JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Effect of Chitosan, *O*-Carboxymethyl Chitosan, and *N*-[(2-Hydroxy-3-*N*,*N*-dimethylhexadecyl ammonium)propyl] Chitosan Chloride on Overweight and Insulin Resistance in a Murine Diet-Induced Obesity

Xiaofei Liu,*[,†,‡] Xiaona Zhi,[†,‡] Yunfei Liu,[†,‡] Bo Wu,[†,‡] Zhong Sun,[§] and Jun Shen[∥]

[†]Department of Polymer Materials Science and Engineering, College of Materials Science and Engineering, and [‡]Tianjin Key Laboratory of Composite and Functional Materials, Tianjin University, Tianjin, 300072, People's Republic of China

[§]Department of Health Statistics and [∥]Department of Sanitary Chemistry, College of Public Health, Tianjin Medical University, Tianjin, 300070, People's Republic of China

ABSTRACT: Two water-soluble chitosan derivatives, *O*-carboxymethyl chitosan (O-CM-chitosan) and *N*-[(2-hydroxy-3-*N*,*N*-dimethylhexadecyl ammonium)propyl] chitosan chloride (N-CQ-chitosan), were prepared, and the therapeutic effects of chitosan, O-CM-chitosan, and N-CQ-chitosan on insulin resistance were simultaneously evaluated by rats fed on a high-fat diet. The parameters of high-fat diet-induced rats indicated that chitosan and its two derivatives not only have low cytotoxicity but can control overnutrition by fat and achieve insulin resistance therapy. However, the results in experiment in vivo showed that the therapeutic degree varied by the molecular weight and surface charge of chitosan, O-CM-chitosan, and N-CQ-chitosan. N-CQ-chitosan with a MW of 5×10^4 decreased body weight, the ratio of fat to body weight, triglyceride, fasting plasma glucose, fasting plasma insulin, free fatty acid, and leptin by 11, 17, 44, 46, 44, 87, and 64% and increased fecal lipid by 95%, respectively.

KEYWORDS: *chitosan, derivatives, high-fat rats, body weight, insulin resistance*

INTRODUCTION

Overweight and obesity have attracted strong attention,[1] which are important contributors to cardiovascular disease,[2] type II diabetes mellitus,[3] and several common cancers.[4] Type II diabetes mellitus is a result of the development of insulin resistance (IR), which is linked to both genetic and environment factors. Obesity, the most important factor, is usually a combination of polygenetic and environmental origins.[5] Therefore, restricted calorie intake, weight reduction, and physical activity can improve insulin sensitivity.[6]

There are many reports concerned with a role for the plasma free fatty acid (FFA) elevation in the development of insulin resistance, which mainly occurred in skeletal muscles and liver.[7−10] Jiao et al.[11] have reported that high-fat diet-induced insulin resistance in Sprague−Dawley rat is closely associated with the plasma FFA elevation as well as heterotopic deposition of triglyceride (TG) in liver and skeletal muscle.

Leptin (16 kDa) is the ob gene product and is produced by adipose tissue. Leptin controls body composition mostly via hypothalamic receptors that regulate food intake and body weight.[12] However, research shows that the leptin level in organisms is positively related with body fat content[13] and insulin levels;[14] that is, leptin synthesis and secretion are markedly increased in obesity in both humans and rodents,[15] and there has been a high incidence of insulin resistance in obesity.

Chitosan is the deacetylated form of the polysaccharide chitin (a byproduct of crustaceans), and it appears to be blind to negatively charged lipids in animal trials, thus reducing the animals' gastrointestinal uptake[16] and lowering their serum cholesterol.[17] However, because of its poor water solubility, the applications of chitosan are limited in medicine and the food industry. Chemical modifications such as carboxylation and quanternization have been adopted to overcome the limited solubility, because of the existence of living amidos and hydroxys.[18] Therefore, we can get negatively charged carboxymethylated chitosan and positively charged quaternized chitosan via carboxylation and quaternization.

In recent years, some reported studies have almost investigated the effect of chitosan on body weight and plasma lipid level,[16,19,20] but its effect on insulin resistance is hardly known.[21] In accordance with obesity is associated with insulin resistance, we hypothesized that chitosan improves insulin resistance. In our study, two water-soluble chitosan derivatives, *O*-carboxymethyl chitosan (O-CM-chitosan), with a negative surface charge, and *N*-[(2-hydroxy-3-*N*,*N*-dimethylhexadecyl ammonium)propyl] chitosan chloride (N-CQ-chitosan), with a positive surface charge, were prepared, and the therapeutic effects of chitosan, O-CM-chitosan, and N-CQ-chitosan in insulin resistance were simultaneously evaluated.

CHEMICALS

Preparation of O-CM-Chitosan and N-CQ-Chitosan. O-CM-chitosan and N-CQ-chitosan were prepared following our previous report.[27] The synthetic process of O-CM-chitosan and N-CQ-chitosan is shown in Figure 1.

