

Differential blood lipid-lowering effects of alkylsulfonated chitosan of different molecular weights in Syrian hamsters *in vivo*

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Received August 2, 2011; Accepted December 9, 2011

DOI: 10.3892/mmr.2011.705

Abstract. This study investigated the effects of alkylsulfonated chitosan of different molecular weights on intestinal lipid absorption, blood lipid profiles and circulating adhesion molecules. Syrian hamsters were fed an AIN-93G-based high-fat diet (HFD) and were orally administered 5 or 10 mg/kg BW of oligomer (6 kDa) chitosan (OC), low-molecular-weight (70 kDa) chitosan (LMC) or high-molecular-weight (200 kDa) chitosan (HMC) four times per week for 12 weeks. Animals receiving 2.5 mg/kg BW lovastatin (LOVA) served as a positive control. The blood lipid profiles of these control animals revealed that all chitosans and LOVA significantly decreased total triglyceride, total cholesterol, low-density lipoprotein (LDL)-cholesterol and very-low-density lipoprotein (VLDL)-cholesterol levels in a dose-dependent manner compared to the HFD-fed controls ($P < 0.05$). The blood lipid lowering effectiveness of the three chitosans followed the order of LMC > OC > HMC. Hamsters receiving 5 and 10 mg/kg LMC ($P < 0.05$) exhibited an increase in fecal fat content. Immunoblot assay revealed that acyl-coen-

zyme A:cholesterol acyltransferase-2 (ACAT-2) expression was suppressed in all chitosan-fed animals compared to the HFD-fed controls ($P < 0.05$). These results suggest that chitosan effectively decreases blood lipid content, and its effectiveness depends on the molecular size of chitosan. The hypolipidemic effect of chitosan is partly attributed to its suppression of intestinal lipid absorption and hepatic ACAT-2 expression.

Introduction

Chitosan is a biopolymer of glucosamine produced by the deacetylation of chitin, which is an aminopolysaccharide found in the exoskeleton of arthropods and the cell wall of fungi. Chitosan is chemically similar to cellulose. Previous studies have shown that chitosan and its oligomers display a variety of biological activities, including antitumor, hypoglycemic, hypolipidemic, antibacterial and eight control effects (1-5). The effectiveness of chitosan in lowering blood lipids and in body weight control allows it to decrease the risk of cardiovascular disease (6-8).

Chitosan is generated from chitin. The extent of hydrolysis and deacetylation produces chitosan with various molecular weights and viscosity. The oligo-form of chitosan, which is generated by the degradation of the chitosan polymer chain, shows better water solubility and antitumor effects than the poly-form of chatoyant (9). Molecular weight and viscosity are critical factors responsible for the hypolipidemic effect of chatoyant (3,6,10). The molecular weight of chitosan ranging from 20 to 2000 kDa displays better potency for lowering blood triglycerides and cholesterol (6,11,12). Establishing the molecular weight and chemical characteristics of chitosan with optimal biological activity remains an elusive target.

Chitosan is insoluble in water and organic solvents. To increase the application of chitosan in the food and medical industry, alkylsulfonation produces a novel modified chatoyant (13,14). This type of alkylsulfonated chitosan

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Key words: acyl-coenzyme A:cholesterol acyltransferase-2, blood lipids, chitosan, hamsters

retains the structure and characteristics of the natural polymer and is a water soluble and anionic polymer as a result of the introduction of a hydrophilic acid functional group.

3-Hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase catalyzes the conversion of HMG CoA to the mevalonate pathway, and is the rate-limiting enzyme of cholesterol biosynthesis (15,16). The inhibition of HMG CoA reductase activity suppresses hepatic cholesterol formation, which in turn decreases blood cholesterol levels. The hypocholesterolemic effects of statins and phytochemicals, including garlic organosulfur compounds and soy isoflavones, have been attributed to their potent inhibition of this enzyme activity (16-19). Acyl-coenzyme A:cholesterol acyltransferase (ACAT) is another key enzyme that regulates plasma cholesterol. ACAT catalyzes cholesteryl esters from cholesterol and fatty acyl-CoA, and is responsible for intestinal cholesterol absorption, hepatic repackaging of very-low density lipoprotein (VLDL), cholesterol storage for steroid hormone biosynthesis (20,21) and the formation of macrophage-derived foam cells in atherosclerotic lesions (22). Bavachin and isobavachalcone inhibition of ACAT activity partly explains the blood cholesterol-lowering effect of *Psoralea corylifolia* (23). In addition to interfering with lipid absorption in the gut, the role of chitosan in modulating HMG-CoA reductase and ACTA activity and expression in hamster livers remains unclear.

