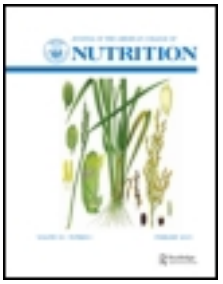

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A Glucomannan and Chitosan Fiber Supplement Decreases Plasma Cholesterol and Increases Cholesterol Excretion in Overweight Normocholesterolemic Humans

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Original Research

A Glucomannan and Chitosan Fiber Supplement Decreases Plasma Cholesterol and Increases Cholesterol Excretion in Overweight Normocholesterolemic Humans

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Key words: chitosan, glucomannan, cholesterol, fecal fat, bile acids, humans

Objective: Both chitosan and glucomannan have demonstrated hypocholesterolemic effects. A recent study in rats indicates that the combination of the two is also a potent hypocholesterolemic agent that increases fecal fat excretion. The objective of the present study was to determine the hypocholesterolemic effect of a supplement containing equal amounts of chitosan and glucomannan on blood lipid concentrations and fecal excretion of fat, neutral sterols and bile acids.

Methods: Twenty-one overweight normocholesterolemic subjects (11 males and 10 females) were fed 2.4 g/day of a supplement containing equal amounts of chitosan and glucomannan. Prior to taking the supplement (initial period) and after 28 days (final period), blood was drawn for measurement of serum lipids and a three-day fecal sample collected for determination of fat, neutral sterol and bile acid excretion. Subjects maintained their normal dietary and activity patterns during the study.

Results: Caloric intake and intake of fat and dietary fiber (excluding the supplement) did not differ between the initial and final periods. Serum total, HDL and LDL cholesterol concentrations were significantly lower ($p < 0.05$) in the final period compared to the initial period. Serum triacylglycerol concentration did not change between periods. There was a trend towards greater fecal excretion of neutral sterols and bile acids ($p = 0.13$ and 0.16, respectively) in the final period. However, fecal fat excretion did not differ between periods.

Conclusions: Serum cholesterol reduction by a chitosan/glucomannan supplement is likely mediated by increased fecal steroid excretion and is not linked to fat excretion.

INTRODUCTION

An elevated plasma cholesterol concentration has long been recognized as an independent risk factor for ischemic heart disease. It is now believed that reducing plasma cholesterol concentration decreases the risk of myocardial infarctions [1]. Water-soluble fibers such as psyllium, guar gum, oat bran and pectin have been shown to reduce plasma cholesterol concentration [2,3]. Although the mechanism by which these fibers have their hypocholesterolemic effect is still uncertain, many studies indicate that increased bile acid excretion and/or decreased cholesterol absorption is responsible [4].

Konjac mannan is a dietary fiber from the tuber *Amorphophallus konjac.* It is a highly branched viscous glucomannan that has a demonstrated hypocholesterolemic effect in animals [5,6] and humans [7,8]. It is highly fermentable within the large intestine. Chitosan, although not derived from plants, is similar to dietary fiber in being a polysaccharide that is indigestible by mammalian digestive enzymes. Chitosan is the deacetylated form of chitin, an aminopolysaccharide found in

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the exoskeleton of arthropods and certain fungi [9]. Several studies have also shown chitosan to be hypocholesterolemic in both animal models [10–13] and humans [14].

Although both glucomannan and chitosan are hypocholesterolemic, few studies have examined the mechanism by which these two materials exert this effect. Maezaki *et al.* [14] reported increased fecal excretion of two bile acids, cholic and chenodeoxycholic acid, in males subjects consuming 3 to 6 g/day of chitosan. In rats, chitosan increased [11] or had no effect [15] on fecal neutral sterol excretion. In a recent study in rats, both chitosan and glucomannan, either alone or in combination, reduced liver cholesterol, with the combination tending to be more effective [16]. Both materials decreased cholesterol absorption, whereas only chitosan led to greater excretion of bile acids, relative to a cellulose-containing diet. Further, fecal fat excretion was greater with chitosan feeding, but not with glucomannan feeding. The greater fecal fat excretion with chitosan feeding is of particular interest in light of studies in humans showing that chitosan supplements accelerate weight loss in subjects consuming hypocaloric diets [17,18].

The objective of the present study was to examine the hypocholesterolemic effect of consuming a supplement containing equal amounts of chitosan and glucomannan in overweight humans. Additionally, we determined whether this supplement would increase the fecal excretion of bile acids, neutral sterols and fat.

METHODS

Subjects

Twenty-two overweight subjects enrolled in the study, which was conducted at the University of Utah, Salt Lake City, Utah. The study was approved by the University of Utah Institutional Review Board. Subject characteristics are shown in Table 1. Subjects ranged in age from 18 to 50 years and had a mean body mass index (± SD) of 28.0 ± 4.6. Twenty-one subjects, 11 male and 10 female, completed the study, as one subject dropped from the study for personal reasons.