Characterization of Chitosan, O-CM-Chitosan, and N-CQ-Chitosan. After it was dried completely at 50 °C, the samples could be used for Fourier transform infrared (FT-IR)

Received: September 22, 2011
Revised: February 28, 2012
Accepted: March 8, 2012
Published: March 8, 2012

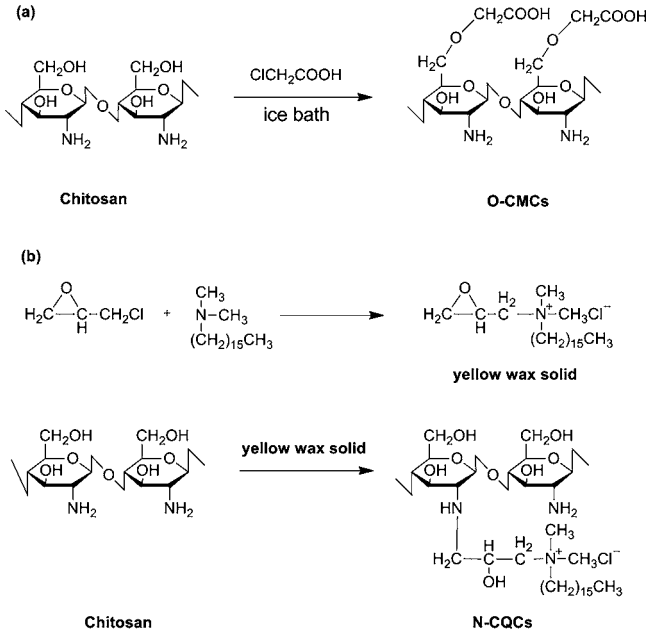

Figure 1. Chemical structures of chitosan, O-CMCs, and N-CQCs.

analysis with the standard KBr pellet method. Figure 2 is the FT-IR spectra of chitosan, O-CM-chitosan, and N-CQ-chitosan. Figure 2a shows the basic characteristics of chitosan at 3455 (O−H stretch), 2867 (C−H stretch), 1654 (N−H bend), 1154 (bridge-O stretch), and 1094 cm^{-1} (C-Ostretch). The FT-IR spectrum of O-CMCs (Figure 2b) showed characteristic absorptions due to the −COOH group at 1737 cm^{-1}, confirming a successful carboxymethylation. The absorption at 1519 cm^{-1} was assigned to $-NH_3^+$. In Figure 2c, the peaks at 2920 and 2870 cm^{-1} were broadened in N-CQ-chitosan, as compared with that of chitosan, which were attributed to the methyl groups and long carbon chain of the quaternary ammonium salt. Characteristic peaks of the hydroxyl and second hydroxyl groups of chitosan between 1160 and 1080 cm^{-1} were unchanged in N-CQ-chitosan, which indicated that no groups were introduced to the C-3 and C-6 during experiment. The N−H bending (1590 cm^{-1}) of the primary amine disappeared due to the change of the primary amine in N-CQ-chitosan. The peak at 1500 cm^{-1} was assigned to NH_3^+. Therefore, it can be concluded that the substitution mainly occurred at the amino groups of chitosan. All of these results proved that the synthesis of carboxymethylated and quaternized chitosan was successful.[27]

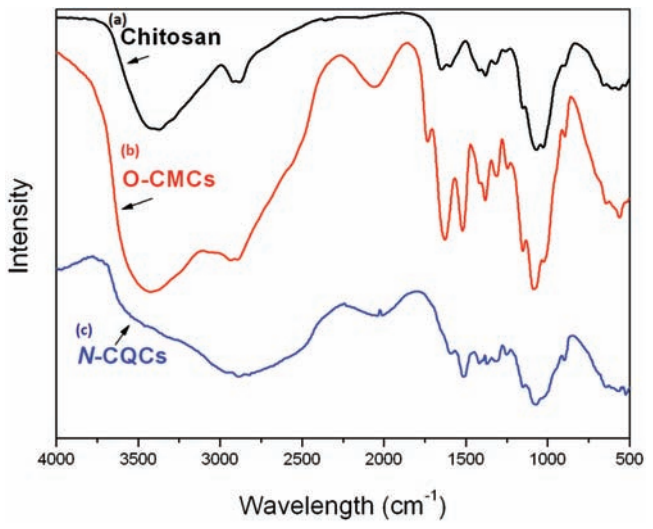

Figure 2. FT-IR spectra of (a) chitosan, (b) O-CMCs, and (c) N-CQCs.

MATERIALS AND METHODS

Materials. Chitosan [weight-average molecular weight (MW) of 5 × 10^4 and 15 × 10^4, with a degree of deacetylation of 0.85], a commercial material, was supplied by Qingdao Medicine Institute, Shandong, China. O-CM-chitosan was prepared in our previous report,[22] and N-CQ-chitosan was prepared from the above raw chitosan with epoxy chloropropane and *N*,*N*-dimethylhexadecyl amine.[23] The degrees of substitution of O-CM-chitosan and N-CQ-chitosan were 0.72 and 0.41.[24,25] All other reagents were analytical grade provided by No. 3 Chemical Reagent Factory of Tianjin, China.

Solubility of Chitosan, O-CM-Chitosan, and N-CQ-Chitosan. The solubility of chitosan, O-CM-chitosan, and N-CQ-chitosan was determined in H_2O, acetic acid (HAc), methanol, ethanol, $CHCl_3$, ether, DMSO, formamide, and DMF.