The experiments in this study assessed three alkylsulfonated chitosans with different molecular weights, i.e. 6, 70, and 200 kDa, for their hypolipidemic activity. Blood triglycerides and cholesterol, fecal fat content and hepatic HMG-CoA reductase and ACAT-2 expression were examined in Syrian hamsters.

Materials and methods

Materials. Alkylsulfonated chitosan was purchased from Taiwan Hopax Chems MFG Co., Ltd. (Kaohsiung, Taiwan). The oligomer chitosan [molecular weight (MW) 6 kDa] and low- and high-molecular-weight polymer chitosan (MW 70 and 200 kDa, respectively) were the experimental materials used in this study. Lovastatin was purchased from Calbiochem Ltd. (San Diego, CA, USA). The anti-HMG CoA reductase, ACAT-2, intracellular adhesion molecule 1 (ICAM-1) or vascular cell adhesion molecule 1 (VCAM-1), and intracellular adhesion molecule 1 (ICAM-1) or vascular cell adhesion molecule 1 (VCAM-1) monoclonal antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

Animals and treatments. Hamsters were treated in compliance with the Guide for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy, Washington D.C., USA and the animal experiments were approved by the Chang Jung Christian University Animal Ethics Committee. Four-week-old male Syrian hamsters were purchased from the National Laboratory Animal Center (Taipei, Taiwan). Following one week of acclimation, hamsters were randomly assigned to each experimental group (n=6) by weight and subjected to a 12-h light cycle. Animals had free access to water and an AIN-93G-based diet (5% soybean oil, ND) or a high-fat diet

Table I. Composition of the experimental diets.

Ingredients	ND (%)	HFD (%)
Corn starch	41.75	26.25
Casein (vitamin-free)	20	20
Maltodextrin	13.2	13.2
Sucrose	10	10
Cellulose	5	5
Soybean oil	5	20
AIN 93G mineral mix	3.5	3.5
AIN 93G vitamin mix	1	1
Cholesterol	0	0.5
L-cystine	0.3	0.3
Choline bitartate	0.25	0.25

AIN-93G (ND, normal diet) and a modified AIN-93G diet containing 20% fat - 0.5% cholesterol (HFD, high-fat diet) were administered.

(20% soybean oil plus 0.5% cholesterol, HFD) (Dyets, Inc., Bethlehem, PA, USA) (Table I). Hamsters fed with the HFD were subdivided into the following treatment groups, and orally administered 5 or 10 mg BW oligomer chitosan (SOC, 10OC), low-molecular-weight chitosan (5LMC, 10 LMC), 5 or 10 mg/kg BW high-molecular-weight chitosan (5HMC, 10HMC) or 2.5 mg/kg BW lovastatin (LOVA, as a positive control). All chitosan products and lovastatin were prepared in phosphate-buffered saline (PBS) and were administered orally 4 times per week for 12 weeks. During the last week, fecal samples from each group were collected in a metabolic cage and were used for total fecal crude fat content examination. Following fasting overnight, hamsters were sacrificed by carbon dioxide euthanasia. Blood was withdrawn using the intracardiac method and serum was prepared for lipid profile analysis. The liver, heart, spleen and kidney were removed and weighed. The largest liver lobes were used for histological examination and the liver remnants were quickly freeze-clamped in liquid nitrogen and stored at -80°C until further analysis. The thoracic aortae were used for ICAM-1 and VCAM-1 determination by immunoblotting assay.

Serum lipid determination. Blood samples were collected and serum was prepared by centrifuging at 1000 x g for 15 min at 4°C. Amounts of total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C) and VLDL-C were measured with commercial kits (Randox Laboratories Ltd., San Diego, CA, USA) according to the manufacturer's instructions.

Histological examination. Liver tissues were fixed in 10% neutral buffered formalin. Sections (5 µm) from the paraffin-embedded tissue were stained with hematoxylin and eosin (H&E). Pathological examination was performed by an expert pathologist in a blinded manner.

Analysis of total fecal crude fat level. Fecal samples were collected in a metabolic cage and were stored at -20°C until

Table II. Growth characteristics of hamsters administered chitosan.