Table 1. Characteristics of study subjects: Combined, males and females[a]

	Combined (n = 21)	Males (n = 11)	Females (n = 10)
Age (years)	28.9 ± 9.8	30.6 ± 9.9	27.7 ± 10.1
Height (cm)	172.9 ± 9.0	179.9 ± 5.4	168.2 ± 7.9
Weight (kg)	84.4 ± 17.7	88.9 ± 17.7	81.3 ± 17.9
Body mass index (kg/m^2)	28.0 ± 4.6	27.4 ± 4.7	28.5 ± 4.7
Body fat (%)	37.2 ± 10.7	27.9 ± 10.5	43.8 ± 3.7
Lean muscle mass (kg)	48.7 ± 11.2	58.5 ± 7.3	41.8 ± 7.8
Fat mass (kg)	31.3 ± 12.7	25.6 ± 14.9	35.3 ± 9.7

[a] Mean ± SD.

Subjects with eating disorders, gastrointestinal disturbances or on chronic drug therapy were excluded from the study, as were pregnant or lactating women. All subjects were given multivitamins while taking the fiber supplement to compensate for any increased loss of fat-soluble vitamins.

Experimental Design

Beginning on day 1, subjects recorded three days of food intake. On days 4 to 6, subjects made a quantitative 72 hour fecal collection. Ingestion of the fiber supplement began on day 6. The fiber supplement was provided in capsules. Subjects were instructed to take five capsules three times a day with a glass of water 30 minutes before breakfast, lunch and dinner for 28 days. Each capsule contained equal amounts of chitosan and glucomannan (Propol™, from *Amorphophallus konjac*). The fifteen capsules taken daily provided 2.4 g of material. The subjects also recorded one day food intakes five times during the period of fiber supplementation, on days 5, 10, 15, 20 and 33. A second 72 hour fecal collection was begun on day 31.

Blood samples were taken via venipuncture to assess blood lipid levels on day 7 (initial period) and day 35 (final period). Subjects fasted for at least 12 hours prior to the blood draw. Blood was drawn at the University of Utah Health Sciences Center outpatient lab by qualified phlebotomists. Analyses of serum total, HDL and LDL cholesterol and serum triacylglycerol concentrations were done by a clinical laboratory by standard methods (ARUP Laboratories, Salt Lake City, Utah).

Feces were collected for analysis of fecal fat, neutral sterols and bile acids. Subjects were provided with airtight plastic containers to defecate in, and gloves were provided to aid in cleanliness. All fecal samples during the 72-hour fecal collection were collected separately in new containers for each defecation. Each subject's daily collection was kept cold on blue ice in an insulated carrier while subjects were away from their residences. Fecal samples were stored refrigerated at the subjects' residence or turned in daily to the nutrition laboratory at the University of Utah.

A percentage moisture analysis was conducted on each sample in each container. Two small (approximately 1 g) portions of feces from different ends of the sample were dried in a drying oven at 120°F for approximately three to four hours. After drying, the samples were removed from the oven and immediately weighed. The two subsamples from each stool sample were averaged and percent moisture calculated as the difference realized between the average wet and dry weights divided by the total wet weight, multiplied by 100.

Each subject's three-day fecal collections were weighed, diluted 1:4 (w/v) with distilled water and homogenized. One aliquot of the homogenate was shipped to the Department of Food Science and Nutrition at the University of Minnesota on dry ice and stored at −20°C until freeze-drying and analyzed

for fecal fat and fecal bile acids. A second aliquot of the homogenate was sent to the Department of Nutritional Science and Dietetics and the University of Nebraska and stored at −20°C until analyzed for fecal neutral steroids.

Analytical Methods

Bile acids were extracted from dried feces using organic solvents [19] and total bile acids measured enzymatically essentially as described by Sheltawy and Lowowsky [20]. Fecal fat was determined gravimetrically after extraction with organic solvents. Fecal neutral steroids were analyzed as previously described [21].

Statistics

Results were analyzed by a paired t test, comparing initial to final period values. Differences due to gender were analyzed by one-way ANOVA. A probability of 0.05 or less was considered statistically significant.

RESULTS

Caloric, fat and dietary fiber intake (exclusive of the supplement) did not differ significantly between the initial and final periods (Table 2). Thus, subjects appeared to maintain their habitual diet during the study. The initial and final weights for all subjects were 84.82 kg and 84.81 kg, respectively, indicating no change in body weight during the course of the study.

There were no statistically significant differences between males and females for any blood lipid or fecal output parameter measured. Therefore, results are reported only for males and females combined.

Both serum total and LDL cholesterol concentrations were significantly lower at the final period of the experiment (day 35) than at the initial period (day 7) (Table 3). Total serum cholesterol was reduced by approximately 7% and LDL cholesterol by 10%. There was a slight but statistically significant reduction of approximately 4% in serum HDL cholesterol in the final period relative to the initial period. However, serum triacylglycerol concentrations did not differ between the initial and final periods of the study.

