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Original Research

Safety Aspects and Cholesterol-Lowering Efficacy of Chitosan Tablets

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Key words: chitosan, carotenoids, fat-soluble vitamins, safety, cholesterol, lipoproteins, glucomannan

Objective: The purpose of this study was to determine the effect of two different doses of chitosan on serum fat-soluble vitamin concentrations, cholesterol concentrations, and other safety parameters.

Methods: A total of 65 men and women consumed 0, 4.5, 6.75 g per day of chitosan or 6.75 g per day glucomannan for eight weeks in a parallel, placebo-controlled, single-blind study. Altogether, 56 participants completed the study.

Results: No differences were detected among the treatments in serum vitamins (vitamin A, vitamin E, 25-hydroxyvitamin D), carotenes (α- and β-carotene), clinical chemistry or hematology measurements. The changes in the total and LDL-cholesterol concentrations among the study groups were not statistically significant.

Conclusion: In the present study, the consumption of chitosan tablets was found to be safe, but there was no significant effect on cholesterol concentration.

INTRODUCTION

Chitosan is a derivative of chitin, natural polymer of glucosamine and N-acetylglucosamine derived from the shells of crustaceans such as crab, lobster and shrimp. Because of its chemical structure, β-1,4-linked polymer of D-glucosamine, chitosan does not get broken down or digested by human gastrointestinal enzymes. It is the most abundant natural polymer after cellulose [1,2].

Chitosan has a positively charged tertiary amino group ($-NH^{3+}$) to which negatively charged molecules like fatty and bile acids can strongly attach [3]. Chitosan also binds neutral lipids like cholesterol and triglycerides through hydrophobic bonds. Several studies have shown that chitosan has cholesterol-lowering properties both in animals and humans [1,4–12]. In humans, dietary chitosan has been reported to reduce serum total cholesterol levels from 5.8% to 42.6% and low-density lipoprotein (LDL) levels from 15.1% to 35.1% [3].

Given chitosan's lipid-binding properties, it is of concern that the absorption of fat-soluble vitamins, which are transported in circulation by lipoprotein particles, might also be reduced in the setting of a chitosan containing diet. In a human study after four weeks of daily chitosan intake, serum vitamin A, D, E and beta-carotene concentrations were not significantly different in those subjects receiving chitosan compared to those receiving placebo while the serum vitamin K concentration increased significantly in the chitosan group compared with placebo [13]. In another study, intake of high dose chitosan for two weeks led to a marked and rapid decrease in serum vitamin E level in rats [14].

No clinically significant adverse effects of chitosan have been reported to date, but mild nausea and constipation have commonly occurred particularly at higher doses [7,13]. Since

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Abbreviations: 25(OH)D = 25-hydroxyvitamin D, LDL = low density lipoprotein, HDL = high density lipoprotein, GLM = general linear model.

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chitosan has potential as a functional food ingredient or supplement, and it also has technological properties in food-processing as a fiber-type ingredient, it's efficacy and safety as it relates to fat-soluble vitamins are of interest.

The primary objective of this study was to determine the effect of two different doses of chitosan (4.5 g and 6.75 g) on serum concentrations of fat-soluble vitamins (vitamin A, α-carotene, β-carotene, vitamin E and vitamin D) compared to the placebo and glucomannan in mildly hypercolesterolemic subjects. The secondary objective was to investigate the effects of two different doses of chitosan on plasma cholesterol concentrations.

MATERIALS AND METHODS

Study Design

This study was a parallel, placebo-controlled trial conducted in a single-blind fashion. Both a placebo and active control (glucomannan) were used. After a two-week run-in period the subjects were assigned randomly to one of the four groups. Men and women were randomized separately. The randomization list consisted of the blocks including codes of all four groups in a randomized order. The subjects were given 0, 4.5, 6.75 g of chitosan per day or 6.75 g of glucomannan per day in form of tablets with main meals for eight weeks. The mean duration of the intervention period was 55 days (min 50, max 58) in the chitosan 4.5 group, 55 days (min 53, max 56) in the chitosan 6.75 group, 54 days (min 50, max 58) in the glucomannan group and 55 days (min 48, max 62) in the placebo group. The study protocol was approved by the ethics committee for human research at the University of Kuopio and the Kuopio University Hospital. Each study subject signed the written consent at the first study visit.