Diet	Food intake g/day	Body weight gain g/12 weeks	Liver	Heart	Kidney	Spleen
ND	8.2±2.1	50±3	5.1±0.5	0.37±0.05	0.97±0.02	0.07±0.01
HFD	8.3±2.7	48±3	5.2±0.6	0.37±0.05	1.00±0.02	0.07±0.01
HFD+5OC	8.1±3.3	51±3	5.1±0.6	0.34±0.05	0.94±0.10	0.10±0.03
HFD+10OC	6.7±2.2	51±3	5.0±0.6	0.34±0.04	0.87±0.06	0.08±0.03
HFD+5LMC	6.8±3.1	50±3	5.4±0.6	0.36±0.06	0.97±0.05	0.10±0.02
HFD+10LMC	7.7±2.3	49±4	5.3±0.5	0.33±0.05	1.00±0.01	0.08±0.02
HFD+5HMC	7.5±2.8	50±5	5.4±0.7	0.35±0.06	0.87±0.06	0.09±0.03
HFD+10HMC	7.8±2.2	50±3	5.5±0.7	0.37±0.07	0.97±0.06	0.10±0.02
HFD+LOVA	7.4±2.7	51±3	5.1±0.7	0.33±0.07	0.90±0.10	0.09±0.03

Hamsters were fed a normal diet (ND) or high-fat diet (HFD), and those fed the HFD were sub-grouped and orally administered 5 or 10 mg/kg BW oligomer chitosan (5OC, 10OC), 5 or 10 mg BW low-molecular-weight chitosan (5LMC, 10 LMC), 5 or 10 mg/kg BW high-molecular-weight chitosan (5HMC, 10HMC) or 2.5 mg/kg BW lovastatin (LOVA). Chitosan was administered four times per week for 12 weeks. Values are presented as the means ± SD, n=5-6.

analysis. Following drying, 5 g of the feces was weighed for crude fat determination using the Soxhlet extractor method (24). Briefly, fecal samples in Soxhlet apparatus were exhaustively extracted by petroleum ether (boiling point range 40–60°C) and allowed to run for 2 h to facilitate at least five cycles of Soxhlet extraction. The extracts were then evaporated to dryness over a steam bath and stored overnight in a desiccator. The added dry weight of the receptor-bottle was the net weight of total crude fat in each fecal sample.

SDS-polyacrylamide gel electrophoresis and immunoblotting. Liver and thoracic aorta tissues were homogenized in four volumes of a buffer (pH 7.4) containing 10 mmol/l potassium phosphate, 150 mmol/l potassium chloride and 1 mM phenylmethylsulfonyl fluoride, and centrifuged at 10,000 x g for 30 min at 4°C. The supernatant was further ultracentrifuged at 105,000 x g for 1 h, and the final cytosolic supernatant was stored at -80°C until analysis. Equal amounts of liver cytosolic fractions of each sample were applied to 10% SDS-polyacrylamide gels for HMG Co A reductase and ACAT-2 expression assay. Cytosolic fractions from the thoracic aorta were used for VCAM-1 and ICAM-1 protein analysis. Following electrophoresis, proteins separated on gels were transferred to polyvinylidene difluoride membranes and immunostained as described by Towbin *et al* (25). The membranes were incubated with 50 g/l non-fat dry milk in 15 mmol/l Tris/150 mmol/l NaCl buffer, pH 7.4, at 4°C overnight to block non-specific binding. The membranes were then incubated with anti-HMG CoA reductase, ACAT-2, ICAM-1 or VCAM-1 antibody at 37°C for 1 h, followed by peroxidase-conjugated secondary antibody. The bands were visualized using hydrogen peroxide/tetrahydrochloride diaminobenzidine or an enhanced chemiluminescent detection kit (Amersham Life Science, Buckinghamshire, UK) and were quantified with an AlphaImager 2000 (Alpha Innotech, San Leandro, CA, USA).

Additional biochemical assays. To examine whether the oral administration of chitosan affects liver function or caused liver toxicity, serum glutamic oxalacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities were determined using commercial assay kits (Randox Laboratories Ltd., San Diego, CA, USA) according to the manufacturer's instructions.

Statistical analysis. Data are expressed as the means ± SD. Statistical analysis was performed using ANOVA (SAS Institute Inc., Cary, NC, USA). Tukey's multiple comparison was used to determine the significant difference among the group means (P<0.05).

