Journal of Food and Drug Analysis, Vol. 14, No. 2, 2006, Pages 183-189 藥物食品分析 第十四卷 第二期

Effect of Chitosan on Plasma Lipids, Hepatic lipids, and Fecal Bile Acid in Hamsters

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(Received: September 7, 2005; Accepted: December 9, 2005)

ABSTRACT

Chitosan is a natural product derived from chitin that possesses hypocholesterolemic properties. However, the mechanism of its cholesterol-lowering effect is still unclear. This study was designed to investigate the effect of chitosan on plasma lipoprotein cholesterol, liver cholesterol and fecal excretion of cholesterol and bile acid in hamsters. Hamsters were fed a high-cholesterol (0.2%) diet containing 4% cellulose (CE) or 4% chitosan (CS) for eight weeks. The hamsters fed the CS diet had significantly lowered plasma total cholesterol (TC), very low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) than those fed the CE diet. CS had no effect on high-density lipoprotein cholesterol (HDL-C) level, but higher ratio of HDL-C to TC was observed in hamsters fed the CS diet when compared with those fed with the CE diet. In addition, CS did not change plasma triglyceride and cholesteryl ester transfer protein (CETP) activity. Hepatic cholesterol contents were significantly lower in hamsters fed CS primarily due to the reduced accumulation of the esterified cholesterol. Fecal excretion of cholesterol and bile acid were significantly increased in hamsters after the CS treatment. Results from this study suggest that the cholesterol-lowering effect of CS may be primarily related to an increase in fecal excretion of cholesterol and bile acid in hamsters.

Key words: chitosan, cholesterol, lipoprotein, hamsters

INTRODUCTION

Epidemiological studies in human populations indicates that hypercholesterolemia is an important risk factor for coronary heart disease[1], and that increased dietary fiber intake is suggested to decrease plasma cholesterol concentration[2,3]. Chitosan, a biopolymer of glucosamine derived from chitin that is chemically similar to that of cellulose, is not digestible by mammalian digestive enzymes and acts as a dietary fiber in gastrointestinal tract[4]. Animal studies have shown that chitosan feeding may inhibit dietary fat digestion[5,6] and decrease plasma cholesterol concentration[4,7-11]. Increased fecal cholesterol accompanied with or without bile acid excretion by interfering intestinal micelle formation is believed to be responsible for its hypocholesterolemic properties[4,7]. However, the exact mechanisms of actions are not fully clear.

Previous rat studies have shown that chitosan remarkably decreases plasma VLDL-C and LDL-C while it increases HDL-C levels, which result in a less atherogenic lipoprotein profile[10,11]. However, the beneficial effect of chitosan on elevating HDL-C or lowering LDL-C is mild or unobserved in recent clinical trials[12-14]. In addition, most of the studies related to chitosan and weight loss have been conducted on rats and the anti-obesity effects of chitosan may be caused by the inhibition of intestinal absorption of dietary fat[5,6]. However, only small change or no effect on human body weight loss and fecal fat excretion was observed due to chitosan supplementation[13,14]. The main reason for these discrepancies seems to be related to the differences in the amount of dietary chitosan ingested, chemical composition of chitosan used and cholesterol metabolism difference between rat and human.

The Golden-Syrian hamsters have been extensively used for investigation of dietary cholesterol on lipoprotein and bile acid metabolism and the mechanism involved because they have similar responses to dietary factors, i.e dietary fiber and cholesterol that are known to alter plasma cholesterol levels in humans[15]. Advantages of this animal model compared with others of comparable size include the presence of LDL receptor-mediated and cholesteryl ester transfer protein (CETP) activities[16]. However, relatively less information is available on the effect of chitosan on lipid metabolism in hamsters[8].

The present study was designed to investigate the effects of chitosan on lipoprotein cholesterol and triglyceride distribution, hepatic cholesterol levels, and fecal excretion of cholesterol and bile acid in hamsters. Plasma hormones such as leptin and insulin that are directly proportional to body fat[17,18] and provide signals to metabolism and energy balance will also be determined. In this study, cellulose was used as a control fiber since it dose not affect plasma lipoprotein cholesterol concentrations in hamsters[19].

MATERIALS AND METHODS

I. *Chitosan and Cellulose*

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The chitosan, prepared from shrimp shell chitin, was generously supplied by the Taiwan Tanabe Seiyaku Co (Taipei, Taiwan). The degree of deacetylation of chitosan was about 90%, and the average molecular weight was about 800 KD. The cellulose used in the study was purchased from Sigma Chemical Co. (St Louis, MO, USA).