Subjects

Altogether, 79 subjects were recruited to take part in the study from the register of Oy Foodfiles Ltd and by an advertisement in a local free paper. To be included in the study, the subjects had to be between 18 and 55 years old and had to have a plasma total cholesterol concentration of 5.0–6.5 mmol/L and have a plasma total triglyceride concentration below 4.0 mmol/L. The exclusion criteria were the presence of liver or kidney disease or disorder, severe lactose-intolerance, gastrointestinal diseases leading to malabsorption of fat-soluble vitamins, history of unstable coronary artery disease (myocardial infarction, unstable angina pectoris, coronary artery bypass graft (CABG), percutaneous transluminal coronary angioplasty (PTCA) within the previous 6 months), stroke, temporal ischemic attack, malignant disease, other serious illness or the history of severe allergic reactions (anaphylactic reaction) when exposed to fish or crustacean. The use of lipid lowering medication, laxatives, guar gum or glucomannan, psyllium or other dietary supplements with laxative or bulking effect, the use of dietary supplements or drugs with fat-soluble vitamins, the chronic use of drugs having the potential to interfere with vitamin absorption and the excessive consumption of alcohol ($>$ 45 g ethanol per day) were also exclusion criteria.

In all, 65 subjects were eligible for randomization and 56 subjects completed the study. The pre-trial characteristics of the study subjects are presented in Table 1. None of the subjects were pregnant or breastfeeding. Seven women used postmenopausal estrogen medication.

Test Products and Diet

Chitosan and glucomannan were provided as tablets. The subjects were instructed to take six tablets three times daily with a glass of water 15 minutes before morning, afternoon, and evening meals. Otherwise, the subjects followed their habitual diet during the study. The timing of the consumption of the tablets (i.e. 15 minutes before the meal) was chosen to enable the chitosan and glucomannan tablets to dissolve and form the gel in stomach before the arrival of the dietary fat and consequently enable effective inhibition of fat absorption.

Each chitosan tablet contained 750 mg ChitoClear® fg 95 chitosan. The chitosan 4.5 group took 6 chitosan tablets and 12 placebo tablets and the chitosan 6.75 group took 9 chitosan and 9 placebo tablets per day. Each glucomannan tablet contained

Table 1. Pretrial Characteristics of the Study Subjects, Mean (SD)

	Chitosan 4.5 g (n = 15)	Chitosan 6.75 g (n = 12)	Glucomannan (n = 15)	Placebo (n = 14)	p-value[a]
Male/Female	7/8	7/5	8/7	5/9	NS
Age (y)	46 (5)	45 (6)	49 (3)	45 (8)	NS
Body mass index[b]	25 (3)	26 (4)	26 (3)	26 (4)	NS
Plasma total cholesterol (mmol/l)	5.8 (0.6)	5.8 (0.5)	5.8 (0.7)	5.8 (0.5)	NS
Plasma LDL-cholesterol (mmol/l)	3.60 (0.65)	3.71 (0.41)	3.49 (0.66)	3.52 (0.81)	NS
Plasma HDL-cholesterol (mmol/l)	1.55 (0.44)	1.40 (0.44)	1.62 (0.40)	1.57 (0.44)	NS
Plasma total triglyceride (mmol/l)	1.38 (0.62)	1.53 (0.89)	1.48 (0.76)	1.46 (0.68)	NS

[a] The significance of the difference among the study groups analyzed with the Chi-Square test of independence (sex), with the univariate analysis of variance (body mass index and plasma lipids) or with the Kruskal-Wallis test (age).

[b] body weight (kg)/height^2 (m).

375 mg of glucomannan. The glucomannan group took 18 glucomannan tablets and the placebo group took 18 placebo tablets per day. The subjects returned the unused tablets to the research unit. The compliance of the treatment was assessed by tablet count.