Cytotoxicity to Hepatocytes with Chitosan and Its Derivatives Analysis. Chitosan and its derivatives were investigated for their possible cytotoxic effects, and the cytotoxicity for hepatocytes, which were obtained from male Wistar Rats (Experimental Animal Center of Tianjin Medical University, Tianjin, China) by collagenase perfusion, was measured by the MTT assay. Cells were seeded in 96-well plates at an initial density of 1 × 10^4 cells/well in 100 μL of growth medium and incubated overnight. The media were replaced by fresh, serum-free media containing chitosan, O-CM-chitosan, and N-CQ-chitosan at various MW. Chitosan, O-CM-chitosan, and N-CQ-chitosan were dissolved in HBBS/HEPES at a concentration of 100 μg/mL. After an additional incubation for another 24 h, 15 μL of MTT (5 mg/mL) solution was added into each well and incubated for 4 h and further dissolved in 150 μL of dimethylsulfoxide (DMSO). All chitosan, O-CM-chitosan, and N-CQ-chitosan were UV sterilized. The absorbance at 490 nm was measured by an enzyme-linked immunosorbent assay (ELISA) plate reader (Bio-Rad, Microplate Reader). The percentage cell viability was calculated according to the following equation: percentage cell viability = $OD_{490(sample)}/OD_{490(control)}$ × 100, where $OD_{490(sample)}$ represents a measurement from a well treated with chitosan, O-CM-chitosan, and N-CQ-chitosan and $OD_{490(control)}$ represents a well treated without any sample.

Animal Experimental Design. Clean male Wistar Rats (n = 120), with a mean mass of 65 ± 15 g, were provided by the Experimental Animal Center of Tianjin Medical University, Tianjin, China. All rats were kept in cages with stainless steel bottoms in a room controlled at 23 ± 1 °C and 55 ± 5% humidity under a 12 h light−dark cycle with lighting from 8:00 a.m. to 8:00 p.m. Rats were allowed to have free access to food and water. All animal protocols were approved by the Institutional Animal Care and Use Committee of Tianjin Medical University.

After acclimation for 7 days, rats were randomly divided into eight groups with 15 rats in each group: group C (normal fat control group), group F (high-fat control group), group TA (chitosan, MW of 5 × 10^4 and 15 × 10^4), group TB (O-CM-chitosan, synthesized from chitosan with MW of 5 × 10^4 and 15 × 10^4), and group TC (N-CQ-chitosan, synthesized from chitosan with MW of 5 × 10^4 and 15 × 10^4). The final concentration of each sample group was 100 mg/L in the mass of diet. Rats of group C were fed on a commercial diet (provided by Tianjin Laboratory Animal Co. Ltd., Tianjin, China). Rats of group F received a high-fat diet containing 10% (w/w) lard, 12% (w/w) reconstituted skim milk, 10% (w/w) yolk powder, and 7% (w/w) casein in commercial diets. The sample groups, including groups TA, TB, and TC, all were fed the same diet as group F but with chitosan, O-CM-chitosan, and N-CQ-chitosan added, respectively.

During the first 6 weeks of the experimental period, all of the rats were fed a high-fat diet to establish the high-fat diet-induced model

Table 1. Solubility of Chitosan, O-CM-Chitosan, and N-CQ-Chitosan in Water and Organic Solvents[a]

	solvent								
sample	H_2O	HAc	methanol	ethanol	$CHCl_3$	ether	DMSO	formamide	DMF
chitosan	−	++	±	±	−	−	−	−	−
O-CM-chitosan	±	++	±	±	−	−	±	−	±
N-CQ-chitosan	±	++	±	±	−	−	±	−	±

[a]++, highly soluble; ±, partially soluble or swollen; and −, insoluble.

except the rats of group C with a commercial diet. Then, in the following 6 weeks, all sample groups were given corresponding diets. During the 12 week experimental period, body weight and food intake were recorded weekly. At the end of the experimental period of continuous feeding in 12 weeks, rats were deprived of food overnight, and their blood was collected from the femoral artery puncture under ether anesthesia. The serum samples were stored in a −20 °C freezer for further analysis.

Ratio of Fat to Body Weight Analysis. The ratio of fat to body weight was calculated according to the formula: the ratio of fat to body weight = (epididymal fat pad weight + perinephrit fat weight)/body weight (g) × 100.

Fecal Lipid Analysis. During the experimental period, the feces excreted were collected every day. Fecal lipids were determined gravimetrically by a modification of the Saxon method.[20]

Assay of Blood Samples. The TG concentration was determined using a enzyme colorimetric assay kit (Zhongsheng Beikong Biotech Co., Ltd., Beijing, China). A glucose oxidase method was employed to measure fasting plasma glucose (FPG), and a rat insulin ELISA kit (Jiancheng Biological Engineering Research Institute, Nanjing, China) was used to measure fasting plasma insulin (FPI). Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as an indicator of insulin resistance according to the formula: HOMA-IR = FPG (mM) × FPI (mIu/L)/22.5.[26] The FFA was detected by using FFA kit (Jiancheng Biological Engineering Research Institute). Leptins were determined using a rat leptin ELISA kit (Aditteram Diagnostic Laboratories, Inc., TX).

Statistical Analysis. The data were expressed as means ± standard deviations (SDs). One-way analysis of variance was carried out, and the statistical comparisons among the groups were performed by Fisher's protected LSD test using a statistical package program to evaluate whether or not there was a significant difference at $p < 0.05$.