II. *Animals and Treatment*

Eighteen four-week-old male hamsters were purchased from the Animal Center of the National Science Council (Taipei, Taiwan). During the adaptation period, hamsters were fed a chow diet (Rodent Laboratory Chow, Ralston Purina, St. Louis, MO, USA) for two weeks. Then the animals were randomly divided into two groups with nine animals in each group; (a) the cellulose group (CE; control group) and (b) the chitosan group (CS group), and the average body weights were 98.1 ± 12.9 g, and 98.3 ± 11.9 g, respectively, for the CE and CS groups. The composition of the experimental diet given to test animals is shown in Table 1. Hamsters were housed in individual stainless cages in a room kept at 23 ± 1°C and 60 ± 5% relative humidity with a 12-hr light and dark cycle (lighting from 8:00 a.m. to 8:00 p.m.). Food and drinking water were available *ad libitum* for eight weeks. Food intake was measured daily, and body weight was measured every week. Feces were collected from each animal during the last 3 days of the experiment, freeze-dried and weighed. 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.

Table 1. Composition of the experimental diet[a] (%)

Ingredient	CE	CS
Casein	20	20
Ching-Shiang oil[b]	3	3
Corn oil	7	7
Vitamin mixture[c]	1	1
Mineral mixture[d]	4	4
Cholesterol	0.2	0.2
Choline chloride	0.2	0.2
L-cystein	0.3	0.3
Cellulose	4	
Chitosan		4
Sucrose	25	25
Corn starch	34.3	34.3
Total	100	100

[a]CE: cellulose group; CS: chitosan group.

[b]Ching-Shiang oil: contained about 26% palmitic acid, 3% stearic acid, 54% oleic acid and 17% linoleic acid.

[c]AIN 76 vitamin mixture: procured from ICN Biochemicals (Costa Mesa, CA).

[d]AIN 76 mineral mixture: procured from ICN Biochemicals (Costa

III. *Collection of Blood and Tissue Samples*

At the end of the experimental period, animals were fasted for 12 hr prior to being sacrificed. Animals were killed 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 (1,750 ×g) at 4°C for 20 min. Liver and the adipose tissues (epididymal + abdominal) from each animal were excised, weighed, and stored at -80°C for analysis.

IV. *Determination of Plasma Lipids Concentration*

Plasma total cholesterol and triglyceride concentrations were determined by an enzymatic method provided by the kits purchased from Audit Diagnostics (Cork, Ireland). The concentrations of VLDL, LDL and HDL in plasma were determined subsequent to plasma ultracentrifugation (194,000 ×g for 3 hr at 10°C)[20]. The total cholesterol and triglyceride levels of the VLDL, LDL and HDL fraction were measured by the same method applied to the plasma.

V. *Determination of Plasma Insulun, Leptin and Glucagon Levels*

The hamster plasma insulin (Mercodia, Uppsala, Sweden), leptin (Assay Designs, Ann Arbor, MI, USA) and glucagon (Linco Research, St. Louis, MO, USA) concentrations were determined according to the procedures provided by the commercially available enzyme immunoassay kits.

VI. *Determination of Plasma Cholesteryl Ester Transfer Protein (CETP) Activity*

Plasma CETP activity was determined flurometrically according to the procedures provided by the kit purchased from Kamiya Biomedical Company (Seattle WA, USA). The excitation wavelength was 465 nm and the emission wavelength was 535 nm.

VII. *Determination of Hepatic Cholesterol and Triglyceride Contents*

Lipids were extracted from liver by the method of Folch *et al.*[21] and solubilized in Triton X-100 according to the method of Carlson and Goldfarb[22]. The hepatic total cholesterol and triglyceride contents were assayed enzymatically by the same method used to measure the concentrations of total cholesterol and triglycerdie in plasma. Free cholesterol concentration in the liver extract was determined according to the procedures provided by the assay kit purchased from Pure Chemical Co., (Osaka,

Japan). The cholesteryl ester concentrations were calculated as the difference between total and free cholesterol.

VIII. *Determination of Fecal Cholesterol and Bile Acid Contents*

Fecal lipids were extracted from dry feces by the method of Folch *et al.*[21]. Fecal cholesterol concentration was assayed by the method used to determine the hepatic total cholesterol concentration. Total fecal bile acids were extracted by the method of Cheng and Lai[23], and quantified enzymatically according to the procedures provided in the assay kit purchased from Randox Laboratories Ltd. (Antrim, UK).

IX. *Statistical Evaluation*

Data were presented as the mean ± standard deviation (SD). Statistical analyses were preformed using Student's *t*-test. The difference was considered significant when $p < 0.05$.