Degree of deacetylation of ChitoClear® fg 95 chitosan was more than 95% and the viscosity was less than 500 mPa•s. Glucomannan was derived from konjac root (*Amorphallus konjac*). The placebo tablets contained cellulose, lactose and small amounts of ferroxide and magnesium stearate. The tablets were packaged in the plastic bottles labeled with the randomization codes.

Measurement Schedule and Methods of Measurements

Previous and current diseases, current medication, alcohol consumption, smoking habits, physical activity, and the use of vitamins and other nutrient supplements were ascertained at the beginning of the study by the interview using a structured questionnaire. The subjects were requested to keep their alcohol and tobacco consumption, physical activity and the use of vitamins and other nutrient supplements constant during the study. In addition, the subjects recorded the changes in their health-status, medication and life-style daily in the diary during the study.

Venous blood samples were collected after an overnight (12 h) fast. Laboratory samples for routine hematology (blood count) and basic laboratory tests (plasma creatinine, plasma urate, plasma gamma-glutamyl transferase, plasma calcium, serum ferritin) were analyzed with standardized methods at the Clinical Chemistry Unit, Kuopio University Hospital.

Serum samples of α- and β-carotene, vitamin A, E and 25-hydroxyvitamin D (25(OH)D) were stored at $-70°C$ until analyzed at end of the study. Serum α- and β-carotene, vitamin A and E were analyzed simultaneously by the HPLC system with internal 4-point calibration [15,16]. Serum 25(OH)D concentrations were determined by RIA-method (Radioimmunometric method, DiaSorin Inc, Stillwater, Minnesota).

Plasma concentrations of lipids were analyzed at the Clinical Chemistry Unit, Kuopio University Hospital using lithium-heparin. Plasma total and HDL-cholesterol and total triglyceride concentrations were measured using commercial reagents with a Konelab 60i Clinical Chemistry Analyzer (Labsystems CLD, Konelab Co, Espoo, Finland). Plasma LDL-cholesterol concentration was calculated by Friedewald formula [17].

Body weight was measured twice at every visit using a digital scale (Scale Seca 707, Vogel & Falke GmpH & Co, Hamburg, Germany). The mean value of the two measurements was used in the analyses. Blood pressure measurements were performed after ten minutes rest in the sitting position with an automatic sphygmomanometer (Omron 711 automatic IS, Omron Matsusaka Co. Ltd, Japan) three times on every occasion. The mean value of the last two measurements was used in the analyses.

The composition of the diet was monitored by a four-day food record: once during the run-in period, and once during the intervention period. The subjects recorded dietary intake of four consecutive days in food records, including one weekend day or the person's day off from work. The serving sizes were estimated with a portion size picture booklet [18,19]. Verbal and written instructions about how to record foods were given to all the subjects personally. At the study visits the nutritionist checked the food records for completion, thus clarifying any missing information. The energy and nutrients were calculated using Micro-Nutrica® (version 2.5) dietary analysis software (the Social Insurance Institution, Turku, Finland), which is based on Finnish and international food composition data.

The RAND 36-Item Health Survey 1.0 [20,21] was used to assess the following eight health concepts: 1) general health, 2) physical functioning, 3) mental health, 4) social functioning, 5) vitality, 6) bodily pain, 7) role functioning/physical and 8) role functioning/emotional.

Each subject completed a structured form on incidence and severity of gastrointestinal (diarrhea, flatulence, abdominal pain, abdominal bloating, constipation, heartburn, nausea, abdominal rumbling, bowel cramps), skin and other symptoms including defecation frequency twice during the study. The severity of symptoms was determined using a four-point category scale.

Statistical Analysis

Statistical analyses were performed with the SPSS Base and Advanced Models 10.1 statistics program (SPSS, Chicago, Illinois). Normal distribution of variables was checked with the Shapiro-Wilk test. Any p-values less than 0.05 were regarded as statistically significant. We were able to detect a 1.35 μmol/L difference in serum β-carotene concentration with an α level of 0.05 and with 0.80 statistical power.