RESULTS

Solubility of Chitosan, O-CM-Chitosan, and N-CQ-Chitosan. The solubility of chitosan and its derivatives was studied in our previous report.[28] The results demonstrated that O-CM-chitosan and N-CQ-chitosan were well soluble in both water and organic solvents (Table 1). The lipotropy of the long carbon chain and the hydrophilicity of the carboxyl group and quaternary ammonium salt group within the molecule changed O-CM-chitosan and N-CQ-chitosan into an amphiphilic polymer.

Cytotoxicity to Hepatocytes with Chitosan and Its Derivatives Analysis. Viability data of hepatocytes are shown in Figure 3. Chitosan and its derivatives (O-CM-chitosan and N-CQ-chitosan) were confirmed to have a lower cytotoxicity to hepatocytes, which were in agreement with previous reports.[29−31] The cell viabilities in the chitosan and its derivatives were 87.4 (chitosan with MW of 5×10^4), 80.9 (O-CM-chitosan with MW of 5×10^4), and 83.5% (N-CQ-chitosan with MW of 5×10^4), at concentration of 100 μg/mL. The cell viability of the two chitosan derivatives was slightly lower than that of chitosan, indicating that the introduction of carboxymethyl and quaternary ammonium salt groups endowed a slightly high cytotoxicity to hepatocytes. Some reports showed that cationic polymers had cytotoxicity caused by

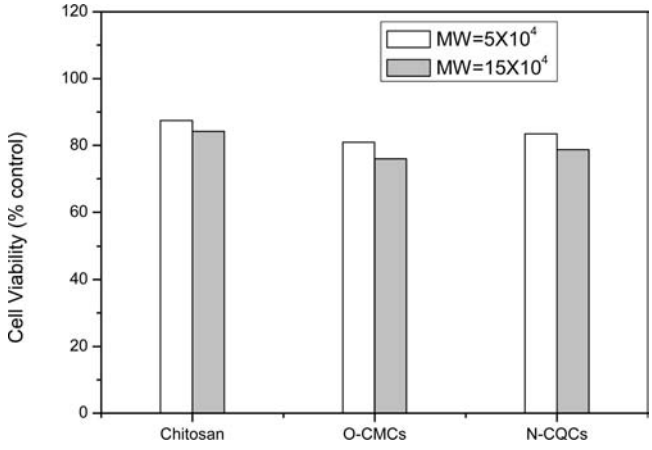

Figure 3. Cell viability of hepatocytes after treatment with chitosan, O-CM-chitosan, and N-CQ-chitosan. Data are presented as means ± SDs, $n = 3$.

polymer aggregation on cell surfaces impairing the important membrane functions.[32]

Effect of Chitosan and Its Derivatives on Overweight. The effects of chitosan and its derivatives on body weight content are shown in Table 2. As compared to group F, chitosan and its derivatives all had a function on decreasing body weight content. By the administration of chitosan and its derivatives for 12 weeks, the content in group TC with MW of 5×10^4 was even lower than that in group C. Chitosan and its derivatives all significantly decreased the body weight. Moreover, chitosan and its derivatives with lower MW had a better lowering body weight effect.

The effect of chitosan and its derivatives on the ratio of fat to body weight was also examined, which is a parameter of evaluating obesity level of the rats. As shown in Table 2, the rat of fat to body weight in each experimental group was all decreased to some degree in comparison with group F. As compared with group F, there are all significant differences in the ratio of fat to body weight among these groups, and the most reduction of the ratio of fat to body weight was found in group TC with MW of 5×10^4.

Then, the content of fecal lipid excretion was examined. The result indicated that chitosan and its derivatives all increased the content of fecal lipid excretion as compared with group F (Table 2). An increase of fecal lipid excretion is a well-known mechanism for the lipid-lowering effect, and there are all significant differences in the content of fecal lipid excretion among these groups. In particular, N-CQ-chitosan with MW of 5×10^4 markedly increased the content of fecal lipid excretion.

Blood Samples Analysis. To investigate the effect of chitosan and its derivatives on insulin resistance, TG, FPG, FPI, FFA, and plasma leptin were measured after the end of the experiment for 12 weeks. With respect to TG (Figure 4), a remarkable reduction of TG content was found in MW of 5 ×

dx.doi.org/10.1021/jf205226r | J. Agric. Food Chem. 2012, 60, 3471−3476

Table 2. Lipid-Lowering Effect of Chitosan and Its Derivatives[a]

group	body weight (g)			ratio of fat to body weight (%)	fecal lipid (%)
	2th week	sixth week	12th week		
group C	169.4 ± 10.9*	319.1 ± 24.3*	419.0 ± 34.6*	2.685 ± 0.18*	2.78 ± 0.56*
group F	181.3 ± 9.4	347.6 ± 15.7	460.0 ± 24.7	4.344 ± 0.33	4.30 ± 0.42
group TA (MW =5 × 10^4)	149.4 ± 27.1*	338.4 ± 11.7*	430.7 ± 27.3*	3.906 ± 0.11*	4.91 ± 0.51*
group TA (MW =15 × 10^4)	144.3 ± 14.4*	348.2 ± 13.8*	442.4 ± 26.9*	4.038 ± 0.23*	4.72 ± 0.64*
group TB (MW =5 × 10^4)	167.8 ± 10.2*	332.3 ± 25.9*	419.4 ± 29.2*	3.868 ± 0.50*	5.74 ± 0.73*
group TB (MW =15 × 10^4)	156.3 ± 14.9*	345.6 ± 11.7*	432.6 ± 30.5*	3.897 ± 0.34*	5.04 ± 0.25*
group TC (MW =5 × 10^4)	167.3 ± 11.9*	333.6 ± 23.6*	409.7 ± 29.3*	3.621 ± 0.28*	8.37 ± 1.58*
group TC (MW =15 × 10^4)	166.2 ± 22.4*	345.5 ± 16.0*	427.8 ± 25.3*	3.858 ± 0.21*	7.02 ± 0.99*

[a]Data are presented as the mean ± SD, n = 15 in each group, *p < 0.05 vs group F.