RESULTS

I. *Body Weight and Tissue Weight*

As shown in Table 2, there was no difference on body weight and adipose tissue (epididymal + abdominal) weight between the two groups of animals fed on chitosan or cellulose diet for eight weeks. However, a slight (8%) but statistically significant reduction on relative liver weight was observed in hamsters fed on the chitosan diet when compared with those fed on the cellulose diet.

II. *Plasma Lipids Concentration*

Hamsters fed on the chitosan diet showed no change on the concentration of plasma TG but plasma TC was reduced by 13% compared to those fed on cellulose (Table 3). Statistically significant decreases in plasma VLDL-C (-38.9%) and LDL-C (-12.7%) that resulted in a significant increase in plasma ratio of HDL-C to TC were observed in the animals fed with chitosan (Table 4). No significant change in lipotrotein triglyceride levels was observed between the two dietary groups. In addition, a lower ratio of VLDL-C to VLDL-TG was observed after the chitosan treatment.

III. *Plasma Concentrations of Insulin, Leptin, Glucagon and CETP Activity*

No significant difference in plasma concentrations of insulin, leptin, glucagon and CETP activity was observed between the two dietary groups (Table 3).

IV. *Hepatic Cholesterol and Triglyceride Levels*

Table 5 shows the contents (mg/liver) and the concentrations (mg/g of liver) of total and free cholesterol,

Table 2. Food intake, final body weight, liver weight and adipose tissue weight of hamsters fed on the chitosan and cellulose diets for 8 weeks[a]

Diet	Body weight	Food intake	Liver weight		Adipose tissue weight	
	(g)	(g/day)	(g)	(g/100g body weight)	(g)	(g/100g body weight)
Cellulose	122.5 ± 13.4	7.9 ± 0.4	6.2 ± 1.1	5.0 ± 0.3	2.5 ± 0.7	2.0 ± 0.3
Chitosan	122.2 ± 13.0	7.8 ± 0.6	5.6 ± 0.6	4.6 ± 0.3*	2.7 ± 0.8	2.2 ± 0.5

[a]Values are expressed as mean ± SD for 9 hamsters per dietary group. *Significant difference from the control group at $p < 0.05$.

Table 3. Plasma total cholesterol, triglyceride, insulin, glucagon, leptin and cholesteryl ester transfer protein (CETP) activity in hamsters fed on the chitosan and cellulose diets for 8 weeks[b]

Diet	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	CETP (nmol/mL/hr)	Insulin (pg/mL)	Leptin (pmol/mL)	Glucagon (pg/mL)
Cellulose	237.6 ± 10.7	97.3 ± 38.3	2.86 ± 0.23	1794.6 ± 655.8	111.4 ± 22.8	279.1 ± 83.6
Chitosan	207.3 ± 22.7*	78.5 ± 26.2	2.71 ± 0.15	1387.4 ± 437.6	122.8 ± 21.2	265.2 ± 27.8

[b]Values are expressed as mean ± SD for 9 hamsters per dietary group. *Significant difference from the control group at $p < 0.05$.

Table 4. Plasma lipoprotein cholesterol and triglyceride levels in hamsters fed on the chitosan and cellulose diets for 8 weeks[a]

Diet	Cholesterol (mg/dL)			Cholesterol (mg/dL)				
	VLDL	LDL	HDL	VLDL	LDL	HDL	HDL-C/TC	VLDL-TC/VLDL-TG
Cellulose	47.5 ± 10.5	69.5 ± 5.9	120.1 ± 9.9	38.2 ± 8.9	27.6 ± 14.9	28.6 ± 7.6	0.51 ± 0.03	1.24 ± 0.16
Chitosan	29.0 ± 10.0*	60.7 ± 7.6*	118.0 ± 13.6	36.2 ± 7.5	15.6 ± 12.5	26.9 ± 3.3	0.57 ± 0.03*	0.80 ± 0.31*

[a]Values are expressed as mean ± SD for 9 hamsters per dietary group. *Significant difference from the control group at $p < 0.05$.

Table 5. Liver lipids content in hamsters fed on the chitosan and cellulose diets for 8 weeks[a]

Diet	Total cholesterol		Free cholesterol		Cholesteryl ester		Triglyceride	
	(mg/g)	(mg/liver)	(mg/g)	(mg/liver)	(mg/g)	(mg/liver)	(mg/g)	(mg/liver)
Cellulose	98.2 ± 6.5	608.2 ± 106.1	19.9 ± 2.9	122.7 ± 20.7	78.3 ± 6.4	485.5 ± 93.6	12.8 ± 8.6	74.9 ± 39.0
Chitosan	57.1 ± 11.0*	332.3 ± 66.9*	18.9 ± 2.3	103.8 ± 15.0	38.2 ± 9.2*	235.3 ± 60.6*	9.6 ± 1.8	56.3 ± 11.5

[a]Values are expressed as mean ± SD for 9 hamsters per dietary group. *Significant difference from the control group at $p < 0.05$.

cholesteryl ester and triglyceride in the liver of hamsters treated with chitosan or cellulose, respectively. Chitosan significantly decreased the concentrations as well as the contents of hepatic total cholesterol and cholesteryl ester but had no effect on free cholesterol and triglyceride when compared with cellulose.