Results

Animal characteristics. Following 12 weeks of feeding, changes in body weight, food intake and the relative visceral weight were determined. Compared to animals fed the 5% soybean oil diet (ND), a HFD (20% soybean oil) caused no changes in food intake, body weight gain nor an increase in the weight percentage of liver, heart, kidney and spleen to body weight. When hamsters were co-administered the three chitosans with either 5 or 10 mg/kg, no effect on food intake, growth performance or the relative weight of the liver, spleen, heart, and kidney was noted (Table II). Moreover, hamsters administered chitosan showed no changes in serum GOT and GPT activities, suggesting that the chitosan dosage used caused no liver toxicity (Table III).

Blood lipid profile. Hamsters fed the HFD exhibited significantly higher serum TG, TC, VLDL-C and LDL-C levels than those noted in the low-fat ND group (Table IV). Upon co-treatment with chitosan and LOVA, three chitosan products resulted in lower serum TG (23-56%), TC (19-48%), VLDL-C (23-25%) and LDL-C (24-44%) in a dose-dependent

Table III. Effects of different types of chitosan on the GOT and GPT in Syrian hamsters fed various diets for 12 weeks.

Diet	GOT (units)	GPT (units)
ND	55±6	77±10
HFD	50±5	75±11
HFD+5OC	54±7	68±11
HFD+10OC	53±6	72±11
HFD+5LMC	52±6	68±12
HFD+10LMC	50±4	73±14
HFD+5HMC	51±5	72±10
HFD+10HMC	53±7	67±13
HFD+LOVA	55±7	63±12

Hamsters were fed either a normal diet (ND) or high-fat diet (HFD) for 12 weeks. Animals fed the HFD were subgrouped and orally administered 5 or 10 mg/kg 6 kDa oligomer chitosan (5OC, 10OC), 5 or 10 mg/kg 70 kDa chitosan (5LMC, 10 LMC), 5 or 10 mg/kg 200 kDa chitosan (5HMC, 10HMC) or 2.5 mg/kg lovastatin (LOVA) four times per week. Values are presented as the means ± SD, n=5-6.

manner compared with the HFD controls ($P<0.05$). The effectiveness of the three chitosans in lowering serum TG and cholesterol levels followed the order of LMC>OC>HMC. Animals administered LOVA exhibited a lower level of TG, TC, VLDL-C and LDL-C than the HFD group ($P<0.05$). Serum contents of HDL-C were not affected by treatment with chitosan and LOVA.

Histological examination. A histological examination was performed to detect fatty liver changes. Feeding mice a HFD for 12 weeks (Fig. 1B) increased fatty liver. When co-administered with 5 or 10 mg/kg OC (Fig. 1C and D), LMC (Fig. 1E and F), or HMC (Fig. 1G and H), fatty liver changes were suppressed. However, LOVA (Fig. 1I) offered minor protection to the fatty changes in liver tissues.

Table IV. Effects of chitosan on blood lipids.

Diet	TG	TC	LDL-C	HDL-C	VLDL-C
ND	144±10 ^c	196±22 ^d	153±16 ^{b,c,d}	72±11	28.7±2.0 ^c
HFD	282±19 ^a	324±13 ^a	236±15 ^a	79±6	56.5±3.8 ^a
HFD+5OC	165±13 ^{b,c}	198±20 ^{c,d}	174±8 ^{b,c}	76±8	33.1±2.6 ^{b,c}
HFD+10OC	150±10 ^c	205±21 ^{c,d}	169±9 ^{b,c,d}	74±8	30.1±1.9 ^c
HFD+5LMC	143±20 ^c	181±12 ^d	138±15 ^{c,d}	71±15	28.7±3.9 ^c
HFD+10LMC	125±11 ^c	168±12 ^d	132±15 ^d	74±9	25.1±2.3 ^c
HFD+5HMC	216±31 ^b	262±27 ^b	180±12 ^b	73±4	43.2±6.1 ^b
HFD+10HMC	180±21 ^{b,c}	249±18 ^{b,c}	174±13 ^{b,c}	75±6	35.9±4.3 ^{b,c}
HFD+LOVA	216±25 ^b	161±15 ^d	130±18 ^d	73±6	43.2±5.1 ^b

Hamsters were fed either a normal diet (ND) or high-fat diet (HFD) for 12 weeks. Animals fed the HFD were orally administered 5 or 10 mg/kg oligomer chitosan (5OC, 10OC), 5 or 10 mg/kg low-molecular-weight chitosan (5LMC, 10 LMC), 5 or 10 mg/kg high-molecular-weight chitosan (5HMC, 10HMC) or 2.5 mg/kg BW lovastatin (LOVA) four times per week. Serum total triglycerides (TG), total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C) and VLDL-cholesterol (VLDL-C) were determined. Values are presented as the means ± SD, n=5-6. ^{a,b,c,d}Group mean values not sharing the same symbols differed significantly, $P<0.05$. Blood lipid values are expressed as mg/dl.