The results are expressed as group means and standard deviations. The equality of variances between the groups was tested with the Levene test for homogeneity of variance and the equality of variance-covariance matrices across the cells was tested with the Box's M test. The general linear model (GLM) repeated measures procedure was used to test between-groups and within-group differences in the repeated continuous variables. If there was a significant overall effect of the between-subjects factor (group), within-subjects factor (time) or interaction of them (group-time), ANOVA with the Bonferroni correction was used in between-group analyses and the paired samples t-test adjusted with the Bonferroni correction for within-group analyses. For the continuous variables, which were not normally distributed after logarithmic or other mathematical transformation the Kruskal-Wallis test (between-groups) and the Wilcoxon tests (within-group) adjusted with Bonferroni

Table 2. Energy and Nutrient Composition of the Background Diet, Mean (SD)

	Chitosan 4.5 g (n = 15)	Chitosan 6.75 g (n = 12)	Glucomannan (n = 15)	Placebo (n = 14)	p-value[a]	p-value[b]
Energy (MJ)					NS	NS
Run-in period	7.7 (1.9)	9.1 (2.4)	8.8 (2.2)	7.8 (2.2)		
Intervention period	7.9 (1.8)	8.6 (2.6)	8.3 (2.4)	8.1 (2.3)		
p-value[c] (NS)						
Protein (E-%)					NS	NS
Run-in period	16.4 (3.2)	17.4 (2.9)	18.0 (3.3)	18.1 (2.5)		
Intervention period	16.1 (2.9)	18.1 (4.8)	18.6 (4.5)	18.9 (4.3)		
p-value[c] (NS)						
Fat (E-%)						0.008
Run-in period	34.5 (5.2)[d]	32.8 (5.2)	27.9 (6.0)	32.4 (3.6)	0.012	
Intervention period	32.2 (5.7)	33.7 (4.7)	32.8 (6.0)	31.8 (4.1)	NS	
p-value[c]	NS	NS	0.028	NS		
Saturated fatty acids (E-%)						0.020
Run-in period	14.3 (2.5)	12.7 (2.7)	11.5 (2.4)	13.2 (2.5)	NS	
Intervention period	13.0 (2.8)	14.1 (2.5)	13.2 (2.5)	12.8 (2.6)	NS	
p-value[c]	NS	NS	NS	NS		
Monounsaturated fatty acids (E-%)						0.032
Run-in period	11.6 (2.4)	10.3 (2.2)	8.9 (2.7)	10.8 (1.4)	0.038	
Intervention period	10.7 (2.9)	10.7 (2.1)	10.9 (2.8)	10.6 (1.6)	NS	
p-value[c]	NS	NS	0.044	NS		
Polyunsaturated fatty acids (E-%)					NS	NS
Run-in period	4.8 (1.0)	5.8 (2.1)	4.2 (1.1)	5.1 (1.0)		
Intervention period	5.0 (1.4)	5.1 (1.6)	5.1 (1.4)	5.1 (1.4)		
p-value[c] (NS)						
Carbohydrates (E-%)						0.002
Run-in period	46.2 (5.6)	48.7 (4.8)	50.3 (7.0)	46.7 (4.7)	NS	
Intervention period	50.0 (7.5)	46.8 (5.7)	44.9 (6.3)	45.5 (6.1)	NS	
p-value[c]	NS	NS	NS	NS		
Alcohol (E-%)						
Run-in period	2.9 (2.8)	1.0 (2.1)	3.9 (5.4)	2.0 (4.1)	NS	
Intervention period	1.8 (2.5)	1.3 (1.5)	3.7 (4.4)	3.8 (5.1)	NS	
p-value[c]	NS	NS	NS	NS		
Cholesterol (mg)						
Run-in period	257 (112)	258 (77)	233 (84)	228 (51)	NS	
Intervention period	278 (151)	264 (106)	252 (95)	254 (65)	NS	
p-value[c]	NS	NS	NS	NS		
Fiber (g)[e]					0.031	NS
Run-in period	19 (7)[f]	27 (7)	26 (6)	24 (7)	0.024	
Intervention period	20 (6)	23 (7)	24 (8)	23 (6)	NS	
p-value[c] (0.046)	NS	NS	NS	NS		
Vitamin A (μg)					NS	NS
Run-in period	1516 (2358)	1728 (1789)	1441 (1549)	793 (298)		
Intervention period	919 (614)	1384 (1121)	784 (264)	1171 (1200)		
p-value[c] (NS)						
Vitamin D (μg)						
Run-in period	6.2 (3.1)	7.7 (3.1)	9.1 (4.6)	5.4 (3.5)	NS	
Intervention period	5.4 (2.5)	6.0 (2.5)	8.8 (8.5)	5.5 (3.5)	NS	
p-value[c]	NS	NS	NS	NS		
Vitamin E (mg)					NS	NS
Run-in period	8.0 (3.0)	10.9 (3.0)	8.7 (3.0)	8.7 (2.1)		
Intervention period	8.0 (2.5)	9.4 (2.4)	9.4 (4.0)	8.6 (2.8)		
p-value[c] (NS)						

