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Chitosan supplementation lowers serum lipids and maintains normal calcium, magnesium, and iron status in hyperlipidemic patients

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Abstract

Chitosan is the most abundant natural amino polysaccharide and is being used as a new source of dietary fiber. The aim of this study was to investigate the effects of water-soluble and water-insoluble chitosan supplementation on blood lipid profiles and mineral status, including calcium, magnesium, and iron, in elderly hyperlipidemic patients. Sixty volunteers with serum total cholesterol concentration of greater than 5.2 mmol/L were randomly divided into 3 groups of 20 each. The treatment groups received oral chitosan, one group receiving water-soluble chitosan and the other receiving water-insoluble chitosan. They received 2 tablets (300 mg/tablet, with each tablet containing 52% chitosan). The third group was the placebo group. Supplements were given 3 times a day, before meals, for 8 weeks with no other dietary restrictions. Serum, 24-hour urine samples, and dietary records were collected and analyzed at 0, 4, and 8 weeks. Total cholesterol significantly declined by 7.5% in the water-soluble and by 8.9% in the water-insoluble chitosan groups over 8 weeks. Significant reductions in serum transferrin levels and mean corpuscular hemoglobin concentrations were observed after 8 weeks of water-soluble chitosan supplementation, but values remained within the reference range. In conclusion, both water-soluble and water-insoluble chitosan supplementation over 8 weeks lowered blood lipids and maintained normal calcium, magnesium, and iron status in elderly hyperlipidemic patients.

Keywords: Chitosan; Hyperlipidemia; Cholesterol; Calcium; Magnesium; Transferring; Elderly; Human

1. Introduction

Serum lipid abnormalities result in increasing vascular risks; hence, aggressive treatment of hyperlipidemia is recommended. Advancing age and hypercholesterolemia have been widely considered cardiovascular risk factors in the elderly [1]. Intervention with lipid management is necessary for people with obesity, diabetes, and renal disease [2-4]. The use of functional foods in diet is a part of the management of dyslipidemia [5].

Chitosan, a polymer glucosamine, is industrially produced by the deacetylation of chitin obtained from crab and prawn shells and is insoluble in water [6]. Chitosan is being used as a new source of dietary fiber because it is biocompatible, has low toxicity in animal organs [7], and its chemical structure is similar to that of cellulose and not cleaved by digestive enzymes in humans [8]. It contains one amino group per residue, which produces high-positive-charge densities in acidic solutions, unlike other dietary fibers. Chitosan is considered a potential ingredient of functional foods because of its beneficial activity in lipid disorders [9].

Several studies have reported that chitosan has a hypocholesterolemic action in animal models [10] and healthy men [11]. In recent years, water-soluble chitosan,

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which is more reactive than water-insoluble chitosan, has been manufactured and applied in many fields [12-14]. However, dietary fiber (containing cellulose, hemicellulose, and lignin) may influence the availability of minerals, such as calcium and magnesium [15]. Animal studies have found that dietary chitosan possibly arrests the absorption of calcium [16-18]. There has been no study on the effects of water-soluble or water-insoluble chitosan in elderly people with mild hyperlipidemia. The objective of the current study was to investigate the 2 different kinds of dietary chitosan supplementation on blood lipid profiles and serum minerals, including calcium, magnesium, and iron, in elderly people with mild hyperlipidemia.

2. Methods and materials

2.1. Subjects

Outpatients who had a fasting serum cholesterol level of 5.2 mmol/L or higher were recruited [19]. The exclusion criteria included having an allergy to crustaceans, gastrointestinal discomfort, poor cardiovascular disease control, and age less than 18 years. Before the start of the study, all lipid-lowering drugs were terminated for a period of 1 week. The study was approved by the Institutional Review Board on Human Subjects Committee at the Taipei Medical University, Taiwan. Informed consent forms were signed by all subjects before starting the study.

2.2. Dietary supplementation

Two kinds of chitosan, with a degree of deacetylation of 92% (Shinera, Taipei, Taiwan), were used in this study. One was water-soluble and had a low molecular weight (average, 30-50 kDa). The other was water-insoluble, with average molecular weight of 100 to 150 kDa. One tablet (300 mg) contained 52% chitosan. The appearance of the placebo tablet was the same as a chitosan tablet and consisted of lactose and starch.

