m. to 8:00 p.m.). Food and drinking water were available *ad libitum* and measured daily. The bodyweight was measured every week. The postprandial experiments were tested after 9 weeks of treatment. To the postprandial experiment, a certain weight of diets containing 10% lard was homogenized with normal saline (1:2, w/v). Rats in each group orally received the homogeneous emulsion [0.8 mL/100 g of body weight (BW)] in the fasted state. Blood samples were obtained at 0 (before diet loading), 2, 3, 4, and 6 h from the tail vein using a heparinized capillary tube. Plasma was isolated immediately by centrifugation at 1570*g* for 20 min at 4 °C and stored at −80 °C until assay.

Table 1. Composition of the Experimental Diets

ingredient (%)	control	HS	HS + 3% chitosan	HS + 5% chitosan	HS + 7% chitosan
casein	20	20	20	20	20
lard	10	10	10	10	10
soybean oil	2	2	2	2	2
vitamin mixture[a]	1	1	1	1	1
mineral mixture[b]	4	4	4	4	4
cholesterol	0.5	0.5	0.5	0.5	0.5
cholic acid	0.2	0.2	0.2	0.2	0.2
choline chloride	0.2	0.2	0.2	0.2	0.2
cellulose	5	5	2		
chitosan[c]			3	5	7
sucrose		57.1	57.1	57.1	55.1
corn starch	57.1				

[a]AIN-93 vitamin mixture. [b]AIN-93 mineral mixture. [c]The average MW of chitosan is about 8.35×10^5 Da.

After the 16 week feeding study, the animals were sacrificed. This study was approved by the Animal House Management Committee of the National Taiwan Ocean University. The animals were maintained in accordance with the guidelines for the care and use of laboratory animals as issued by the Animal Center of the National Science Council.

Collection of Blood and Tissue Samples. At the end of the experimental period, animals fasted for 12 h prior to being sacrificed (at 10:00 a.m.) by exsanguinations via the abdominal aorta while under diethyl ether anesthesia. Heparin was used as the anticoagulant. Plasma was separated from the blood by centrifugation (1750*g*) at 4 °C for 20 min. The liver and soleus muscle from each animal were excised and weighed.

Determination of Plasma Glucose, Insulin, Adiponectin, Leptin, and Tumor Necrosis Factor-α (TNF-α). Plasma glucose was determined with a kit purchased from Audit Diagnostics Co. (Cork, Ireland). Plasma insulin was determined using a rat insulin enzyme-linked immunosorbent assay (ELISA) kit (Mercodia AB, Uppsala, Sweden). Plasma adiponectin, leptin, and TNF-α levels were determined using the rat ELISA kits (Assay Designs, Inc., Ann Arbor, MI).

Determination of Plasma Lipid Concentration. Plasma TC, TG, and free fatty acid levels were determined by an enzymatic method provided by the kits purchased from Audit Diagnostics (Cork, Ireland). The TG-rich lipoproteins from a separate aliquot of plasma were isolated by density gradient ultracentrifugation (Hitachi, SP 85G, RPL 42T Rotor, Tokyo, Japan) by 194000*g* at 10 °C for 3 h, and the lipoproteins were recovered by tube slicing.[17]

Lipolysis Rate Measurement. Adipose tissue (0.2 g) was minced into small pieces and placed in 2 mL of 25 mM *N*-tris-(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) buffer containg 1 μM isoproterenol and incubated at 37 °C. Isoproterenol produced a dose-dependent increase in lipolysis. There was a time-dependent increase in lipolysis. After 1, 2, and 3 h of incubation, 0.2 mL of medium was used to measure the levels of glycerol by a commercial reagent (RANDOX GY105, Amtrim, U.K.), and then the absorbance at 405 nm was recorded using a spectrophotometer. The lipolysis rate was indicated by micromoles of glycerol released per gram of tissue per hour.

Adipose Tissue Lipoprotein Lipase (LPL) Activity. LPL activity in adipose tissue was measured as described previously by Cook et al.[18] Adipose tissue (0.1 g) was minced into small pieces and placed in Krebs−Ringer bicarbonate buffer (pH 7.4) in the presence of heparin (10 units/mL) for 60 min at 37 °C. The heparin solution was mixed with an equal volume of 2 mM *p*-nitrophenyl butyrate (pNPB). Absorbance at 405 nm was recorded following a 10 min incubation using a spectrophotometer. LPL activity was recorded as the amount of *p*-nitrophenol product formed over the 10 min incubation.