Total fecal crude fat levels. The total fecal fat content was measured to determine whether chitosan lowered lipid TG and cholesterol levels by interfering with fat absorption in the gastrointestinal tract. As shown in Fig. 2, following 12 weeks of 5 or 10 mg/kg LMC treatment, a 15 and 21% higher fecal fat content was noted, respectively. This was higher than that in the HFD-fed controls ($P<0.05$). However, administration of OC, HMC and LOVA did not change the feces fat content.

Hepatic HMG CoA reductase and ACAT-2 protein expression. Hepatic ACAT-2 and HMG CoA reductase protein levels were determined by immunoblot assay. The results indicated that HFD produced a higher ACAT-2 expression than that noted in ND-fed animals, although the difference was insignificant (Fig. 3A). When hamsters were co-treated with 5 or 10 mg/kg OC, LMC and HMC or 2.5 mg/kg LOVA, hepatic ACAT-2 content significantly decreased ($P<0.05$). Regarding hepatic HMG CoA reductase, its expression was enhanced in hamsters fed the HFD diet ($P<0.05$) (Fig. 3B). However, chitosan and LOVA produced no changes in the hepatic content of this rate-limiting enzyme responsible for cholesterol synthesis.

VCAM-1 and ICAM-1 expression in thoracic aorta. Hyperlipidemia is associated with chronic inflammation in the vessel walls. Due to the effectiveness of chitosan at lowering blood lipids, this study examined the effect of chitosan suppression on the expression of ICAM-1 and VCAM-1, two adhesion molecules that are highly induced by inflammatory stimuli in the thoracic aorta. Unlike the ND-fed group, the HFD-fed animals exhibited much more VCAM-1 protein ($P<0.05$) (Fig. 4A). However, LMC, HMC, OC or LOVA treatment significantly suppressed HFD-induced VCAM-1 expression ($P<0.05$). Among the different types of chitosan, LMC and OC displayed greater potency than HMC ($P<0.05$). Regarding the changes in ICAM-1 in the thoracic aorta, a slight increase was produced by HFD feeding (Fig. 4B), but its expression was significantly decreased in hamsters treated with 5 and 10 mg/kg LMC and 5 mg/kg OC ($P<0.05$). Note that HMC and LOVA did not change the ICAM-1 level.