2.3. Experimental design

The study was single blinded. Patients were randomly divided into 3 groups of 20 each. The treatment groups received oral chitosan, one group receiving water-soluble chitosan and the other water-insoluble chitosan, and the third group received placebo tablets. The protocol consisted of taking 2 tablets, 3 times a day before meals for 8 weeks. Patients were asked to continue their normal living routines without restricting their diets or altering their eating habits. We collected blood centrifuged at 3500 rpm for 15 minutes at 4°C and supernatants for analysis at 0, 4, and 8 weeks after subjects had fasted for at least 8 hours.

2.4. Analyses of anthropometry and serum lipid profiles

Height was recorded to the nearest 0.5 cm at the beginning of the study. Body weight was measured by segmental, multifrequency bioelectrical impedance analysis (InBody 3.0, Biospace, Seoul, Korea) at each visit. Blood was analyzed for total cholesterol (TC), triacylglycerol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) in serum. The TC and triacylglycerol concentrations were measured enzymatically [20,21] on a Hitachi 7450 analyzer (Hitachi, Japan). The LDL-C concentrations were determined by homogeneous turbidimetric test [22], and HDL-C concentrations were determined by homogeneous enzymatic colorimetric assay [23] on the same analyzer.

2.5. Determination of serum and urinary mineral status

To monitor minerals in serum, the following laboratory parameters were determined at 0, 4, and 8 weeks: urinary calcium and urinary magnesium from urine samples; and serum calcium, serum magnesium, and iron status including serum iron, ferritin, transferrin, transferrin saturation, total iron-binding capacity (TIBC), hemoglobin, and mean corpuscular hemoglobin concentration (MCHC) from blood samples. Serum calcium and urinary calcium were measured by the *O*-cresolphthalein complex colorimetric method; serum magnesium and urinary magnesium were measured by the xylidyl blue method; and serum iron was measured by the Nitroso-PSAP method [24] using a Hitachi 7170S autoanalyzer. Ferritin was determined by an immunoturbidimetric assay on the same analyzer. Transferrin was measured by nephelometry. Transferrin saturation was calculated from the monthly blood tests and was based on the formula: (serum iron / TIBC) × 100. Serum iron plus the unsaturated iron-binding capacity equals TIBC.

2.6. Assessment by nutritional survey

Nutrition was assessed from a 3-day diet record for 2 weekdays and 1 weekend day in weeks 0, 4, and 8, and information was collected on the day the subject returned. We mainly assessed the dietary intake of total energy, carbohydrates, protein, and fat to confirm that subjects did not change their eating habits. Dietary calcium, magnesium, iron, and vitamin C intake were analyzed at the same time.

Table 1
Characteristics of hyperlipidemic patients in the treatment and placebo groups

Parameter	Treatment group		
	Water-soluble	Water-insoluble	Placebo
No. of patients (male, female)	20 (6, 14)	20 (6, 14)	20 (6, 14)
Age (y)	61.1 ± 2.5	61.6 ± 2.7	63.9 ± 2.6
Weight (kg)			
Week 0	66.2 ± 2.3	67.9 ± 2.8	67.2 ± 2.1
Week 8	65.9 ± 3.9	67.4 ± 2.9	66.7 ± 2.1
BMI (kg/m^2)			
Week 0	27.1 ± 0.9	26.9 ± 0.8	27.6 ± 0.9
Week 8	27.0 ± 1.6	26.8 ± 0.8	27.6 ± 1.0

Values are presented as mean ± SEM.
BMI indicates body mass index.
Analyses by 2-way ANOVA followed by LSD test showed no significant change.

Dietary record data were measured by the Nutritional Chamberlain Line, Nutritionist Edition, version 2002, E-Kitchen Business Corp, Taiwan, which is referred to in the 1998 database of the Department Of Health, Taiwan.