Western Blot Analysis. Total protein containing 50−100 μg was separated on 8% sodium dodecyl sulfate (SDS)−polyacrylamide minigels and transferred to nitrocellulose membranes (Amersham). After blocking, blots were incubated with antibodies for α-tubulin, VLDL receptor (VLDLR), angiopoietin-like 4 (ANGPTL4) (Santa Cruz), and apolipoprotein B (apoB)48/apoB100 (Meridian Life Science) in phosphate-buffered saline (PBS)/Tween-20 for 1 h, followed by two washes in PBS/Tween-20, and then incubated with horseradish-peroxidase-conjugated goat anti-mouse IgG for 30 min. Moreover, α-tubulin served as a control for sample loading and integrity. The antibody-reactive bands were revealed by the enhanced chemiluminescence kit (Amersham) and were used to expose to Kodak radiographic film. The amount of polypeptide was quantitated by integrated densitometric analysis of the film (Kodak Gel Logic-100 Imaging System).

Statistical Evaluation. Results are given as the mean ± standard deviation (SD). Statistical differences among groups were calculated by analysis of variance (ANOVA) (SAS Institute, Cary, NC), and group means were considered to be significantly different at $p < 0.05$ as determined by Duncan's multiple range test.

RESULTS

Effects of Chitosan on Postprandial Lipid Responses in HS-Diet-Fed Rats. The body weights of HS-diet-fed rats were significantly higher than control rats (Figure 1). Supplementation of 5 and 7% chitosan could significantly decrease the increased body weights in HS-diet-fed rats (Figure 1). The rats fed a HS diet for 9 weeks significantly increased the postprandial plasma TC, TG, and TG-rich lipoprotein TG levels (Figures 2 and 3). Rats fed a HS diet supplemented with chitosan (3, 5, and 7%) had lower plasma cholesterol levels at 0, 3, and 6 h after feeding with postprandial experimental diets (Figure 2). However, supplementation of chitosan in the diet

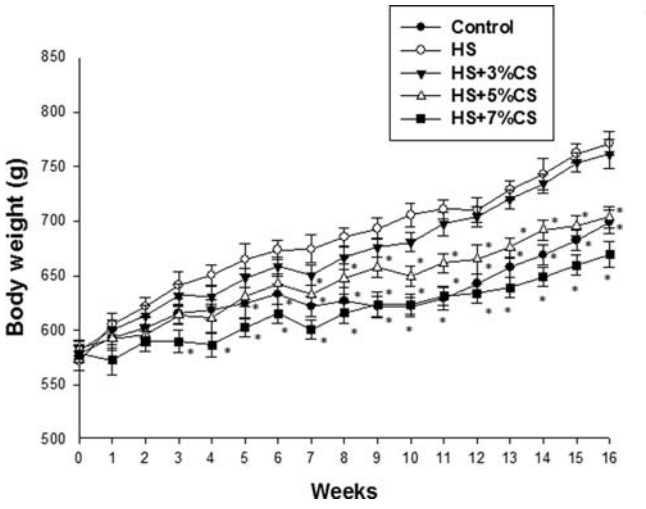

Figure 1. Changes of body weights in rats fed with various experimental diets during the treatment period. Rats were fed with HS diets with or without chitosan (3, 5, and 7%) supplementation. Control rats were fed with normal rodent diets. Results are expressed as the mean ± SD for each group (n = 8−10). (*) $p < 0.05$ compared to the HS-diet alone group.

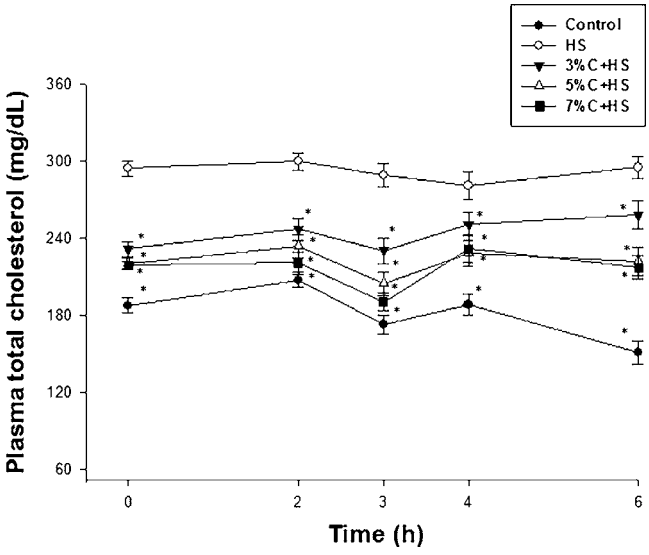

Figure 2. Effect of chitosan on postprandial plasma TC levels in rats fed with a HS diet for 9 weeks. Results are expressed as the mean ± SD for each group (n = 8−10). (*) $p < 0.05$ compared to the HS-diet alone group.

did not reverse the increased plasma TG (Figure 3A) and TG-rich lipoprotein TG (Figure 3B) in HS-diet-fed rats. Unexpectedly, the plasma TG and TG-rich lipoprotein TG levels in the HS + 7% chitosan group were higher than the HS-diet alone group during the postprandial experiment (Figure 3). Moreover, supplementation of chitosan significantly decreased the postprandial ratio of apoB48/apoB100 in TG-rich lipoprotein fractions of HS-diet-fed rats (Figure 4).