[a] The significance of the overall difference among the study groups analyzed with the GLM for repeated measures or with the Kruskal-Wallis test (alcohol, cholesterol and vitamin D).

[b] The significance of the time*group interaction analyzed with the GLM for repeated measures.

[c] The significance of the within-group difference analyzed with the GLM for repeated measures or with the Wilcoxon test (alcohol, cholesterol and vitamin D).

[d] Indicates the significant difference ($p < 0.05$) compared to the Glucomannan group.

[e] chitosan and glucomannan are not included.

[f] Indicates the significant difference ($p < 0.05$) compared to the Chitosan 6.75 g and Glucomannan groups.

correction were used. The symptoms were analyzed with the Pearson Chi-Square test. The exact *p*-values of Pearson's statistic were used.

The standardized concentrations of serum fat soluble vitamins and carotenes for total plasma lipids (total cholesterol + total triglycerides) were calculated. The percentage changes in the total and LDL-cholesterol concentration were calculated by comparing the end measurement (8 wk) to the baseline measurement (0 wk) in each study group.

RESULTS

Treatment Compliance

The mean intake of chitosan was 4.28 ± 0.31 g (95 ± 7% of the target amount) in the chitosan 4.5 group and 6.43 ± 0.38 g (95 ± 6% of the target amount) in the chitosan 6.75 group. The mean intake of glucomannan was 6.46 ± 0.29 g (96 ± 4% of the target amount) in the glucomannan group.

During the run-in period, there were some differences in the nutrient intake among the groups (Table 2). The fat intake was lowest in the glucomannan group and fiber intake was lowest in the chitosan 4.5 group. However, there were no statistically significant differences in energy and nutrient intake among the groups during the intervention period.

Serum Carotenes and Fat-Soluble Vitamins

Serum α-carotene concentration seemed to decrease in every study group during the intervention period, but the change was not significant (Table 3). There were no differences in serum vitamin A, α-carotene, β-carotene, vitamin E or 25(OH)D responses among the four study groups (Table 3) and the results were same after standardization for serum total lipid concentration. However, in the glucomannan group an increase of 44 ± 69% in standardized 25(OH)D concentration reached statistical significance ($p = 0.032$).

Other Safety Measurements

There were no significant differences in clinical chemistry or hematological measurements among the study groups (Table 4). However, the hematocrit increased in the glucomannan group and plasma calcium concentration decreased in every study group. The increase in hematocrit was statistically but not clinically significant.

Plasma Lipids

There were no statistically significant differences in absolute plasma lipid concentrations among the study groups (Table 5). During the intervention period, plasma total and LDL-cholesterol concentration decreased significantly only in the glucomannan group ($p = 0.044$ and $p < 0.004$, respectively). There were no significant differences in percentage change in plasma total or LDL-cholesterol concentration among the groups.

Body Weight and Blood Pressure

The body weight and blood pressure remained stable during the intervention period in every study group.