2.7. Statistical analysis

Results are presented as mean ± SEM. Two-way analysis of variance (ANOVA) was performed separately for each of the variables to assess mean differences across time (baseline, 4 weeks, and 8 weeks) and group (water-soluble, water-insoluble, and placebo) and to calculate Fisher's least significant difference (LSD) in SAS, version 8.2 (SAS Institute, Cary, NC, USA). Differences were considered significant if P value is less than .05.

3. Results

3.1. Patients' characteristics in treatment and placebo groups

Sixty patients (18 men and 42 women), with average age more than 60 years, completed the study. Baseline characteristics were not significantly different for patients in the 2 chitosan groups compared with placebo (Table 1). Neither body weight nor body mass index of the patients changed significantly in the 3 groups throughout the test period. There were no significant differences between men and women in anthropometric measurements or serum lipid profiles. Therefore, results are combined for both sexes.

3.2. Effects of dietary chitosan ingestion on serum lipid levels

Water-insoluble chitosan significantly reduced TC levels compared with the placebo ($P < .05$) (Table 2). At 8 weeks, the TC level decreased by 7.5% in the water-soluble chitosan group and 8.9% in the water-insoluble chitosan group (both $P < .05$). Serum LDL-C levels decreased by about 10% and 6%, respectively, in these groups over 8 weeks. There were no significant differences in serum HDL-C and triacylglycerol levels among 3 groups during the experiment. The TC/HDL-C ratio decreased from 5.07 ± 0.26 to 4.63 ± 0.18 over 8 weeks with water-soluble chitosan supplement but did not change with ingestion of water-insoluble chitosan.

Table 2
Effect of water-soluble and water-insoluble chitosan on serum lipid profiles in hyperlipidemic patients

Parameter and week	Treatment group		
	Water-soluble	Water-insoluble	Placebo
TC (mmol/L)			
0	6.29 ± 0.16	6.88 ± 0.12	6.07 ± 0.14
8	5.82 ± 0.15*	5.53 ± 0.19†	6.08 ± 0.17
Triacylglycerol (mmol/L)			
0	2.45 ± 0.34	1.98 ± 0.24	2.03 ± 0.25
8	2.25 ± 0.27	1.73 ± 0.24	1.93 ± 0.26
LDL-C (mmol/L)			
0	3.93 ± 0.16	3.63 ± 0.11	3.83 ± 0.13
8	3.53 ± 0.16	3.42 ± 0.17	3.82 ± 0.16
HDL-C (mmol/L)			
0	1.28 ± 0.07	1.46 ± 0.07	1.32 ± 0.09
8	1.28 ± 0.06	1.28 ± 0.08	1.41 ± 0.07
TC/HDL-C			
0	5.07 ± 0.26	4.26 ± 0.19	4.77 ± 0.24
8	4.63 ± 0.18	4.55 ± 0.21	4.45 ± 0.22

Values are presented as mean ± SEM.

* $P < .05$ for difference from baseline. Within a column, difference from baseline was analyzed by 2-way ANOVA followed by LSD test.

† $P < .05$ for differences between treatment groups. Within a row, differences between the chitosan groups and the placebo group were analyzed by 2-way ANOVA followed by LSD test.

Table 3
Effect of water-soluble and water-insoluble chitosan on the calcium, magnesium, and iron levels in serum of hyperlipidemic patients