Effects of Chitosan on Lipid Metabolism in HS-Diet-Fed Rats. Rats were fed with a HS diet for 16 weeks in the presence or absence of chitosan (3, 5, and 7%). The food intake, drinking water intake, and urine volume in rats fed with a HS diet with or without supplementation of chitosan for 16 weeks were not altered (Table 2; $p > 0.05$). However, as shown in Table 3, the levels of fasting plasma glucose and homeostasis model assessment equation of insulin resistance (HOMA-IR) in control-diet-fed rats were significantly lower than the HS-diet-fed rats. Supplementation of 7% chitosan in the diet significantly reversed the increased plasma glucose and HOMA-IR levels in HS-diet-fed rats. The plasma insulin levels in HS-diet-fed rats supplemented with 5 and 7% chitosan were significantly lower than the HS-diet-fed rats without chitosan. Moreover, HS-diet-fed rats supplemented with 5 and 7% chitosan had lower plasma levels of TC, LDL-C + VLDL-C, TC/HDL-C ratio (Table 4), leptin, and TNF-α (Table 5) compared to the HS-diet alone group. The increased plasma TG levels in HS-diet-fed rats were enhanced by the supplementation of 7% chitosan in the diet (Table 4). Supplementation of 7% chitosan in the diet could also increase the plasma free fatty acid levels in HS-diet-fed rats (Table 5).

On the other hand, it was noticed that the VLDLR protein expression in skeletal muscles of HS-diet-fed rats was significantly decreased, which could be significantly reversed by supplementation of 5 and 7% chitosan (Figure 5). Moreover, the LPL activity and TG level in epididymal fats of HS-diet-fed rats were increased, although the lipolysis rate was unchanged (Figure 6). Supplementation of 7% chitosan in the diet significantly elevated the lipolysis rate (Figure 6A) and decreased the accumulation of TG (Figure 6C) but did not affect the LPL activity (Figure 6B) in epididymal fats of HS-diet-fed rats. Nevertheless, the plasma ANGPTL4 protein expression was not affected in HS-diet-fed rats but was significantly increased in 7% chitosan-supplemented HS-diet-fed rats (Figure 7).

DISCUSSION

A previous study has found that long-term feeding with a HS diet triggers hypertriglyceridemia and hyperglycemia, in which blood insulin concentrations remain unchanged and unable to compensate for the increased demands of the developing metabolic changes.[2] Del Zotto et al. have further shown that the number of pancreatic islets/unit area and mass of β-cell are elevated in HS-diet-fed rats.[19] In the present study, we found that long-term HS-diet feeding produces the impairment of glucose and lipid metabolism in rats, including the induction of hyperglycemia, insulin resistance, and hyperlipidemia. Therefore, this HS-diet-fed animal model shows similar symptoms of type 2 diabetes. The present work further showed that supplementation of chitosan in the diet can improve the increased body weight, hyperglycemia, hypercholesterolemia, and increased HOMA-IR induced by HS-diet feeding in rats. These results indicate that chitosan possesses the ability to improve the impairment of glucose and lipid metabolism in a HS-diet-fed rat model.

A study has reported that type 2 diabetic patients have abnormal postprandial lipoprotein and lipid metabolism;[20] it found that VLDL secretion is higher, and the capacity of LPL to minimize postprandial hyperlipidemia may be reduced in type 2 diabetic patients. The postprandial lipoprotein abnormalities have also been inferred to confer an increased risk of developing cardiovascular disease in diabetic patients.[21] In the present study, the rats fed a HS diet significantly increased the postprandial plasma TC, TG, and TG-rich lipoprotein TG levels, indicating that HS-diet feeding could affect the postprandial lipoprotein and lipid metabolism. Supplementation of chitosan in the diet significantly reversed the increased plasma cholesterol level but did not reverse the increased plasma TG and TG-rich lipoprotein TG levels in HS-diet-fed