V. *Fecal Excretion of Cholesterol and Bile Acid*

Hamsters consuming the chitosan diet had higher fecal cholesterol concentration, as compared to hamsters fed the cellulose diet, at the end of experimental period (2.9 ± 1.0 mg/g for cellulose group; 9.6 ± 2.3 mg/g for chitosan group). In addition, chitosan induced an increase in fecal bile acid concentration in hamsters. The fecal bile acid concentration for the hamsters fed on chitosan diet (4.0 ± 1.6 μmol/g) was significantly higher than that of the animals that received the cellulose diet (1.4 ± 0.7 μmol/g). The average daily fecal excretion of cholesterol and bile acid was significantly enhanced in hamsters fed on the chitosan diet compared to those fed on the cellulose diet (Figures 1 and 2).

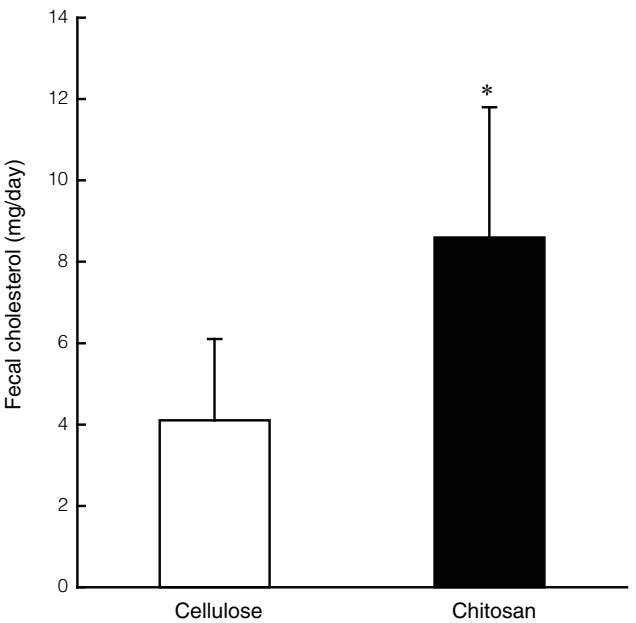

Figure 1. Daily fecal excretion of cholesterol in hamsters fed on a diet containing cellulose or chitosan for 8 weeks. Values are expressed as mean ± SD for 9 hamsters. *Significant difference from the control group at $p < 0.05$.

DISCUSSION

The present study confirms the consistently reported cholesterol-lowering effect of chitosan. In this study, chitosan lowered plasma total cholesterol, VLDL-C and LDL-C concentration in hamsters compared to the cellulose-treated control group. This finding is in agreement with the results of our previous studies in rats[10,11], although with less degree of cholesterol-lowering effect. In addition to the plasma cholesterol-lowering effect, the present study also showed that chitosan significantly enhanced fecal excretion of cholesterol and bile acid and reduced hepatic cholesteryl ester.

Hamsters[24] or rats[25] fed with high cholesterol diet may enhance hepatic cholesterol accumulation that result in increased esterification and storage. Hepatic cholesterol accumulation caused by high intake of dietary cholesterol may down-regulate the activity of 3-hydroxyl-3-methylglutaryl-coenzyme A (HMG-CoA) reductase[26] and the mRNA levels for apoB/E receptor that are associated with decreases in LDL uptake[27]. This study showed that daily fecal excretion of cholesterol was significantly increased by chitosan resulting in a decrease in hepatic cholesterol level primarily in the form of