Parameter and week	Treatment group		
	Water-soluble	Water-insoluble	Placebo
Calcium (mg/dL)			
0	9.6 ± 0.1	9.3 ± 0.1	9.5 ± 0.1
4	9.4 ± 0.1	9.6 ± 0.1	9.5 ± 0.1
8	9.5 ± 0.1	9.5 ± 0.1	9.5 ± 0.1
Magnesium (mg/dL)			
0	2.1 ± 0.0	2.1 ± 0.0	2.1 ± 0.0
4	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
8	2.0 ± 0.0	2.1 ± 0.0†	2.0 ± 0.0
Iron, serum iron (μg/dL)			
0	89.3 ± 5.3	97.7 ± 8.1	95.5 ± 7.4
4	89.7 ± 5.1	100.3 ± 6.2	92.8 ± 7.4
8	96.5 ± 6.2	102.0 ± 9.8	101.3 ± 7.0
Ferritin (ng/dL)			
0	177.1 ± 26.4	191.9 ± 26.8	186.4 ± 29.6
4	157.6 ± 19.2	182.1 ± 25.0	181.5 ± 28.8
8	167.8 ± 18.5	204.1 ± 34.9	200.9 ± 28.1
Transferrin (mg/dL)			
0	259.0 ± 9.4	254.1 ± 9.2	243.0 ± 7.5
4	231.3 ± 8.9*,†	247.5 ± 7.2	258.7 ± 8.9
8	249.7 ± 9.7	266.8 ± 11.0	262.5 ± 6.4
Transferrin saturation (%)			
0	27.58 ± 1.98	31.89 ± 3.34	27.46 ± 2.41
4	26.59 ± 1.60	28.87 ± 1.87	28.09 ± 2.28
8	28.91 ± 1.90	29.52 ± 2.57	32.67 ± 3.37
TIBC (μg/dL)			
0	330.8 ± 11.4	329.9 ± 17.6	360.1 ± 14.8
4	341.2 ± 11.8	351.3 ± 10.2	332.5 ± 7.7
8	337.8 ± 11.4	345.3 ± 12.5	336.7 ± 17.8
Hemoglobin (g/dL)			
0	13.44 ± 0.31	13.10 ± 0.33	13.39 ± 0.23
4	13.39 ± 0.32	13.22 ± 0.35	13.51 ± 0.24
8	13.52 ± 0.30	13.33 ± 0.39	13.48 ± 0.37
MCHC			
0	35.37 ± 0.25	34.13 ± 0.24	35.28 ± 0.36
4	34.35 ± 0.25*,†	34.63 ± 0.23	33.54 ± 0.23†
8	33.90 ± 0.17†	34.57 ± 0.22	33.91 ± 0.21†

Values are presented as mean ± SEM.

* $P < .05$ for difference from baseline. Within a column, difference from baseline was analyzed by 2-way ANOVA followed by LSD test.

† P Within a row, differences between the chitosan groups and the placebo group were analyzed by 2-way ANOVA followed by LSD test ($P < .05$).

3.3. Effect of dietary chitosan ingestion on the mineral status in serum

Table 3 shows the effects of water-soluble and water-insoluble chitosan ingestion on the serum mineral level. Serum calcium levels did not differ among the 3 groups at

the beginning or the end of the study period. Serum magnesium concentrations were marginally higher in the water-insoluble chitosan group than in the placebo group at 8 weeks ($P = .049$). After week 4, the water-soluble chitosan group had significantly lower serum transferrin ($P = .03$) and significantly higher MCHC ($P = .03$) levels than the placebo group. Both serum transferrin and MCHC levels were significantly reduced after ingestion of water-soluble chitosan for 4 weeks ($P < .05$) but remained within the normal clinical range, and the change in serum transferrin was no longer significant at week 8. The placebo group had significantly decreased MCHC after 4 weeks. No differences in either chitosan group were observed for serum iron, ferritin, transferrin saturation, TIBC, or hemoglobin when compared with values of the placebo group or values at week 0. We noted no differences in urinary calcium excretion or urinary magnesium excretion after ingestion of the 2 kinds of chitosan for 8 weeks (Fig 1).

3.4. Results of the nutritional survey

There were no changes in the dietary intake of total energy, carbohydrates, protein, fat, cholesterol, or dietary fiber (except for chitosan) at baseline and during the supplementation periods in the 3 groups (data not shown). Analyses of the intake of dietary calcium, magnesium, and iron, as well as vitamin C, which can assist the absorption of dietary iron, indicated that they did not significantly differ among 3 groups for the 8 weeks of supplementation (Table 4). Throughout the test period, no clinically problematic symptoms such as diarrhea or constipation were observed.