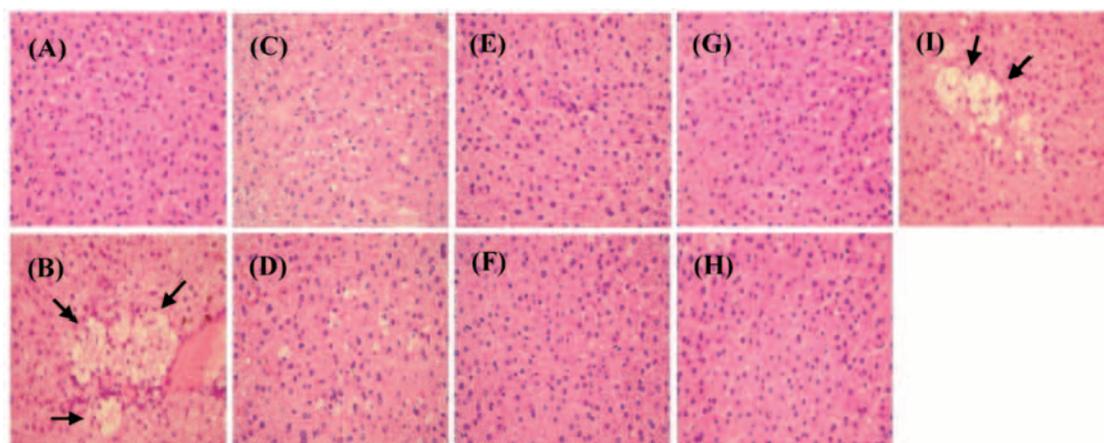


Figure 1. Fatty liver formation in Syrian hamsters fed a high-fat diet and chitosan of different molecular weights. Animals were fed (A) normal or (B) high-fat diets for 12 weeks. Hamsters in the high-fat diet were further subgrouped and orally administered (C and D) oligomer chitosan, (E and F) 5 or 10 mg/kg low-molecular-weight chitosan, (G and H) high-molecular-weight chitosan, or (I) 2.5 mg/kg lovastatin four times per week. Tissue sections were stained with hematoxylin and eosin (magnification, x200). The typical fatty changes are indicated by arrows.

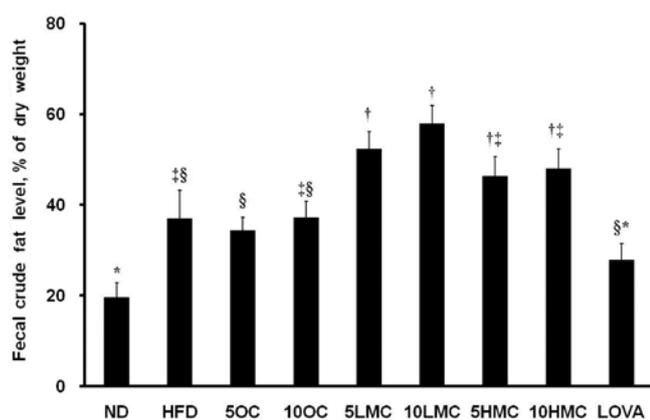


Figure 2. Changes in the fecal fat content by chitosans. Hamsters were fed either a low-fat diet (ND) or high-fat diet (HFD). Animals fed the HFD were sub-grouped and orally administered 5 or 10 mg/kg oligo-form chitosan (5OC or 10OC), low-molecular-weight chitosan (5LMC or 10LMC), high-molecular-weight chitosan (5HMC or 10HMC), or 2.5 mg/kg lovastatin (LOVA) for 12 weeks. Feces were collected during the week prior to animal sacrifice, and the crude feces fat content was determined as described in Materials and methods. Values are presented as the means \pm SD, n=5-6. $^{\dagger}\ddagger\text{§}$ Values not sharing the same letters differed significantly, $P < 0.05$.

Discussion

Recent studies indicate that chitosan is effective at decreasing food lipid and cholesterol absorption in the gastrointestinal tract. As a result, chitosan is a functional anti-obesity food, and is capable of lowering blood triglycerides and cholesterol (3,10,26). Chitosan, a biopolymer of glucosamine, is produced by the deacetylation of chitin and consists of polymers with various molecular weights. The hypolipidemic activity of chitosan is related to a number of chemical characteristics, including molecular weight, degree of acetylation and viscosity (6,27). Regarding the effect of the differential molecular weight, feeding an obese mouse model a HFD showed that chitosan with 46 kDa molecular weight is more effective at attenuating body weight gain and lowering blood total triglycerides and total cholesterol and LDL-cholesterol