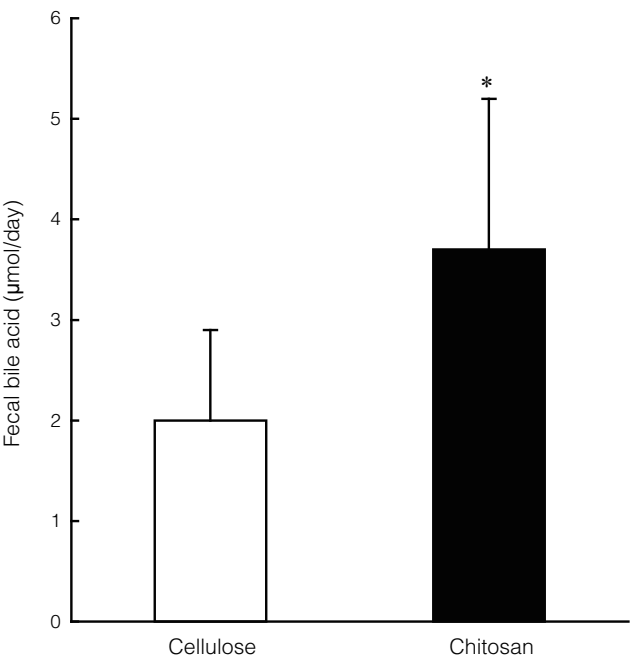

Figure 2. Daily fecal excretion of bile acid in hamsters fed on a diet containing cellulose or chitosan for 8 weeks. Values are expressed as mean ± SD for 9 hamsters. *Significant difference from the control group at $p < 0.05$.

cholesteryl ester. These data were consistent with previous findings in rat that chitosan reduced intestinal absorption of cholesterol[4,28] and reduced hepatic cholesteryl ester accumulation in rats[10,11]. In addition, fecal bile acid excretion, the major degradation of endogenous cholesterol, was enhanced after chitosan feeding to hamsters might stimulate the synthesis of bile acid using cholesterol as the precursor. It has been proposed that in hamsters, cholesterol used for bile acid synthesis is preferentially derived from plasma rather than newly synthesized cholesterol[29]. Although we did not measure hepatic HMG-CoA reductase activity of hamsters in this experiment, LeHoux and Grondin[30] demonstrated lack of significant difference on the hepatic HMG-CoA reductase activity in rats fed a diet enriched in cholesterol and chitosan. Taken together, these results suggested that the hypocholesterolemic effect of chitosan in hamsters may be related to lower absorption of cholesterol and bile acids, resulting in a decrease in hepatic cholesterol accumulation and in an increase in hepatic cholesterol catabolism, and thus leading to an increase in hepatic uptake of plasma cholesterol via the LDL receptor.

Previous studies have reported that the *in vitro* bile acid binding capacity and plasma and liver cholesterol-lowering effect of chitosan were of a magnitude approximately equivalent to that of cholestyramine[31]. Chitosan is dissolved in the aqueous acidic fluid of stomach and the amino groups ($-NH_2$) of chitosan take on hydrogen ions (H^+) to become positively charged tertiary amino group ($-NH_3^+$), as well as leads to an unique intrinsic property of chitosan in term of binding with negatively charged molecules, i.e fatty and bile acids[32]. However, as chitosan enters into the intestine with weak alkaline environment, its hydrogen ions (H^+) may be lost and subsequently diminish its bile acid binding capacity. This might be one of the reasons to explain why chitosan did not increase fecal bile acid excretion in several studies[31,33]. It is possible that the physiochemical properties of chitosan, such as molecular weight, deacetylation and viscosity, may also determine its effect on fecal cholesterol and bile acid excretion. Therefore, the mechanism action of cholesterol-lowering effects between chitosan and cholestyramine appears to be different.

Although the hypolipidemic properties of chitosan are well known, fewer investigators have focus on its effect on lipoprotein metabolism. Many studies have clearly demonstrated that chitosan reduces plasma cholesterol without lowering plasma triglyceride[11,13,14,34,35]. In this study, hamsters fed chitosan significantly decreased plasma VLDL-C without lowering VLDL-TG, and resulted in lower ratio of cholesterol/triglyceride in VLDL fraction, suggesting that the livers from these animals secreted VLDL that reflected their hepatic lipid composition and hepatic VLDL lipid composition may be modified by chitosan. These findings are in line with our previous rat studies; the hypocholesterolemic effect of chitosan was due mainly to a decrease in cholesterol carried in VLDL fraction[10,11]. On the other hand, chitosan feeding in rats have shown to stimulate hepatic fatty acid synthetase activity, which is possibly resulted from the decreased absorption of dietary fat[11]. The increment of de novel fatty acid synthesis may provide substrate for further hepatic TG synthesis and VLDL assembly. In this study, no significant difference in hepatic TG and VLDL-TG was observed between hamsters fed on cellulose diet and chitosan diet. Therefore, it appears that hepatic VLDL secretion is not impaired in hamsters after chitosan treatment. In addition, it is expected that VLDL with a low cholesterol/triglyceride ratio will be efficiently hydrolysed by lipoprotein lipase (LPL), thus leading to formation of a smaller particle with atherogenic potential[36]. However, lower LDL-C concentration was found in hamsters fed on chitosan diet than in those fed on cellulose diet despite a decrease in cholesterol/triglyceride ratio in VLDL caused by chitosan. Plasma cholesterol lowering effect of chitosan in hamsters may be possibly associated with faster VLDL fractional catabolic rates and removed from the circulation by hepatic receptors, since reduced hepatic cholesterol accumulation and enhanced fecal bile acid excretion by chitosan may up regulate hepatic LDL receptor.