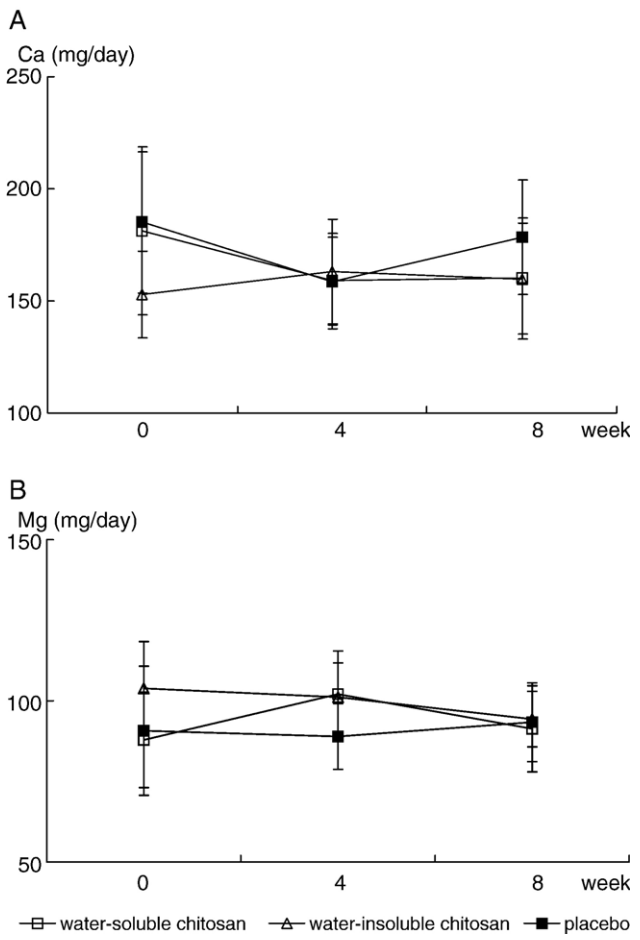

Fig. 1. Urine excretion of calcium (A) and magnesium (B) levels after administration of water-soluble chitosan, water-insoluble chitosan, and placebo in hyperlipidemic patients. Values are mean ± SEM.

Table 4
Daily dietary nutrient intake of all hyperlipidemic patients

Parameter and week	Treatment groups		
	Water-soluble	Water-insoluble	Placebo
Calcium (mg)			
0	479 ± 88	691 ± 153	625 ± 94
4	356 ± 46	415 ± 61*	455 ± 43
8	309 ± 49	336 ± 40*	521 ± 93
Magnesium (mg)			
0	227 ± 22	211 ± 18	249 ± 25
4	205 ± 17	210 ± 19	223 ± 20
8	193 ± 20	183 ± 14	216 ± 21
Iron (mg)			
0	8 ± 1	9 ± 1	9 ± 1
4	7 ± 0	9 ± 1	9 ± 1
8	7 ± 1	8 ± 1	9 ± 1
Vitamin C (mg)			
0	141 ± 45	124 ± 18	170 ± 39
4	99 ± 19	140 ± 30	136 ± 18
8	127 ± 25	178 ± 35	141 ± 25

Values are presented as mean ± SEM.
* $P < .05$ for difference from baseline. Within a column, difference from baseline was analyzed by 2-way ANOVA followed by LSD test.

4. Discussion

Both water-soluble and water-insoluble chitosan reduced TC and LDL-C levels in these older-aged patients with hypercholesterolemia. A possible reason for the observation of decreased cholesterol is that both types of chitosan greatly increase fecal elimination of steroids, which has been reported in animal and human studies [11,25-27]. As the absorption of cholesterol from diet and from bile acids decreases, the hepatic bile acid pool is depleted, and more cholesterol is diverted to produce bile acids, thus reducing blood lipid levels. Many studies have reported that water-soluble dietary fiber can reduce the serum cholesterol level because of excretion of bile acids and lipids [28,29].