Table 3. Serum Fat-Soluble Vitamin and Carotene Concentrations during the Study, Mean (SD)

	Chitosan 4.5 g (n = 15)	Chitosan 6.75 g (n = 12)	Glucomannan (n = 15)	Placebo (n = 14)	p-value[a]	p-value[b]
Vitamin A (μmol/l)					NS	NS
0 wk	3.51 (0.89)	3.65 (0.86)	3.74 (0.99)	3.68 (0.55)		
8 wk	3.98 (1.11)	3.81 (1.07)	3.75 (0.98)	3.87 (0.74)		
p-value[c] (0.029)	NS	NS	NS	NS		
α-carotene (μmol/l)						
0 wk	1.20 (0.75)	1.24 (1.07)	1.43 (1.78)	1.17 (0.54)	NS	
8 wk	0.90 (0.53)	1.07 (0.94)	1.12 (1.52)	1.07 (0.47)	NS	
p-value[c]	NS	NS	NS	NS		
β-carotene (μmol/l)					NS	NS
0 wk	2.11 (1.17)	2.13 (1.55)	2.53 (3.09)	2.22 (1.40)		
8 wk	2.05 (1.58)	2.16 (1.43)	2.19 (2.83)	2.15 (1.36)		
p-value[c] (NS)						
Vitamin E (μmol/l)					NS	NS
0 wk	35.18 (4.18)	33.08 (4.42)	34.95 (6.33)	33.92 (6.02)		
8 wk	35.14 (3.87)	32.22 (5.77)	34.27 (7.06)	34.66 (7.84)		
p-value[c] (NS)						
25(OH)D (nmol/l)					NS	NS
0 wk	63.0 (23.1)	61.5 (31.2)	56.1 (27.6)	60.6 (20.8)		
8 wk	67.5 (19.9)	74.1 (31.1)	69.8 (33.1)	65.4 (18.4)		
p-value[c] (0.001)	NS	NS	NS	NS		

[a] The significance of the overall difference among the study groups analyzed with the GLM for repeated measures or with the Kruskal-Wallis test (α-carotene).

[b] The significance of the time*group interaction analyzed with the GLM for repeated measures.

[c] The significance of the within-group difference analyzed with the GLM for repeated measures or with the Wilcoxon test (α-carotene).

Table 4. Hematology and Clinical Chemistry, Mean (SD)

	Chitosan 4.5 g (n = 15)	Chitosan 6.75 g (n = 12)	Glucomannan (n = 15)	Placebo (n = 14)	p-value[a]	p-value[b]
Hemoglobin (g/l)					NS	NS
−2 wk	139 (13)	140 (15)	141 (14)	134 (10)		
8 wk	140 (15)	139 (13)	144 (13)	133 (11)		
p-value[c] (NS)						
Hematocrit					NS	NS
−2 wk	0.40 (0.04)	0.40 (0.04)	0.41 (0.04)	0.39 (0.02)		
8 wk	0.41 (0.04)	0.41 (0.04)	0.42 (0.04)	0.40 (0.03)		
p-value[c] (< 0.001)	NS	NS	0.032	NS		
Leucocytes (10^9/l)						
−2 wk	6.9 (2.4)	6.0 (1.6)	5.5 (1.1)	5.3 (0.9)	NS	
8 wk	5.8 (1.5)	5.9 (1.6)	5.3 (1.5)	5.5 (1.0)	NS	
p-value[c]	NS	NS	NS	NS		
Plasma creatinine (μmol/l)					NS	NS
−2 wk						
8 wk	85 (11)	89 (10)	86 (9)	86 (10)		
p-value[c] (NS)	84 (11)	88 (9)	87 (11)	83 (10)		
Plasma urate (μmol/l)					NS	NS
−2 wk	269 (74)	295 (77)	287 (80)	269 (47)		
8 wk	278 (90)	276 (85)	281 (95)	264 (64)		
p-value[c] (NS)						
Plasma gamma-glutamyl transferase (U/l)					NS	NS
−2 wk						
8 wk	29 (18)	33 (11)	33 (26)	32 (20)		
p-value[c] (NS)	29 (16)	32 (12)	32 (22)	32 (19)		
Serum ferritin (μg/l)					NS	NS
0 wk	77 (38)	86 (73)	128 (156)	79 (79)		
8 wk	72 (43)	84 (70)	129 (167)	73 (71)		
p-value[c] (NS)						
Plasma calcium (mmol/l)					NS	NS
0 wk	2.31 (0.06)	2.37 (0.07)	2.36 (0.09)	2.36 (0.08)		
8 wk	2.27 (0.07)	2.30 (0.07)	2.29 (0.07)	2.26 (0.06)		
p-value[c] (< 0.005)	0.020	0.016	< 0.005	< 0.005		