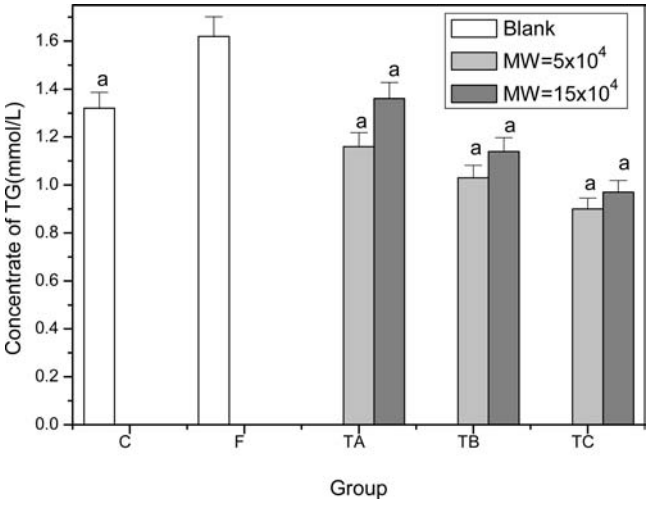

Figure 4. Effect of chitosan and its derivatives on TG after treatment with chitosan, O-CM-chitosan, and N-CQ-chitosan. Data are presented as the mean ± SD, n = 15 in each group, (a) p < 0.05 vs group F.

10^4 (lower MW) groups. Besides, the TG concentration in serum was significantly decreased when compared with group F, and the content of TG in each sample group was almost lower than that in group C except group TA with a MW of 15 × 10^4.

As shown in Table 3, the levels of FPG and FPI in group F were significantly higher than those in groups TA, TB, and TC.

Table 3. Effect of Chitosan and Its Derivatives on IR[a]

group	FPG (mM)	FPI (mIu/L)	HOMA-IR
group C	1.74 ± 0.44*	6.36 ± 0.43*	0.50 ± 0.16*
group F	4.70 ± 0.22	9.82 ± 0.69	2.06 ± 0.24
group TA (MW = 5 × 10^4)	3.57 ± 0.54*	6.31 ± 0.94*	1.02 ± 0.30*
group TA (MW = 15 × 10^4)	3.62 ± 0.26**	6.47 ± 1.02*	1.05 ± 0.24*
group TB (MW = 5 × 10^4)	3.35 ± 0.95**	5.54 ± 0.37*	0.84 ± 0.29*
group TB (MW = 15 × 10^4)	3.48 ± 0.56*	5.78 ± 0.33*	0.91 ± 0.20*
group TC (MW = 5 × 10^4)	2.55 ± 0.37**	5.46 ± 0.27*	0.62 ± 0.12*
group TC (MW = 15 × 10^4)	3.14 ± 0.36**	5.51 ± 0.33*	0.78 ± 0.14*

[a]Data are presented as the mean ± SD, n = 15 in each group, *p < 0.05 vs group F, and **p < 0.01 vs group F.

It can be concluded that chitosan and its two derivatives had down-regulated effects on FPG and FPI, and the order of the improved effects was as follows: N-CQ-chitosan > O-CM-chitosan > chitosan. It is probably due to amphiphilicity and solubility of N-CQ-chitosan and O-CM-chitosan. Meanwhile, HOMA-IR was calculated (Table 3) according to the formula as described previously.[26] It can be seen that HOMA-IR was significantly decreased after treatment with chitosan and its two derivatives for 12 weeks, which achieved the therapeutic result.

Then, the effects on the content of FFA and leptin in the rats' blood serum were investigated, which were effective among all sample groups (TA, TB, and TC) from Figures 5 and

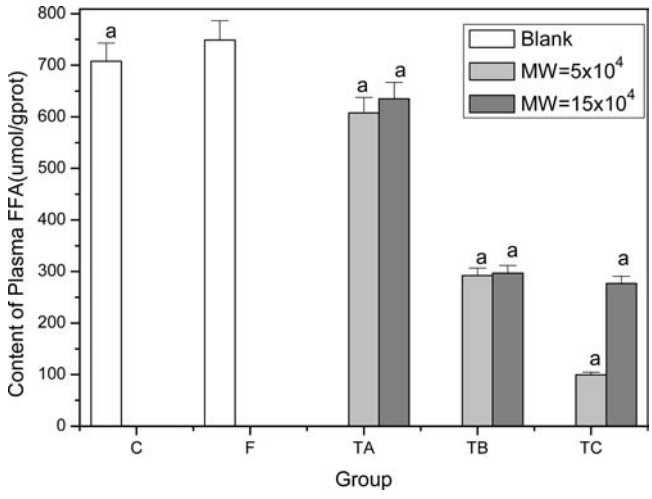

Figure 5. Effect of chitosan and its derivatives on the content of FFA in the rats' blood serum after treatment with chitosan, O-CM-chitosan, and N-CQ-chitosan. Data are presented as the mean ± SD, n = 15 in each group, (a) p < 0.05 vs group F.