In the present study, we expected that the serum hypocholesterolemic effect of water-soluble chitosan would be better than that of the water-insoluble form. However, water-soluble chitosan reduced the serum cholesterol level by 7.5%, and water-insoluble chitosan reduced it by 8.9%. Holme [30] showed that for every 1% reduction in cholesterol, an estimated 2.5% reduction in coronary heart disease incidence is indicated, but cholesterol reduction must be at least 8% to 9% to be effective in lowering total mortality. As to the effect on coronary heart disease incidence, water-insoluble chitosan supplementation, with

a reduction of 8.9%, may be better than water-soluble chitosan. In addition, our results also showed that decreases in LDL-C were 10% and 6% after consumption of water-soluble and water-insoluble chitosan, respectively; although there was no significant difference. Water-soluble chitosan supplementation also reduced the TC/HDL-C ratio from 5.07 to 4.63; a ratio of 5.0 or more is a risk factor for cardiovascular disease [31]. These data suggest that water-soluble chitosan has greater effectiveness in reducing serum LDL-C levels, a cardiovascular risk factor. Dietary chitosan has been shown to improve blood lipid profiles in diabetic and renal disease patients [32,33] and may prevent atherogenesis in vessels [34]. Therefore, we propose that both water-soluble chitosan and water-insoluble chitosan could alter serum TC and LDL-C in elderly hyperlipidemic patients and serve as a useful dietary supplement for preventing hyperlipidemia.

The present study is the first to investigate the effect of dietary chitosan supplementation on the calcium and magnesium status in elderly hyperlipidemic patients. Deuchi et al [16] reported that 5% chitosan fed to rats decreased bone mineral content and mineral absorption, including calcium, magnesium, and iron, in comparison with rats fed cellulose. Wada et al [17] noted that the whole-body retention of ^{47}Ca decreased, and the urinary excretion of ^{47}Ca significantly increased in rats fed a 5% chitosan diet compared with rats fed a cellulose diet. Yang et al [18] demonstrated that the habitual intake of dietary chitosan with a low Ca intake reduced bone mass by increasing urinary Ca excretion in ovariectomized stroke-prone spontaneously hypertensive rats. These studies, mainly conducted in animals, suggest that dietary chitosan may affect calcium metabolism. In our study, serum magnesium levels of the water-insoluble chitosan group significantly differed from those of the placebo group but remained in the reference range. It is known that urinary calcium excretion is an indicator of body calcium retention [35] and that hypomagnesemia or magnesium deficiency may be a risk factor for coronary artery disease [36]. In the present study, we detected no change in serum or urine calcium and magnesium or in the dietary intake between the 2 chitosan groups. These results indicate that the chitosan supplementation that was provided in this study does not influence calcium and magnesium in blood.

The effect of dietary chitosan on the serum iron status of humans is unclear. The iron levels of subjects with hypercholesterolemia were still in a reference range after ingestion of water-soluble or water-insoluble chitosan according to Herbert [37], who summarized the sequential stages of the iron status range from iron overload to iron-deficiency anemia. Although significant decreases in both serum transferrin and MCHC levels in the water-soluble chitosan group were observed, the levels of serum transferrin and MCHC remained in the reference range. Serum transferrin levels can also reflect short-term storage of the iron pool in tissue, and minute amounts of circulating ferritin are closely correlated with body iron stores. The relationship of transferrin and MCHC can be explained by erythroblasts receiving iron from transferrin by endocytosis. The transferrin level is low, and then the MCHC level increases. In our study, MCHC values were influenced by ingestion of water-soluble chitosan after 4 weeks but remained within the reference range.

An in vitro study showed that iron was selectively absorbed by chitosan, with 100% deacetylation, at a lower pH [38]. One clinical study reported that ingestion of chitosan (average molecular weight 27 kDa, 89% deacetylation) effectively improved serum hemoglobin levels of patients with chronic renal failure [33]. Conversely, hemoglobin and serum iron were not altered in rats fed chitosan with a molecular weight of 500 to 1000 kDa and 70% deacetylation. In the present study, we thought that water-insoluble chitosan affected the absorption of some minerals but observed differences in serum transferrin and MCHC levels in subjects ingesting water-soluble 925 deacetylated chitosan of average molecular weight of 30 to 50 kDa. Water-insoluble chitosan, with an average molecular weight of 100 to 150 kDa and 92% deacetylated, had no effect on iron status. Possibly, the molecular weight of dietary chitosan might influence the absorption and metabolism of iron. Therefore, the effect of dietary chitosan on iron metabolism in humans needs more studies in the future to investigate the iron bioavailability of different chitosan supplements. We considered that short-term ingestion of water-soluble and water-insoluble chitosan may not influence serum iron status.