[a] The significance of the overall difference among the study groups analyzed with the GLM for repeated measures or with the Kruskal-Wallis test (leucocytes).

[b] The significance of the time*group interaction analyzed with the GLM for repeated measures.

[c] The significance of the within-group difference analyzed with the GLM for repeated measures or with the Wilcoxon test (leucocytes).

RAND-36

There were no differences among the groups or significant changes within-groups in the scores for eight RAND-36 subscales (i.e., General health, Physical functioning, Mental Health, Social Functioning, Vitality, Bodily pain, Role Functioning/Physical, Role functioning/Emotional).

Symptoms

There were no differences in defecation frequency among the groups. Four subjects in the chitosan 6.75 group and one subject in the Glucomannan group discontinued the study due to transient adverse events. Common gastrointestinal symptoms (loose feces, constipation, abdominal pain, repeated flatulence, abdominal bloating and abdominal rumbling) were reported in each group. There was a significant association between the study groups and the incidence of constipation ($p = 0.021$), heartburn ($p = 0.044$) and nausea ($p = 0.023$) during the first 4-wk period and between the study groups and nausea ($p = 0.016$) during the second 4-wk period. The incidence of constipation seemed to be greatest in the chitosan 4.5 group during the first four weeks of ingestion of chitosan. The incidence of heartburn and nausea seemed to be greatest in the both chitosan groups. However, there were no significant differences between the groups after performing pair-wise comparisons.

Three subjects in the chitosan 4.5 and chitosan 6.75 group and one subject in the placebo group reported skin symptoms during the intervention period. No one reported skin symptoms in the glucomannan group.

DISCUSSION

The safety and efficacy of two different doses of chitosan were studied in mildly hypercholesterolemic men and women. The tested amounts of chitosan were 4.5 g (actual intake 4.28 g)

Table 5. Plasma Lipids during the Study, Mean (SD)

	Chitosan 4.5 g (n = 15)	Chitosan 6.75 g (n = 12)	Glucomannan (n = 15)	Placebo (n = 14)	p-value[a]	p-value[b]
Total cholesterol (mmol/l)					NS	NS
0 wk	5.5 (0.7)	5.6 (0.6)	5.6 (0.7)	5.5 (0.6)		
4 wk	5.4 (0.4)	5.2 (0.8)	5.3 (0.8)	5.6 (0.6)		
8 wk	5.4 (0.6)	5.3 (0.7)	5.4 (0.6)	5.6 (0.7)		
p-value[c] (0.014)	NS	NS	0.044	NS		
LDL-cholesterol (mmol/l)					NS	NS
0 wk	3.39 (0.68)	3.57 (0.49)	3.40 (0.76)	3.38 (0.67)		
4 wk	3.34 (0.38)	3.22 (0.64)	2.99 (0.66)	3.45 (0.69)		
8 wk	3.32 (0.52)	3.36 (0.67)	3.10 (0.66)	3.47 (0.58)		
p-value[c]	NS	NS	< 0.004	NS		
HDL-cholesterol (mmol/l)					NS	NS
0 wk	1.58 (0.43)	1.40 (0.46)	1.66 (0.42)	1.60 (0.43)		
4 wk	1.54 (0.52)	1.41 (0.49)	1.69 (0.44)	1.63 (0.46)		
8 wk	1.55 (0.45)	1.38 (0.48)	1.76 (0.44)	1.61 (0.32)		
p-value[c] (NS)						
Total triglycerides (mmol/l)					NS	NS
0 wk	1.20 (0.48)	1.34 (0.65)	1.23 (0.56)	1.17 (0.48)		
4 wk	1.21 (0.58)	1.31 (0.86)	1.28 (0.73)	1.10 (0.32)		
8 wk	1.18 (0.62)	1.29 (0.54)	1.11 (0.41)	1.16 (0.43)		
p-value[c] (NS)						

[a] The significance of the overall difference among the study groups analyzed with the GLM for repeated measures.