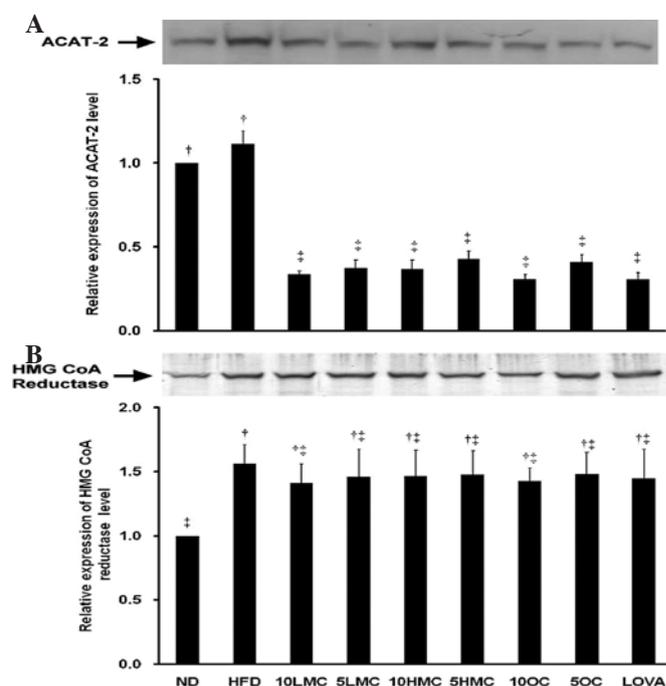


Figure 3. Hepatic HMG CoA reductase and ACAT-2 protein expression in hamsters treated with various chitosans. Following 12 weeks of feeding ND or HFD or HFD plus 5 or 10 mg/kg OC, LMC, or HMC, or 2.5 mg/kg lovastatin (LOVA), (A) HMG CoA reductase and (B) ACAT-2 expression in liver tissues was measured by immunoblotting assay. HMG CoA reductase and ACAT-2 expression in the ND group was treated as 1. Values are presented as the means \pm SD, n=5-6. $^{\dagger}\ddagger\text{§}$ Group mean values not sharing the same letters differed significantly, $P < 0.05$.

than 650 kDa chatoyant (6). This finding agrees with the current study, which shows that lower molecular weight chitosan (70 kDa) displays better hypolipidemic and hypocholesterolemic activity than 200 kDa chitosan (Table IV). Moreover, low-molecular-weight chitosan results in a higher lipid contents in feces than the high-molecular-weight product (Fig. 2). The increase in fecal fat content caused by chitosan is attributed to the formation of agglomerates with fatty acids

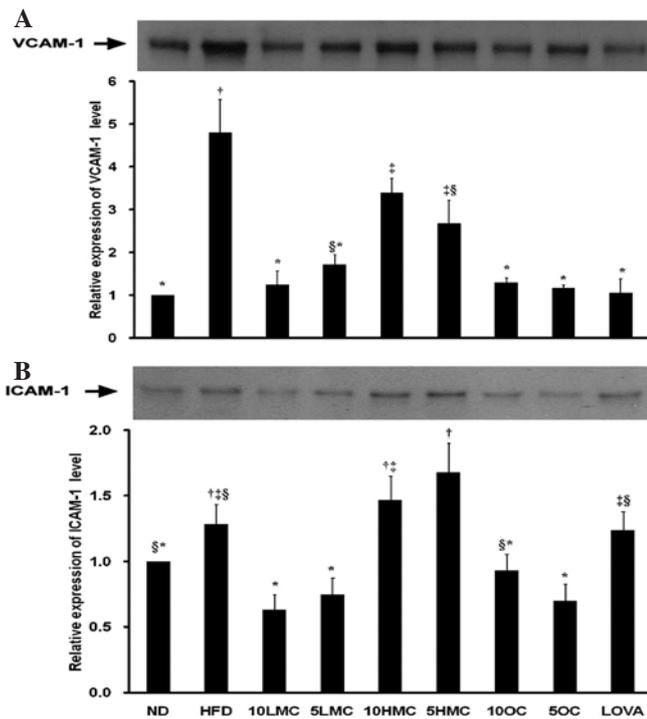


Figure 4. Effects of chitosan on thoracic aortic VCAM-1 and ICAM-1 protein expression. Hamsters were fed a diet with 5% soybean oil (ND) or 20% soybean oil/0.5% cholesterol (HFD). Animals fed the HFD were subgrouped and orally administered 5 or 10 mg/kg OC, LMC or HMC, or 2.5 mg/kg lovastatin (LOVA) for 12 weeks. Thoracic aortic (A) VCAM-1 and (B) ICAM-1 protein levels were measured by immunoblotting assay. VCAM-1 and ICAM-1 expression in the ND group was treated as 1. Values are presented as the means \pm SD, $n=5-6$. * Group mean values not sharing the same letters differed significantly, $P<0.05$.

and cholesterol in the gastrointestinal tract (12,28). These findings suggest that the differential potency on the decrease in dietary lipid absorption explains, at least partly, the difference in the blood lipid lowering effect of 70 kDa and 200 kDa chitosan (12,28-30). In addition, the chitosan decrease of fatty liver (Table IV) suggests that glucosamine polymers may affect lipid metabolism in the liver.