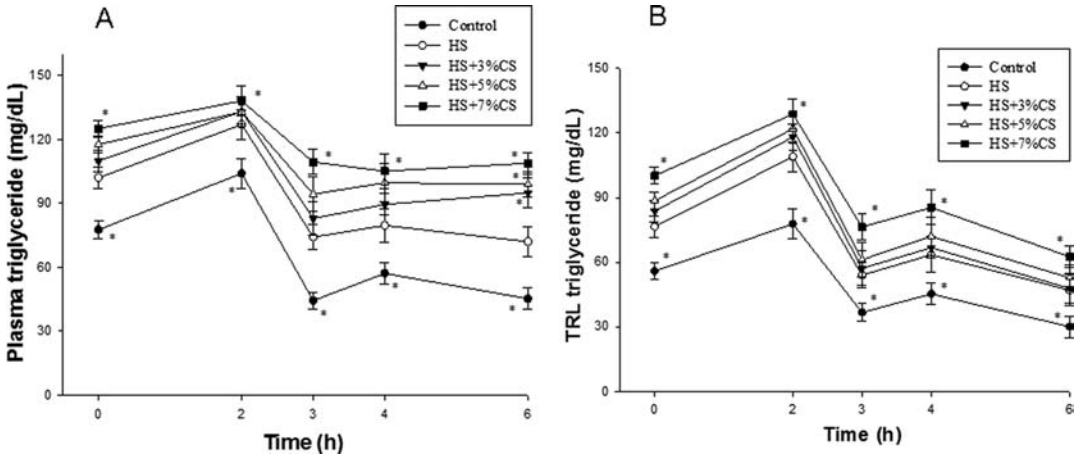

Figure 3. Effects of chitosan on postprandial plasma TG and TG-rich lipoprotein fraction TG levels in rats fed with a HS diet for 9 weeks. Results are expressed as the mean ± SD for each group (n = 8−10). (∗) $p < 0.05$ compared to the HS-diet alone group.

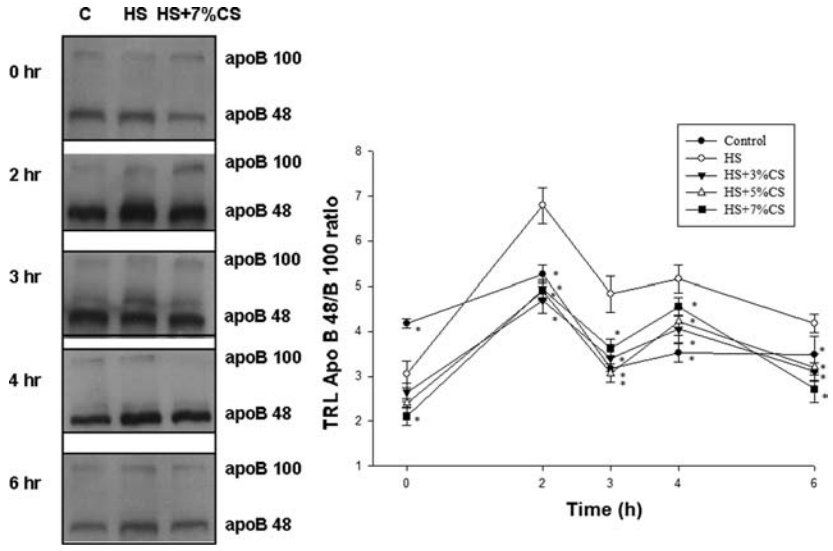

Figure 4. Effect of chitosan on postprandial TG-rich lipoprotein fraction apoB48/apoB100 protein ratio in rats fed with a HS diet for 9 weeks. The protein expressions of apoB48 and apoB100 were determined by western blotting. Results are expressed as the mean ± SD for each group (n = 8−10). (∗) $p < 0.05$ compared to the HS-diet alone group.

Table 2. Changes of Food Intake, Drinking Water Intake, and Urine Volume in Rats Fed with Different Experimental Diets for 16 Weeks[a]

	control	HS	3HS[b]	5HS[c]	7HS[d]
food intake (g/day)	26.3 + 5.3	24.3 ± 32	25.7 ± 2.6	22.7 ± 3.5	20.7 ± 4.5
drinking volume (mL/day)	21.5 ± 3.8	25.4 ± 8.3	24.4 ± 5.2	24.5 ± 6.3	23.6 ± 5.8
urine volume (mL/day)	10.7 ± 2.3	12.2 ± 3.8	10.9 ± 3.9	10.2 ± 3.8	9.4 ± 1.7

[a]Results are expressed as the mean ± SD for each group of rats (n = 8−10). [b]3HS = high sucrose + 3% chitosan diet. [c]5HS = high sucrose + 5% chitosan diet. [d]7HS = high sucrose + 7% chitosan diet.