Cholesterol ester transfer protein (CETP), is a protein that transfers cholesterol ester from HDL to VLDL and LDL[37], is thought to participate in a pathway of reverse cholesterol transport in which cholesterol, esterified within HDL by the action of lecithin: cholesterol acyltransferase, is transported from peripheral tissue to the liver[38,39]. Some investigators have indicated that chitosan had lowered plasma TC and elevated HDL-C concentration[10,11,34,40]. There was, however, no significant difference in CETP activity between the chitosan group and the cellulose group, whereas animals fed on chitosan had lower plasma TC concentration and higher HDL-C/TC ratio. Thus, the hypocholesterolemic effect of chitosan may not be directly related to its effect on CETP activity.

On the other hand, some previous rat studies showed that chitosan feeding over 7% reduced food intake and increased fecal fat excretion, resulted in reduced average daily energy intake and reduced body weight gain[4,11]. Similar results are found in hamsters, though these effects are diminished when chitosan supplemetation is reduced from 8% to 4%[8]. In this study, however, no significant difference existed in food intake, body weight and adipose tissue weight in hamsters between the two dietary groups. We also found that 4% chitosan feeding in hamsters had no significant effect on plasma hormones, such as leptin, insulin and glucagon levels that are known as a regulator on food intake and energy homeostasis[41,42], suggesting that the energy balance is not altered after long-term feeding of chitosan. Therefore, the long-term chitosan supplementation appears to be useful to induce or to maintain weight loss under unrestricted condition. Since these hormones cannot only influence on body weight

and energy balance but also provide important signals to regulate lipid metabolism as well[43,44] our study first demonstrated that the hypocholesterolemic effect of chitosan also may not be directly related to its effect on hormone regulations.

In conclusion, the findings of this study emphasize the cholesterol-lowering potential of chitosan in hamsters. Chitosan feeding in hamsters may significantly reduce hepatic cholesteryl ester accumulation and lower plasma VLDL cholesterol, which is similar to rats although its plasma cholesterol-lowering effect is lessened. Our study further suggests that these effects of chitosan may be mediated by enhancing fecal excretion of cholesterol and bile acid.

ACKNOWLEDGEMENTS

This study was supported by a grant (NSC 89-2313-B-019-068-A24) from the National Science Council, ROC.