In conclusion, we found that water-soluble or water-insoluble chitosan supplementation lowers blood lipid profiles in hypercholesterolemic patients. In addition, the chitosan did not influence calcium, magnesium, and iron status during the 8-week study.

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References

[1] Davidson MH, Kurlandsky SB, Kleinpell RM, Maki KC. Lipid management and the elderly. Prev Cardiol 2003;6:128-33.
[2] Orzano AJ, Scott JG. Diagnosis and treatment of obesity in adults: an applied evidence-based review. J Am Board Fam Pract 2004;17:359-69.
[3] Scheen AJ. Management of the metabolic syndrome. Minerva Endocrinol 2004;29:31-45.
[4] Logar CM, Herzog CA, Beddhu S. Diagnosis and therapy of coronary artery disease in renal failure, end-stage renal disease, and renal transplant populations. Am J Med Sci 2003;325:214-27.
[5] Thompson GR. Management of dyslipidaemia. Heart 2004;90:949-55.
[6] Muzzarelli AAA. Enzymatic synthesis of chitin and chitosan, occurrence of chitin. In: Muzzarelli AAA, editor. Chitin. New York: Pergamon Press; 1977. p. 5-17.

[7] Arai K, Minumari K, Fujita T. On the toxicity of chitosan. Bull Tokai Reg Fish Lab 1968;56:889-92.
[8] Furda I. Aminopolysaccharides—their potential as dietary fiber. In: Furda I, editor. Unconventional sources of dietary fiber. ACS Symposium Series 214. Washington (DC): American Chemical Society; 1983. p. 105-22.
[9] Koide SS. Chitin-chitosan: properties, benefits and risks. Nutr Res 1998;18:1091-101.
[10] Sugano M, Fujikawa Y, Hiratsuji K, Nakashima N, Fukuda N, Hasegawa Y. A novel use of chitosan as a hypocholesterolemic agent in rats. Am J Clin Nutr 1980;33:787-93.
[11] Maezaki Y, Tsuji K, Nakagawa Y, Kawai Y, Akimoto M, Tsugita T, et al. Hypocholesterolemic effect of chitosan in adult males. Biosci Biotechnol Biochem 1993;57:1439-44.
[12] Takimoto H, Hasegawa M, Yagi K, Nakamura T, Sakaeda T, Hirai M. Proapoptotic effect of a dietary supplement: water soluble chitosan activates caspase-8 and modulating death receptor expression. Drug Metab Pharmacokinet 2004;19:76-82.
[13] Kim MS, Sung MJ, Seo SB, Yoo SJ, Lim WK, Kim HM. Water-soluble chitosan inhibits the production of pro-inflammatory cytokine in human astrocytoma cells activated by amyloid beta peptide and interleukin-1beta. Neurosci Lett 2002;321:105-9.
[14] Richardson SC, Kolbe HV, Duncan R. Potential of low molecular mass chitosan as a DNA delivery system: biocompatibility, body distribution and ability to complex and protect DNA. Int J Pharm 1999;178:231-43.
[15] Reinhold JG, Faradji B, Abadi P, Ismail-Beigi F. Decreased absorption of calcium, magnesium, zinc and phosphorus by humans due to increased fiber and phosphorus consumption of wheat bread. J Nutr 1976;106:493-503.
[16] Deuchi K, Kanauchi O, Shizukuishi M, Kobayashi E. Continuous and massive intake of chitosan affects mineral and fat-soluble vitamin status in rats fed on a high-fat diet. Biosci Biotechnol Biochem 1995; 59:1211-6.
[17] Wada M, Nishimura Y, Watanabe Y, Takita T, Innami S. Accelerating effect of chitosan intake on urinary calcium excretion by rats. Biosci Biotechnol Biochem 1997;61:1206-8.
[18] Yang CY, Oh TW, Nakajima D, Maeda A, Naka T, Kim CS, et al. Effects of habitual chitosan intake on bone mass, bone-related metabolic markers and duodenum CaBP D9K mRNA in ovariectomized SHRSP rats. J Nutr Sci Vitaminol (Tokyo) 2002;48: 371-8.