[b] The significance of the time*group interaction analyzed with the GLM for repeated measures.

[c] The significance of the within-group difference analyzed with the GLM for repeated measures.

and 6.75 g (actual intake 6.43 g). A randomized, placebo-controlled, single-blind, parallel study design was used for this study, in which glucomannan was used as the control agent. The chitosan and placebo tablets were packaged in different bottles for technical reasons and consequently it was necessary for the study personnel to know which one is which to advise subjects to take the correct amount of both tablets (especially in the chitosan 4.5 group). However, all laboratory analyses were double blinded.

Chitosan did not have any significant effect on serum concentrations of vitamin A, α-carotene, β-carotene, vitamin E and 25-hydroxyvitamin D during 8 weeks consumption. This observation is consistent with the results of the studies of Pittler et al. [13] and Colombo & Sciutto [5]. In both studies serum vitamin A, β-carotene, vitamin D and E concentrations remained stable during the 4-week ingestion of chitosan in overweight subjects consuming normal or hypocaloric diet. In the present study, serum 25-hydroxyvitamin D concentration increased slightly, but the increase was not statistically significant in any study group. This is probably due to the seasonal variation (chronobiology) of vitamin D synthesis in the skin. The present study was conducted from April to June and vitamin D_3 synthesis in the skin is less in spring than in summer [22].

In summary, in this study, chitosan was determined to be safe to be used as a cholesterol-lowering ingredient with no observable effect on serum vitamin levels.

The slight decrease of plasma calcium concentration found in all study groups was not clinically significant and can probably be explained by the change in albumin concentrations rather than the decrease in the absorption of calcium. As plasma albumin concentration decreases the calcium concentration decreases because half of the calcium in the circulation is bound by albumin.

We found no differences in plasma cholesterol concentrations among the study groups. Our finding is similar to that of other studies, in which 1.2–2.4 g daily of chitosan had slight or negligible effect on serum cholesterol concentrations during a two to three month ingestion period [9,23,24]. On the other hand, previous studies have shown that chitosan together with a hypocaloric diet decreases serum total and LDL-cholesterol concentrations over 10% more than a hypocaloric diet alone in obese subjects [4,5,7].

The changes in cholesterol levels observed in the present study did not reach statistical significance due to the small sample size. A group size of 22 would have been required to achieve a power of 0.8 and a $p = 0.05$ to detect a cholesterol reduction of 0.3 ± 0.5 mmol/L. For this reason, the authors suggest a larger study be carried out.

Based on subject's food diaries, it is possible that the intake of dietary fiber may have affected subject's cholesterol concentrations. It has been shown that soluble dietary fiber can result in cholesterol reductions in those subjects with initially higher blood cholesterol concentrations [25,26]. We found no change in body weight or food intake. This is interesting in that it has been reported in a rodent study that there was an observed increased in satiety and

consequent reduction in food intake resulting in a cholesterol-lowering effect attributed to chitosan [27].

Transient adverse effects, specifically gastrointestinal symptoms, were reported to some extent by all groups. There were more reports of constipation, heartburn and nausea in the chitosan groups compared to the placebo. Discontinuation of the study was more frequent in the group receiving 6.75 g of chitosan, consequently amounts greater than 6.75 g of chitosan cannot be recommended.

CONCLUSION

In conclusion, two different doses (4.5 g and 6.75 g) of chitosan in the form of tablets, consumed daily by mildly hypercholesterolemic Finnish subjects eating a typical Finnish diet, did not affect serum concentrations of vitamin A, α-carotene, β-carotene, vitamin E, 25-hydroxyvitamin D, or other safety parameters. Modest reduction in plasma cholesterol concentrations did not reach statistical significance in this study.

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