Table 3. Changes of Plasma Glucose, Insulin Concentrations, and HOMA-IR Index in Rats Fed with Different Experimental Diets for 16 Weeks[a]

	control	HS	3HS[b]	5HS[c]	7HS[d]
glucose (mg/dL)	172.1 ± 19.5[e]	206.7 ± 18.3	203.9 ± 11.4	200.6 ± 9.1	180.6 ± 15.1[e]
insulin (μg/L)	1.11 ± 0.50	1.24 ± 0.47	1.14 ± 0.56	0.78 ± 0.39[f]	0.70 ± 0.21[f]
HOMA-IR[g]	7.54 ± 1.97[f]	12.0 ± 4.68	11.5 ± 6.05	6.73 ± 1.26[f]	6.50 ± 2.13[e]

[a]Results are expressed as the mean ± SD for each group of rats (n = 8−10). [b]3HS = high sucrose + 3% chitosan diet. [c]5HS = high sucrose + 5% chitosan diet. [d]7HS = high sucrose + 7% chitosan diet. [e]$p < 0.01$ compared to the HS group. [f]$p < 0.05$ compared to the HS group. [g]HOMA-IR = fasting plasma glucose concentration (mmol/L) × fasting plasma insulin (mU/L)/22.5.

Table 4. Changes of Plasma Lipid Concentrations in Rats Fed with Different Experimental Diets for 16 Weeks[a]

	control	HS	3HS[b]	5HS[c]	7HS[d]
TC (mg/dL)	136.2 ± 35.6[e]	215.7 ± 49.8	165.8 ± 73.4	144.1 ± 29.9[e]	143.5 ± 58.1[f]
HDL-C (mg/dL)	44.6 ± 14.8	39.2 ± 18.1	46.8 ± 17.3	46.1 ± 13.9	55.3 ± 25.9
LDL-C + VLDL-C (mg/dL)	84.8 ± 31.5[e]	176.6 ± 58.6	119.0 ± 60.8	98.0 ± 24.1[e]	88.2 ± 41.5[e]
TC/HDL-C ratio	2.60 ± 0.75[e]	5.11 ± 1.59	3.40 ± 0.67[f]	3.25 ± 0.66[e]	3.17 ± 1.05[e]
HDL-C/(LDL-C + VLDL-C) ratio	0.56 ± 0.30[f]	0.25 ± 0.12	0.44 ± 0.23	0.49 ± 0.85[e]	0.50 ± 0.20[e]
triglyceride (mg/dL)	48.7 ± 12.8[f]	61.8 ± 9.41	63.5 ± 14.3	68.9 ± 15.4	83.7 ± 21.8[f]

[a]Results are expressed as the mean ± SD for each group of rats (n = 8−10). [b]3HS = high sucrose + 3% chitosan diet. [c]5HS = high sucrose + 5% chitosan diet. [d]7HS = high sucrose + 7% chitosan diet. [e]$p < 0.01$ compared to the HS group. [f]$p < 0.05$ compared to the HS group.

Table 5. Changes of Plasma Free Fatty Acid, Leptin, Adiponectin, and TNF-α Concentrations in Rats Fed with Different Experimental Diets for 16 Weeks[a]

	control	HS	3HS[b]	5HS[c]	7HS[d]
free fatty acid (mequiv/L)	0.68 ± 0.09	0.68 ± 0.10	0.63 ± 0.23	0.65 ± 0.17	0.83 ± 0.14[e]
leptin (ng/mL)	8.53 ± 2.84[e]	13.11 ± 4.39	13.05 ± 3.24	8.72 ± 2.92[e]	6.65 ± 1.50[f]
adiponectin (μg/mL)	15.49 ± 3.93[e]	11.54 ± 2.51	14.89 ± 4.91	14.25 ± 3.48	13.30 ± 3.92
TNF-α (pg/mL)	27.9 ± 16.1	32.2 ± 11.7	21.5 ± 11.7	14.2 ± 4.49[f]	13.3 ± 7.31[f]

[a]Results are expressed as the mean ± SD for each group of rats (n = 8−10). [b]3HS = high sucrose + 3% chitosan diet. [c]5HS = high sucrose + 5% chitosan diet. [d]7HS = high sucrose + 7% chitosan diet. [e]$p < 0.05$ compared to the HS group. [f]$p < 0.01$ compared to the HS group.