6. When groups TA, TB, and TC are compared with group F, there are all significant reductions in the content of FFA in the serum, and the plasma FFA content in each sample group was all lower than that in group C. It is thus clear that chitosan and its derivatives have a strong effect on the content of FFA. Furthermore, each sample group with MW of 5 × 10^4 markedly decreased the content of FFA in the plasma.

On the other hand, chitosan and its derivatives were proved to cause a significant decrease in the content of leptin in the serum except group TA with MW of 15 × 10^4 (Figure 6). Also, N-CQ-chitosan had a better improved effect than O-CM-chitosan and chitosan, and it may be due to the quaternary ammonium cation of N-CQ-chitosan.

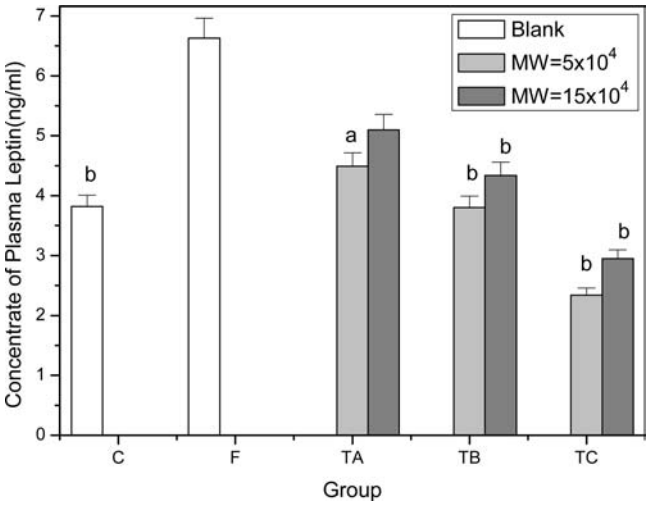

Figure 6. Effect of chitosan and its derivatives on the content of leptin in the rats' blood serum after treatment with chitosan, O-CM-chitosan, and N-CQ-chitosan. Data are presented as the mean ± SD, $n = 15$ in each group, (a) $p < 0.05$ vs group F, and (b) $p < 0.01$ vs group F.

DISCUSSION

The result of the present investigation made clear that chitosan had the potential ability to decrease body weight. Some studies have demonstrated that chitosan could bind to negatively charged lipids, thus reducing their gastrointestinal uptake.[16,20] Nauss et al.[33] have suggested that a soluble form of chitosan would be able to interfere with intraluminal lipid absorption through the interaction with micelle formation or emulsification of lipids in the enteric phase. On the other hand, it has also been suggested that the effect of chitosan on body weight is substantially less and unlikely to be of clinical significance.[19] It is therefore noteworthy to find that chitosan is an effective treatment for overweight and obesity.

According to our experiment in vivo, groups with chitosan and its derivatives had different inhibiting effects on body weight of the diet-induced rats. Furthermore, the lipid-lowering effect of N-CQ-chitosan with MW of 5×10^4 was better. As compared with the high-fat control group, there was a reduction in body weight by 11%, in the rate of fat to body weight by 17%, and it elevated fecal lipid excretion by 95%. Therefore, we may consider that feeding chitosan and its derivatives resulted in down-regulated effects on lipid and improved obesity, which was in accordance with the above studies.[16,20] The phenomenon that chitosans form highly viscous solutions in dilute acids may cause distension of the duodenum in animals and thereby increase satiety.[34] The effect could account for the lower body weights observed for rats fed on chitosan and its derivatives in the present experiment in vivo.

Moreover, it was found that a lower MW of chitosan and its derivatives had a better therapeutic effect from all our data, which is probably because lower MW of them were absorbed by rats easily.[35] Chitosan (MW of 5×10^4) reduced TG and FFA by 28 and 19%, O-CM-chitosan (MW of 5×10^4) decreased TG and FFA by 36 and 61%, and N-CQ-chitosan (MW of 5×10^4) reduced TG and FFA by 44 and 87%, respectively. It was probably that lipid was probably bound to chitosan and its derivatives and excreted with feces, which was in agreement with our above analysis. The order of lipid-lowering in vivo is as follows: N-CQ-chitosan > O-CM-chitosan > chitosan. This is probably because N-CQ-chitosan and O-CM-chitosan are amphiphilic polymer and have better solubility than chitosan, and the positive surface charge of N-CQ-chitosan made it easy to bind with negtively charge lipid.

In addition to those observations, the levels of FPG, FPI, and leptin in the rats' blood serum were also significantly decreased. Treatment with chitosan (MW = 5×10^4), O-CM-chitosan (MW = 5×10^4), and N-CQ-chitosan (MW = 5×10^4) for 12 weeks resulted in a 24, 29, and 46% decrease in FPG level, a 36, 43, and 44% decrease in FPI, and a 31, 42, and 64% decrease in plasma leptin, respectively. The order of therapeutic effect is as follows: N-CQ-chitosan > O-CM-chitosan > chitosan. It may be due to the amphiphilicity, solubility, and surface charge of chitosan and its two derivatives. Indeed, leptin is generally believed to have an insulin-sensitizing effect.[14] Thus, the reduced FPG and leptin were the result of the alleviation of insulin resistance. Moreover, leptin also contributes to preventing excess lipid accumulation.[15] So, this phenomenon indicated that chitosan and its derivatives could decrease body fat through controlling the level of leptin in the serum and reduce fat toxicity, improve insulin sensitivity,[7−10] and then reduce the incidence of type II diabetes mellitus and some complications related to obesity.