REFERENCES

1. Pekkanen, J., Linn, S., Heiss, G., Suchindran, C. M., Leon, A., Rifkind, B. M. and Tyroler, H. A. 1990. Ten-year mortality from cardiovascular disease in relation to cholesterol level among men with and without pre-existing cardiovascular disease. N. Engl. J. Med. 322: 1700-1707.
2. Brown, L., Rosner, B., Willett, W. W. and Sacks, F. M. 1999. Cholesterol-lowering effects of dietary fiber: A meta-analysis. Am. J. Clin. Nutr. 69: 30-42.
3. Fernandez, M. L. 2001. Soluble fiber and nondigestible carbohydrate effects on plasma lipids and cardiovascular risk. Curr. Opin. Lipidol. 12: 35-40.
4. Gallaher, C. M., Munion, J., Hesslink, R., Wise, J. and Gallaher, D. D. 2000. Cholesterol reduction by glucomannan and chitosan is mediated by change in cholesterol absorption and bile acid and fat excretion in rats. J. Nutr. 30: 2753-2759.
5. Kanauchi, O., Deuchi, K., Imasato, Y., Shizukuishi, M. and Kobayashi, E. 1995. Mechanism for the inhibition of fat digestion by chitosan and for the synergistic effect of ascorbate. Biosci. Biotechnol. Biochem. 59: 786-790.
6. Deuchi, K., Kanauchi, O., Imasato, Y. and Kobayashi, E. 1995. Effect of the viscosity or deacetylation degree of chitosan on fecal fat excreted from rats fed on a high-fat diet. Biosci. Biotechnol. Biochem. 59: 781-785.
7. Sugano, M., Fujikawa, Y., Hiratsuji, K., Nakashima, N. and Hasegawa, Y. 1980. A novel use of chitosan as a hypocholesterolemic agent in rats. Am. J. Clin. Nutr. 33: 787-793.
8. Trautwein, E. A., Jurgeensen, U. and Erbersdobler, H. F. 1997. Cholesterol-lowering effect and preventing action of chitosans with different degrees of deacetylation in hamsters fed cholesterol-rich diets. Nutr. Res. 17: 1053-1065.
9. Ormrod, D. J., Holmes, C. C. and Miller, T. E. 1998. Dietary chitosan inhibits hypercholesterolaemia and atherogenesis in the apolipoprotein E-deficient mouse model of atherosclerosis. Atherosclerosis 138: 329-334.
10. Chiang, M. T., Yao, H. T. and Chen, H. C. 2000. Effect of dietary chitosans with different viscosity on plasma lipids and lipid peroxidation in rats fed on a diet enriched with cholesterol. Biosci. Biotechnol. Biochem. 64: 965-971.
11. Yao, H. T. and Chiang, M. T. 2002. Plasma lipoprotein cholesterol in rats fed a diet enriched in chitosan and cholesterol. J. Nutr. Sci. Vitaminol. 48: 379-383.
12. Bokura, H. and Kobayashi, S. 2003. Chitosan decreases total cholesterol in women: A randomized, double-blind, placebo-controlled trial. Eur. J. Clin. Nutr. 57: 721-725.
13. Mhurchu, C. N., Poppitt, S. D., McGill, A. T., Leahy, F. E., Bennett, D. A., Lin, R. B., Ormrod, D., Ward, L., Strik, C. and Rodgers, A. 2004. The effect of the dietary supplement, chitosan, on body weight: A randomised controlled trial in 250 overweight and obese adults. Int. J. Obes. Relat. Metab. Disord. 28: 1149-1156.
14. Pittler, M. H., Abbot, N. C., Harkness, E. F. and Ernst, E. 1999. Randomized, double-blind trial of chitosan for body weight reduction. Eur. J. Clin. Nutr. 53: 379-381.
15. Fernandez, M. L., Wilson, T. A., Conde, K., Vergara-Jimenez, M. and Nicolosi, R. J. 1999. Hamsters and guinea pigs differ in their plasma lipoprotein cholesterol distribution when fed diets varying in animal protein, soluble fiber, or cholesterol content. J. Nutr. 129: 1323-1332.
16. Dorfman, S. E., Smith, D. E., Osgood, D. P. and Lichtenstein, A. H. 2003. Study of diet-induced changes in lipoprotein metabolism in two strains of Golden-Syrian hamsters. J. Nutr. 133: 4183-4188.
17. Bagdade, J. D., Bierman, E. L. and Porte, D. Jr. 1967. The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and non-diabetic subjects. J. Clin. Invest. 46: 1549-1557.
18. Havel, P. J., Kasim-Karakas, S., Mueller, W., Johnson, P. R., Gingerich, R. L. and Stern, J. S. 1996. Relationship of plasma leptin to plasma insulin and adiposity in normal weight and overweight women: Effects of dietary fat content and sustained weight loss. J. Clin. Endocrinol. Metab. 81: 4406-4413.
19. Daggy, B. P., O'Connell, N. C., Jerdack, G. R., Stinson, B. A. and Setchell, K. D. 1997. Additive hypocholesterolemic effect of psyllium and cholestyramine in the hamster: Influence on fecal sterol and bile acid profiles. J. Lipid Res. 38: 491-502.
20. Takehisa, F. and Suzuki, Y. 1990. Effect of guar gum and cholestyramine on plasma lipoprotein cholesterol in rats. J. Jpn. Soc. Nutr. Food Sci. 43: 269-274.
21. Folch, J., Lees, M. and Sloane-Stanley, G. M. 1957. A purification of total lipid from animal tissue. J. Biol. Chem. 226: 497-509.