To determine the optimal range of chitosan molecular weight on hypolipidemia, this study also tested an oligo-form chitosan of 6 kDa. Recent *in vitro* experiments indicate that the oligo-form of chitosan displays anti-tumorigenesis, anti-adipogenesis and apoptosis activity. For instance, 1-3 kDa chitosan was found to inhibit the inflammatory response in HT-29 cells induced by cytokines (31) and to suppress adipogenesis of 3T3-L1 preadipocytes (32). In addition, 3-5 kDa oligo-form chitosan inhibited colon cancer formation by increasing quinone reductase and glutathione S-transferase activities and glutathione levels, and inhibited ornithine decarboxylase activity and cyclooxygenase-2 expression in HT-29 cells (33). However, the results of this study clearly indicate that the effects of 6 kDa oligo-form chitosan on lowering blood lipids (Table IV) and inhibiting intestinal fat absorption (Fig. 2) are inferior to 70 kDa chitosan. These results suggest that, in an *in vivo* model, chitosan with an optimal molecular weight is required for approaching the best blood lowering activity.

ACAT-2 plays a significant role in hepatic cholesterol metabolism (20,21). In the liver, ACAT catalyzes the intra-

cellular cholesteryl ester production from esterification of cholesterol and is involved in the production and release of apoB-containing lipoproteins (34). Higher ACAT activity speeds up the release of VLDL from the liver, leading to higher blood cholesterol levels. The hypocholesterolemic effect of a number of phytochemicals has been attributed to their action on inhibiting ACAT activity and expression. For instance, the effects of pinitol and naringenin on lowering total blood cholesterol and LDL-cholesterol levels in hamsters and rats fed a HFD is explained partly by their effectiveness at down-regulating ACAT expression in liver tissue (35,36). Bavachin and isobavachalcone are potent ACAT inhibitors in HepG2 cells (23). This study shows that in addition to lowering blood VLDL- and LDL-cholesterol levels, either low- or high-molecular-weight chitosan or oligomer chitosan significantly decreased hepatic ACAT-2 expression in hamsters (Fig. 3A). By contrast, the hypocholesterolemic effect of chitosan is likely to be independent of the modulation of HMG CoA reductase expression (Fig. 3B).

In the early stage of atherosclerosis, endothelial cells express a variety of cellular adhesion molecules, such as VCAM-1 and ICAM-1. These molecules play a significant role in recruiting circulating leukocytes to the vascular endothelium and facilitating their penetration into the vascular intima (37). The interruption of the abnormal induction of adhesion molecules under certain circumstances, such as chronic inflammation, hypercholesterolemia and oxidative stress, ameliorates the progression of this blood vessel disease (17,38). The expression of ICAM-1 and VCAM-1 in endothelial cells is highly inducible under certain stimuli, including proinflammatory cytokines and oxidative stress. Higher ICAM-1 and VCAM-1 levels are associated with hypercholesterolemia (39,40). Oxidized LDL stimulates endothelial cells to produce MCP-1, ICAM-1, VCAM-1 and E- and P-selectin and increases monocyte recruitment and infiltration into the lesions of the endothelium. The suppression of abnormal adhesion molecule expression in vascular endothelium is thought to protect against the incidence of atherosclerosis (41,42). It is interesting to examine whether chitosan decreases adhesion molecule expression. This study revealed a 4-fold increase in the VCAM-1 level in the thoracic aorta of hamsters fed the HFD compared to the ND diet (Fig. 4A). After co-administration with chitosan, VCAM-1 expression was significantly suppressed, with 70 kDa and 7 kDa chitosan displaying higher inhibitory potency than that of 200 kDa high-molecular-weight chitosan. A similar inhibition on HFD-induced ICAM-1 expression was also noted by dosing with 70 kDa and 7 kDa chitosan (Fig. 4B). The similar pattern of decreasing blood lipid levels and vascular VCAM-1 and ICAM-1 expression among the three chitosans of different molecular weight suggests that the hypolipidemic effect of chitosan is through the amelioration of chronic inflammation and oxidative stress, which in turn attenuates the adhesion molecules induced by a HFD (43,44).

In conclusion, the results of this study indicate that chitosan effectively lowers blood triglycerides, total cholesterol and VLDL- and LDL-cholesterol and further suppresses vascular adhesion molecule expression in hamsters fed a HFD. This effect is partly attributable to the chitosan inhibition of intestinal lipid absorption and hepatic ACAT-2

expression. Moreover, the hypolipidemic potency depends on the molecular weight of chitosan.

Acknowledgements

This study was supported by Grants FY95-RD-003 from Fooyin University and Taiwan Hopax Chemicals Mfg. Co., Ltd., Kaohsiung, Taiwan, and NSC 96-2320-B-309-005-MY3 from the National Science Council, Taiwan.

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