It has been confirmed in this study that such a low weight gain was not caused by growth retardation due to any toxicity of chitosan and its derivatives. With regard to the mechanism, it is considered that the chitosan and its derivatives dissolved in the stomach to form an emulsion with intragastric oil droplets and would begin to precipitate in the small intestine.[20] Moreover, chitosan and its derivatives also reduced the levels of TG, FPG, FPI, FFA, and leptin in the rats' blood serum, which actually improved insulin resistance, and the different surface charge and MW of chitosan and its derivatives had different effects on these parameters. These results imply that a suitable chitosan and its derivatives intake would be useful to control overnutrition by fat and to improve insulin resistance.

AUTHOR INFORMATION

Corresponding Author

*Tel: +86 22 2740 8099. Fax: +86 22 2740 4724. E-mail: liuxf315@yahoo.cn.

Funding

The work was financially supported by National Natural Science Foundation of China.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Flegal, K. M.; Carroll, M. D.; Ogden, L. G.; Johnson, C. L. Prevalence and trends in obesity among US adults, 1999−2008. *J. Am. Med. Assoc.* **2010**, *303*, 235−241.

(2) Whitlock, G.; Lewington, S.; Ni, M. C. Coronary heart disease and body mass index: A systematic review of the evidence from large prospective cohort studies. *Semin. Vasc. Med.* **2002**, *2*, 369−381.

(3) Carey, V. J.; Walters, E. E.; Colditz, G. A.; Solomon, C. G.; Willett, W. C.; Rosner, B. A.; Speizer, F. E.; Manson, J. E. Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women. *Am. J. Epidemiol.* **1997**, *145*, 614−619.

(4) Calle, E. E.; Rodriguez, C.; Walker-Thurmond, K.; Thun, M. J. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N. Engl. J. Med.* **2003**, *348*, 1625−1638.

(5) Cummings, D. E.; Schwartz, M. W. Genetics and pathophysiology of human obesity. *Annu. Rev. Med.* **2003**, *54*, 453−471.