22. Carlson, E. and Goldford, S. 1979. A sensitive enzymatic method for determination of free and esterified tissue cholesterol. Clin. Chem. Acta. 79: 575-582.
23. Cheng, H. H. and Lai, M. H. 2000. Fermentation of resistant rice starch produces propionate reducing serum and hepatic cholesterol in rats. J. Nutr. 130: 1991-1995.
24. Billett, M. A., Bruce, J. S., White, D. A., Bennett, A. J. and Salter, A. M. 2000. Interactive effects of dietary cholesterol and different saturated fatty acids on lipoprotein metabolism in the hamster. Br. J. Nutr. 84: 439-447.
25. Fungwe, T. V., Cagen, L., Wilcox, H. G. and Heimberg, M. 1992. Regulation of hepatic secretion of very low density lipoprotein by dietary cholesterol. J. Lipid Res. 33: 179-191.
26. Spady, D. K. and Dietschy, J. 1988. M. Interaction of dietary cholesterol and triglycerides in the regulation of hepatic low density lipoprotein transport in the hamster. J. Clin. Invest. 81: 300-309.
27. Horton, J. D., Cuthbert, J. A. and Spady, D. K. 1993. Dietary fatty acids regulate hepatic low density lipoprotein (LDL) transport by altering LDL receptor protein and mRNA levels. J. Clin. Invest. 92: 743-749.
28. Vahouny, G. V., Satchithanandam, S., Cassidy, M. M., Lightfoot, F. B. and Furda, I. 1983. Comparative effects of chitosan and cholestyramine on lymphatic absorption of lipids in the rat. Am. J. Clin. Nutr. 38: 278-284.
29. Scheibner, J., Fuchs, M., Hormann, E., Tauber, G. and Stange, E. F. 1994. Biliary cholesterol secretion and bile acid formation in the hamster: The role of newly synthesized cholesterol. J. Lipid Res. 35: 690-697.
30. LeHoux, J. G. and Grondin, F. 1993. Some effects of chitosan on liver function in the rat. Endocrinology 132: 1078-1084.
31. Sugano, M., Fujikawa, Y., Hiratsuji, K., Nakashima, N., Fukada, N. and Hasegawa, Y. 1980. A novel use of chitosan as a hypocholesterolemic agent in rats. Am. J. Clin. Nutr. 33: 787-793.
32. Ylitalo, R., Lehtimen, S., Wuolijoki, E., Ylitalo, P. and Lehtimaki, T. 2002. Cholesterol-lowering properties and safety of chitosan. Arzneimittelforschung 52: 1-7.
33. Fukada, Y., Kimura, K. and Ayaki, Y. 1991. Effect of chitosan feeding on intestinal bile acid metabolism in rats. Lipids 26: 395-399.
34. Ausar, S. F., Morcillo, M., Leon, A. E., Ribotta, P. D., Masih, R., Vilaro Mainero, M., Amigone, J. L., Rubin, G., Lescano, C., Castagna, L. F., Beltramo, D. M., Diaz, G. and Bianco, I. D. 2003. Improvement of HDL- and LDL-cholesterol levels in diabetic subjects by feeding bread containing chitosan. J. Med. Food 6: 397-399.
35. Metso, S., Ylitalo, R., Nikkila, M., Wuolijoki, E., Ylitalo, P. and Lehtimaki, T. 2003. The effect of long-term microcrystalline chitosan therapy on plasma lipids and glucose concentrations in subjects with increased plasma total cholesterol: A randomised placebo-controlled double-blind crossover trial in healthy men and women. Eur. J. Clin. Pharmacol. 59: 741-746.
36. Schreier, L., Berg, G., Zago, V., Gonzalez, A. I. and Wikinski, R. 2002. Kinetics of *in vitro* lipolysis of human very low-density lipoprotein by lipoprotein lipase. Nutr. Metab. Cardiovasc. Dis. 12: 13-18.
37. Tall, A. R. 1993. Plasma cholesteryl ester transfer protein. J. Lipid Res. 34: 1255-1274.
38. Czarnecka, H. and Yokoyama, S. 1995. Lecithin:cholesterol acyltransferase reaction on cellular lipid released by free apolipoprotein-mediated efflux. Biochemistry 34: 4385-4392.
39. Tsutsumi, K., Hagi, A. and Inoue, Y. 2001. The relationship between plasma high density lipoprotein cholesterol levels and cholesteryl ester transfer protein activity in six species of healthy experimental animals. Biol. Pharm. Bull. 24: 579-581.
40. Maezaki, Y., Keisuke, T., Nakagawa, Y., Kawai, Y., Akimoto, M., Tsugita, T., Takekawa, W., Terada, A., Hara, H. and Mitsuoka, T. 1993. Hypocholesterolemic effect of chitosan in adult males. Biosci. Biotechnol. Biochem. 57: 1439-1444.
41. Woods, S. C., Seeley, R. J., Porte, D. Jr. and Schwartz, M. W. 1998. Signals that regulate food intake and energy homeostasis. Science 280: 1378-1383.
42. Baile, C. A., Della-Fera, M. A. and McLaughlin, C. L. 1983. Hormones and feed intake. Proc. Nutr. Soc. 42: 113-127.
43. Guettet, C., Mathe, D., Navarro, N. and Lecuyer, B. 1989. Effects of chronic glucagon administration on rat lipoprotein composition. Biochim. Biophys. Acta. 1005: 233-238.
44. Shimamura, M., Matsuda, M., Ando, Y., Koishi, R., Yasumo, H., Furukawa, H. and Shimomura, I. 2004. Leptin and insulin down-regulate angiopoietin-like protein 3, a plasma triglyceride-increasing factor. Biochem. Biophys. Res. Commun. 322: 1080-1085.