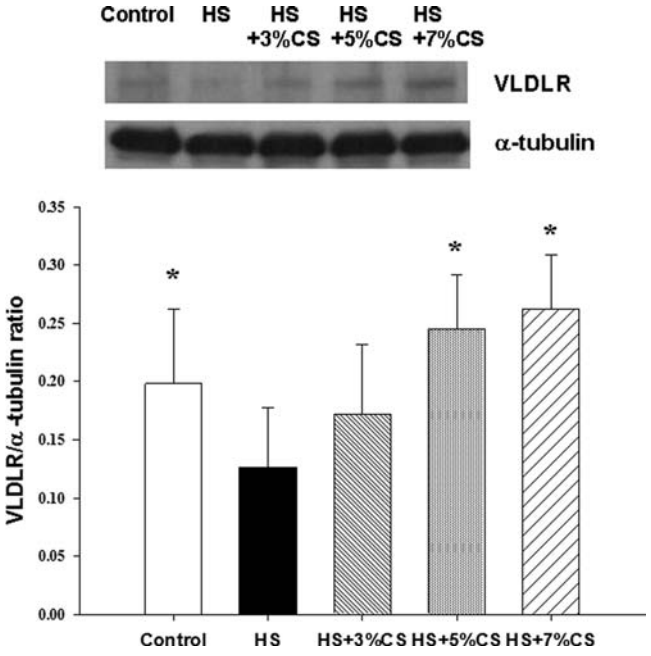

Figure 5. Effect of chitosan on the protein expression of muscle VLDLR in rats fed with a HS diet for 16 weeks. The protein expression of VLDLR was determined by western blotting. Results are expressed as the mean ± SD for each group (n = 8−10). (*) $p < 0.05$ compared to the HS-diet alone group.

rats. Moreover, apoB48 mainly presents intestinal chylomicrons and VLDL lipoproteins in rats,[22] and apoB100 is generated in the liver.[23] The determination of plasma apoB48 and apoB100 expressions may reflect the source of postprandial plasma TG-rich lipoprotein particles.[24] The results of the present work showed that the fasting plasma TG-rich lipoprotein apoB48/apo B100 ratio is lower than the postprandial ratio in HS-diet-fed rats, indicating that the liver source of TG-rich lipoprotein particles in fasting HS-diet-fed rats is higher than the postprandial situation. Supplementation of chitosan in the diet markedly decreased the increased postprandial TG-rich lipoprotein apoB48/apoB100 ratios in HS-diet-fed rats, indicating that chitosan can regulate the source of postprandial plasma TG-rich lipoprotein particles during HS-diet feeding.

Supplementation of chitosan in the diet has been found to lower plasma TC, VLDL-C, and LDL-C levels in high-cholesterol-diet-fed hamsters by increasing the fecal excretion of cholesterol and bile acid.[11] A study has also shown that chitosan significantly reverses the increased plasma TC and LDL-C levels in cholesterol-enriched-diet-fed rats by upregulating the liver LDL receptor expression and increasing the excretion of fecal bile acids.[25] Yao et al. have also found that supplementation of chitosan in the diet for 7 weeks reduces the plasma glucose, TC, and free fatty acid levels in streptozotocin-induced diabetic rats.[26] In these studies, the plasma TG levels were not affected by chitosan. Similarly, the present study showed that supplementation of 5 and 7% chitosan significantly decreased the increased plasma levels of TC, LDL-C + VLDL-C, and TC/HDL-C ratio in HS-diet-fed rats. We also found that the expression of VLDLR proteins in skeletal muscles of HS-diet-fed rats was significantly decreased, which could be significantly reversed by supplementation of 5 and 7% chitosan. VLDLR, a member of the LDL receptor family, has been shown to bind and internalize TG-rich lipoproteins, such as VLDL and chylomicron.[27] TG-rich lipoproteins and TG-derived free fatty acids are transported into peripheral tissues via this VLDLR pathway. Therefore, the inhibition of VLDLR protein expression in skeletal muscles by a HS diet reflects that the transport of TG-rich lipoproteins or free fatty acids into skeletal muscles is decreased and may cause hyperlipidemia; in contrast, supplementation of 5 and 7% chitosan in the diet significantly reversed the inhibition of VLDLR protein expression by HS-diet feeding, indicating that chitosan can enhance the removal of plasma lipids through the VLDLR pathway.