[19] International Task Force for Prevention of Coronary Heart Disease. Prevention of coronary heart disease: scientific background and new clinical guidelines. Nutr Metab Cardiovasc Dis 1992;2:113-156.
[20] Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20: 470-5.
[21] Spayd RW, Bruschi B, Burdick BA, Dappen GM, Eikenberry JN, Esders TW, et al. Multilayer film elements for clinical analysis: applications to representative chemical determinations. Clin Chem 1978;24:1343-50.
[22] Rifai N, Warnick GR, McNamara JR, Belcher JD, Grinstead GF, Frantz Jr ID. Measurement of low-density-lipoprotein cholesterol in serum: a status report. Clin Chem 1992;38:150-60.
[23] Sugiuchi H, Uji Y, Okabe H, Irie T, Uekama K, Kayahara N, et al. Direct measurement of high-density lipoprotein cholesterol in serum with polyethylene glycol-modified enzymes and sulfated alpha-cyclodextrin. Clin Chem 1995;41:717-23.
[24] Ohno N, Sakai T. Spectrophotometric determination of iron in boiler and well water by flow injection analysis using 2-nitroso-5-(*N*-propyl-*N*-sulfopropylamino) phenol. Analyst 1987;112:1127.
[25] Sugano M, Fujikawa T, Hiratsuji Y, Hasegawa Y. Hypocholesterolemic effects of chitosan in cholesterol-fed rats. Nutr Rep Int 1978; 18:531-7.
[26] Fukada Y, Kimura K, Ayaki Y. Effect of chitosan feeding on intestinal bile acid metabolism in rats. Lipid 1991;26:395-9.
[27] Gallaher DD, Gallaher CM, Mahrt GJ, Carr TP, Hollingshead CH, Hesslink R, et al. A glucomannan and chitosan fiber supplement decreases plasma cholesterol and increases cholesterol excretion in overweight normocholesterolemic humans. J Am Coll Nutr 2002;21: 428-33.
[28] Council on Scientific Affairs. Dietary fiber and health. J Am Med Assoc 1989;262:542-6.
[29] Kishimoto Y, Wakabayashi S, Takeda H. Hypocholesterolemic effect of dietary fiber: relation to intestinal fermentation and bile acid excretion. J Nutr Sci Vitaminol (Tokyo) 1995;41:151-61.
[30] Holme I. An analysis of randomized trials evaluating the effect of cholesterol reduction on total mortality and coronary heart disease incidence. Circulation 1990;82:1916-24.
[31] The expert's panel. Summary of the national guidelines for the diagnosis and management of lipid disorders in Taiwan. Chin Med J 1995;55:1-14.
[32] Ausar SF, Morcillo M, Leon AE, Ribotta PD, Masih R, Vilaro Mainero M, et al. Improvement of HDL- and LDL-cholesterol levels in diabetic subjects by feeding bread containing chitosan. J Med Food 2003;6:397-9.
[33] Jing SB, Li L, Ji D, Takiguchi Y, Yamaguchi T. Effect of chitosan on renal function in patients with chronic renal failure. J Pharm Pharmacol 1997;49:721-3.
[34] Ormrod DJ, Holmes CC, Miller TE. Dietary chitosan inhibits hypercholesterolaemia and atherogenesis in the apolipoprotein E-deficient mouse model of atherosclerosis. Atherosclerosis 1998; 138:329-34.
[35] Weaver CM. Assessing calcium status and metabolism. J Nutr 1990; 120:1470-3.
[36] Wannasilp N, Poungvarin N, Pokum S, Leowattana W, Mahanonda N. Serum magnesium in Thai coronary artery disease patients. J Med Assoc Thai 2001;84:S645-9.
[37] Herbert V. Everyone should be tested for iron disorders. J Am Diet Assoc 1992;92:1502-9.
[38] Baba Y, Noma H, Nakayama R, Matsushita Y. Preparation of chitosan derivatives containing methylthiocarbamoyl and phenylthiocarbamoyl groups and their selective adsorption of copper(II) over iron(III). Anal Sci 2002;18:359-61.