(6) Greenfield, J. R.; Campbell, L. V. Insulin resistance and obesity. *Clin. Dermatol.* **2004**, *22*, 289−295.
(7) Itani, S. I.; Ruderman, N. B.; Schmieder, F.; Boden, G. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and I kappa B-alpha. *Diabetes* **2002**, *51*, 2005−2011.
(8) Lam, T. K.; Yoshii, H.; Haber, C. A.; Bogdanovic, E.; Lam, L.; Fantus, I. G. Free fatty acid-induced hepatic insulin resistance: a potential role for protein kinase C-delta. *Am. J. Physiol. Endocrinol. Metab.* **2002**, *283*, 682−691.
(9) Santomauro, A. T.; Boden, G.; Silva, M. E.; Rocha, D. M.; Santos, R. F.; Ursich, M. J. Overnight lowering of free fatty acids with Acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects. *Diabetes* **1999**, *48*, 1836−1841.
(10) Boden, G.; She, P.; Mozzoli, M.; Cheung, P.; Gumireddy, K.; Reddy, P.; Xiang, X. Q.; Luo, Z. J.; Ruderman, N. Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor-kappa B pathway in rat liver. *Diabetes* **2005**, *54*, 3458−3465.
(11) Jiao, K.; Liu, H.; Chen, J. K.; Tian, D. M.; Hou, J. F.; Kaye, A. D. Roles of plasma interleukin-6 and tumor necrosis factor-α and FFA and TG in the development of insulin resistance induced by high-fat diet. *Cytokine* **2008**, *42*, 161−169.
(12) Temelkova-Kurktschiev, T. S.; Koehler, C.; Henkel, E.; Leonhardt, W.; Fuecker, K.; Hanefeld, M. Postchallenge plasma glucose and glycemic spikes are more strongly associated with atherosclerosis than fasting glucose or HbA1c level. *Diaberes Care* **2000**, *23*, 1830−1834.
(13) Nagi, D. K.; Bennett, P. H.; Knowler, W. C. Intact proinsulin,des 31,32 proinsulin, and specific insulin concentrations among nondiabetic and diabetic subjects in populations at varying risk of type 2 diabetes. *Diabetes Care* **1998**, *21*, 127−133.
(14) Takekoshi, K. Leptin directly stimulates catecholamine secretion and synthesis in cultured porcine adrenal medullary chromaffin cells. Biochem. Biophys. *Res. Commun.* **1999**, *261*, 426−431.
(15) Campbell, F. M.; Gordon, M. J.; Hoggard, N.; Dutta-Roy, A. K. Interaction of Free Fatty Acids with Human Leptin. *Diabetes* **1998**, *247*, 654−658.
(16) Deuchi, K.; Kanauchi, O.; Imasato, Y.; Kobayashi, E. Effect of the viscosity or deacetylation degree of chitosan on fecal fat excreted from rats fed on a high fat diet. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 781−785.
(17) Ormrod, D. J.; Holmes, C. C.; Miller, T. E. Dietary chitosan inhibits hypercholesterolaemia and atherogenesis in the apolipoprotein deficient mouse model of atherosclerosis. *Atherosclerosis* **1998**, *138*, 329−334.
(18) Mohamed, E. B. Chemical modification of chitosan: synthesis and biological activity of new heterocyclic chitosan derivatives. *Polym. Int.* **2008**, *57*, 254−261.
(19) Nauss, J. L.; Thompson, J. L.; Nagyvary, J. The binding of micellar lipids to chitosan. *Lipids* **1983**, *18*, 714−719.
(20) Flourie, B.; Vidon, N.; Florent, C. H.; Bernier, J. J. Effect of pectin on jejunal glucose absorption and unstirred layer thickness in normal man. *Gut* **1984**, *25*, 936−941.
(21) Hernandez-Gonzales, S. O.; Gonzalez-OOrtiz, M.; Martinez-Abundis, E.; Robles-Cervantes, J. A. Chitosan improves insulin sensitivity as determined by the euglycemic-hyperinsulinemic clamp technique in obese subjects. *Nutr. Res. (N.Y.)* **2010**, *30*, 392−395.
(22) Liu, X. F.; Song, L.; Li, L. Antibacterial effects of chitosan and its water-soluble derivatives on E. coli, plasmids DNA and mRNA. *J. Appl. Polym. Sci.* **2007**, *103*, 3521−3528.
(23) Guo, Z. Y.; Xing, R.; Liu, S.; Zhong, Z.; Ji, X.; Wang, L.; Li, P. C. The influence of molecular weight of quaternized chitosan on antifungal activity. *Carbohydr. Polym.* **2007**, *71*, 694−697.
(24) Sang-Hoon, L.; Samuel, M. H. Synthesis and antimicrobial activity of a water-soluble chitosan derivative with a fiber-reactive group. *Carbohydr. Res.* **2004**, *339*, 313−319.
(25) Sun, S. L.; Wang, A. Q. Adsorption kinetic chitosan of Cu(II) ions using N,O-carboxymethyl-chitosan. *J. Hazard. Mater.* **2006**, *131*, 103−111.
(26) Iwai, H.; Ohno, Y.; Aoki, N. The effect of leptin, tumor necrosis factor-α (TNF-α), and nitric oxide (NO) production on insulin resistance in otsuka long-evans fatty rats. *Endocr. J.* **2003**, *50*, 673−680.
(27) Liu, X. F.; Yang, F.; Song, T.; Zeng, A. R.; Wang, Q.; Sun, Z.; Shen, J. Synthesis of carboxymethylated and quaternized chitosans and their therapeutic effect on nonalcoholic fatty liver disease. *J. Agric. Food Chem.* **2011**, *59*, 10683−10692.
(28) Liu, X. F.; Yang, F.; Song, T.; Zeng, A. R.; Wang, Q.; Sun, Z.; Shen, J. Therapeutic effect of carboxymethylated and quanternized chitosan on insulin resistance in high-fat-diet-induced rats and 3T3-L1 adipocytes. *J. Biomater. Sci.* **2011**, 1−14, DOI: 10.1163/092050611X579771.
(29) Thanou, M.; Florea, B. I.; Geldof, M.; Junginger, H. E.; Borchard, G. Quaternized chitosan oligomers as novel gene delivery vectors in epithelial cell lines. *Biomaterials* **2007**, *23*, 153−159.
(30) Anitha, A.; Rani, V. V. D.; Krishna, R.; Sreeja, V.; Selvamurugan, N.; Nair, S. V.; Tamura, H.; Jayakumar, R. Synthesis, characterization, cytotoxicity and antibacterial studies of chitosan,O-carboxymethyl and N,O-carboxymethyl chitosan nanoparticles. *Carbohydr. Polym.* **2009**, *78*, 672−677.
(31) Zhang, Y. F.; Yin, P.; Zhao, X. Q.; Wang, J.; Wang, J.; Wang, C. D.; Ren, L.; Zhang, Q. Q. O-Carboxymethyl-chitosan/organosilica hybrid nanoparticles as non-viral vectors for gene delivery. *Mater. Sci. Eng.* **2009**, *29*, 2045−2049.
(32) Wong, K.; Sun, G.; Zhang, X.; Dai, H.; Liu, Y.; He, C.; Leong, K. W. PEI-g-chitosan, a novel gene delivery system with transfection efficiency comparable to polyethylenimine in vitro and after Liver Administration in vivo. *Bioconjugate Chem.* **2009**, *17*, 152−158.
(33) Mhurchi, C. N.; Dunshea-Mooji, C.; Bennrtt, D.; Rodegers, A. Effect of chitosan on weight loss in overweight and obese individuals: a systematic review of randomized of controlled trials. *Obes. Rev.* **2005**, *6*, 35−42.
(34) Sugano, M.; Watanabe, S.; Kishi, A.; Izume, M.; Ohtakara, A. Hypocholesterolemic action of chitosans with different viscosity in rats. *Lipids* **1988**, *23*, 187−191.
(35) Liu, X. F.; Zeng, A. R.; Li, L.; Yang, F.; Wang, Q.; Sun, Z.; Shen, J. Synthesis of a novel amphiphilic quaternized chitosan and its distribution in rats. *J. Biomater. Sci.* **2011**, *22*, 1115−1130.