Adipose tissue LPL is known as an important enzyme responsible for the hydrolysis of circulating TG in chylomicron and VLDL.[28,29] LPL activator NO-1886 has been found to suppress fat accumulation and insulin resistance in high-fat-diet-fed rats.[29] ANGPTL4 is characterized as a secretory protein, and its expression is under control of the peroxisome proliferator-activated receptor (PPAR) family and fatty acids.[30] Several studies have shown that ANGPTL4 can trigger

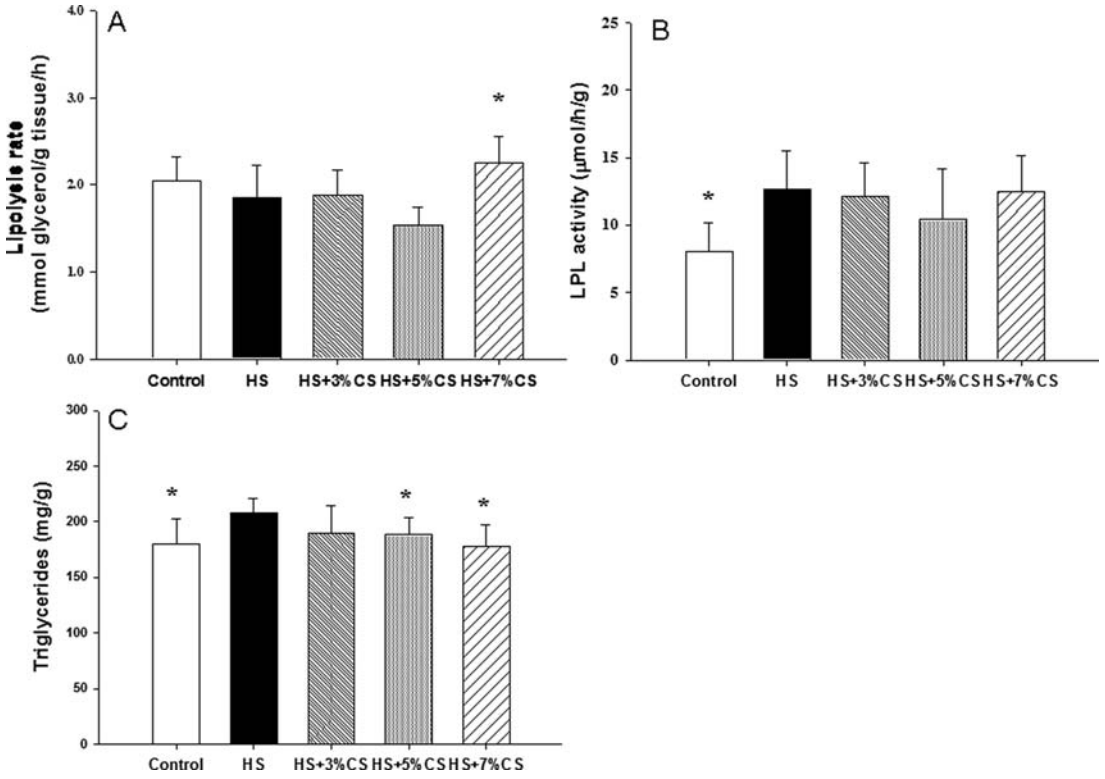

Figure 6. Effects of chitosan on the LPL activity, lipolysis rate, and TG levels in epididymal fat pads in rats fed with a HS diet for 16 weeks. Results are expressed as the mean ± SD for each group (n = 8−10). (*) $p < 0.05$ compared to the HS-diet alone group.

lipolysis in adipose tissue.[31−34] Yoshida et al. have found that recombinant ANGPTL4 protein injection rapidly enhances the circulating plasma lipids in obese mice with diabetes (KK/San mice).[32] ANGPTL4 has also been shown to be a lipoprotein lipase and hepatic lipase inhibitor, can decrease the dietary fatty acid uptake in the liver, and further impairs plasma TG clearance.[35,36] PPARα was known to upregulate hepatic ANGPTL4 expressions and plasma ANGPTL4 concentrations that act as a LPL activity inhibitor.[37] ANGPTL4-related LPL activity inhibition caused the inhibition of LPL-dependent VLDL lipolysis, leading to hypertriglyceridemia.[34] PPARα has also been shown to stimulate the hepatic expression of the VLDLR.[34] Recently, our previous study has demonstrated that chitosan feeding can increase the hepatic PPARα expression in diabetic rats.[38] In the present study, we found that HS-diet feeding increases the LPL activity and TG level in epididymal fats, but the lipolysis rate is unchanged. Supplementation of 7% chitosan in the diet significantly elevated the lipolysis rate and decreased the accumulation of TG in epididymal fats of HS-diet-fed rats. However, supplementation of 7% chitosan in the diet significantly enhanced the plasma free fatty acid and TG levels in HS-diet-fed rats. Supplementation of 7% chitosan in the diet could also significantly increase the plasma ANGPTL4 protein expression in HS-diet-fed rats, but HS-diet feeding alone did not affect the ANGPTL4 protein expression. Taken together, these results suggest that 7% chitosan supplementation increases ANGPTL4 expression, which may be upregulated by PPARα, and then enhances the lipolysis rate in epididymal fat tissues, resulting in the increase of plasma free fatty acid levels. The increased ANGPTL4 expression could also change the plasma LPL activity, which induces the decrease in hydrolysis of TG-rich lipoproteins, causing the increase of plasma TG levels.

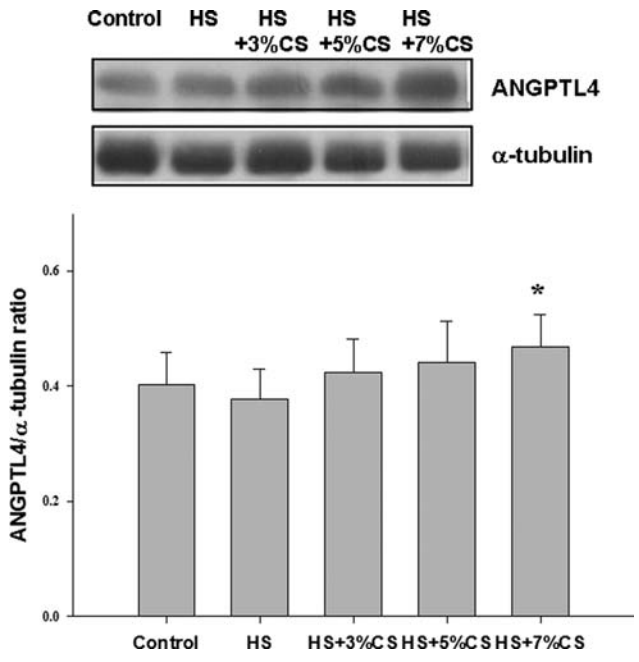

Figure 7. Effect of chitosan on the protein expression of plasma ANGPTL4 in rats fed with a HS diet for 16 weeks. The protein expression of ANGPTL4 was determined by western blotting. Results are expressed as the mean ± SD for each group (n = 8−10). (*) $p < 0.05$ compared to the HS-diet alone group.

Cani and colleagues have demonstrated that metabolic endotoxemia triggers body weight gain and diabetes in a mouse model, and lowering plasma lipopolysaccharide (LPS, endotoxin) levels may be a valuable strategy for the control of

metabolic diseases.[39] Recently, Delzenne and colleagues have also mentioned that gut microbes can interact with the tissues of the host and trigger metabolic disorders via several mechanisms.[40] Gut microbes have been found to enhance the adipose LPL-controlled fatty acid storage by blunting the ANGPTL4 expression in the intestine.[40] Chitosan was known to exert antibacterial activity *in vitro*.[41] It has also been shown that endotoxins can bind completely with chitosan with the formation of LPS−chitosan and LPS−protein−chitosan complexes and a decrease in acute toxicity of LPS is observed on their binding with chitosan.[42] Therefore, chitosan may improve the impaired glucose and lipid metabolism in obesity or diabetes via its antibacterial or endotoxin removal activities.

Chitosan has been found to disrupt intercellular tight junctions in a drug absorption model of the Caco-2 cell monolayer, indicating that chitosan may increase the permeability of an epithelium.[43] An *in vivo* study for absorption of chitosans (low to high MW) has also shown that chitosan can be absorbed from the gastrointestinal tract into blood circulation in mice after oral administration, and these absorbed chitosan molecules could further distribute to organs, such as the liver, kidney, spleen, thymus, heart, and lung.[44] On the other hand, high-MW chitosan could imbed fat in the intestine, causing that fat to not be absorbed and increase fecal fat excretion.[45] These findings infer that chitosan regulates lipid metabolism through direct and indirect pathways. Our present work showed that supplementation of chitosan in the diet can improve the impairment of glucose and lipid metabolism in a HS-diet-fed rat model. Chitosan markedly decreased the increased postprandial TG-rich lipoprotein apoB48/apoB100 ratios in HS-diet-fed rats, indicating that chitosan can regulate the source of postprandial plasma TG-rich lipoprotein particles during HS-diet feeding. Chitosan could also enhance the removal of plasma lipids through a skeletal muscle VLDLR pathway. However, high-dose chitosan (7%) significantly increased the plasma ANGPTL4 protein expression in HS-diet-fed rats, which enhances the lipolysis rate in epididymal fat, resulting in the increase of plasma free fatty acid levels; it could also change the plasma LPL activity and induce the decrease of TG-rich lipoproteins hydrolysis, causing an increase in plasma TG levels. These findings suggest that long-term feeding of chitosan can regulate the postprandial lipid responses and lipid-related metabolic changes in rats with HS-diet-impaired glucose tolerance.

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Notes

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