Ingestion of the supplement resulted in a strong trend towards a greater fecal dry weight ($p = 0.052$) (Table 4). There were also tendencies towards greater daily fecal excretion of both bile acids ($p = 0.16$), neutral sterols ($p = 0.13$), and total steroid excretion ($p = 0.13$) in the final period compared to the initial period. In particular, there was a strong trend toward increased fecal excretion of cholesterol ($p = 0.064$), whereas excretion of other neutral sterols was essentially unchanged. The supplement did not significantly alter daily fecal fat excretion.

Table 2. Intake during baseline and study periods of calories, fat and dietary fiber[1]

	Period	
	Initial	Supplement
Calories (kcal/day)	2050 ± 200	1985 ± 149
Fat (g/day)	71.9 ± 8.6	70.3 ± 6.5
Dietary fiber (g/day)[2]	15.7 ± 1.9	14.5 ± 1.2

[1] Values are means ± SEM. There were no statistically significant differences in intake between baseline and study periods for any parameter.

[2] Excludes dietary fiber from supplement.

Table 3. Serum lipid concentrations[1]

	Period		*p* value[2]
	Initial	Final	
Total cholesterol (mmol/L)	4.29 ± 0.22	4.00 ± 0.18	0.002
HDL cholesterol (mmol/L)	1.11 ± 0.05	1.06 ± 0.05	0.008
LDL cholesterol (mmol/L)	2.61 ± 0.19	2.36 ± 0.15	0.003
Triacylglycerol (mmol/L)	1.19 ± 0.14	1.27 ± 0.16	0.358

[1] Values are means ± SEM, n = 21.

[2] Probability of difference between initial and final period.

DISCUSSION

In this study, overweight subjects with initial serum cholesterol concentrations within the normal clinical range consumed 2.4 g/day of a supplement containing equal amounts of chitosan and glucomannan for twenty-eight days in a non-placebo-controlled study. Consumption of the supplement resulted in significant reductions of serum total, HDL and LDL cholesterol concentration, but no change in serum triacylglycerol concentration. These results are consistent with the hypocholesterolemic effect of chitosan and glucomannan reported in other human studies [7,8,14]. It is notable that in the present study

Table 4. Daily fecal excretion[1]

	Period		*p* value[2]
	Initial	Final	
Dry wt (g/day)	64.5 ± 6.8	79.7 ± 10.7	0.052
Fat (g/day)	13.8 ± 1.4	14.3 ± 1.7	0.764
Total bile acids (μmol/day)	2569 ± 299	3251 ± 488	0.161
Coprostan-3-ol (μmol/day)	863 ± 179	868 ± 225	0.220
Coprostan-3-one (μmol/day)	111 ± 63	207 ± 93	0.495
Dihydrocholesterol (μmol/day)	312 ± 93	182 ± 82	0.455
Cholesterol (μmol/day)	922 ± 174	1613 ± 344	0.064
Total neutral sterols (μmol/day)	2448 ± 337	3080 ± 524	0.134
Total steroids (μmol/day)	5263 ± 611	6551 ± 958	0.126

[1] Values are means ± SEM, n = 15–16.

[2] Probability of difference between initial and final period.

these reductions were obtained with lower amounts of material than has been used in other studies. The two previous studies in humans using glucomannan employed amounts of 3 to 3.9 g/day [7,8], whereas the previous chitosan feeding study gave 3 to 6 g/day [14]. There does appear, however, to be a lower limit to the efficacy of chitosan. Subjects consuming approximately 1.2 g/day of chitosan for four weeks found supplementation to be ineffective in lowering serum cholesterol [22], whereas an eight week supplementation with 2.4 g/day of chitosan led to only a marginally significant reduction in LDL cholesterol and no reduction in total cholesterol [23]. From the present study it cannot be determined which of the two materials, glucomannan or chitosan, is more potent in lowering cholesterol; however, our recent studies in rats suggest that the two materials are equipotent on a weight basis [16].

Relative to other soluble dietary fiber sources, the supplement used in this study appears to be a more potent hypocholesterolemic agent. Brown *et al.* [24], in a meta-analysis of the cholesterol lowering effect of various dietary fibers, reported net changes per g of soluble fiber of −0.029 mmol/L/g LDL cholesterol (95% CI: −0.035, −0.022). In the present study, the change in LDL cholesterol was −0.104 mmol/L/g. Our study did not utilize a placebo group, unlike the studies cited in the meta-analysis of Brown *et al.* [24]. However, examination of changes in total serum cholesterol in 20 of the studies used in their analysis indicates an average change of only 0.16% in the groups given a placebo, a value not different from zero (data not shown). Thus, inclusion of a placebo group would not likely have resulted in a significant adjustment in the final cholesterol concentrations.

It is of interest that the relatively large reduction in serum total and LDL cholesterol was obtained using subjects that are likely relatively resistant to diet-induced changes in their serum cholesterol, that is, overweight subjects with initially normal serum cholesterol concentrations. Both these factors appear to independently make individuals resistant to the effects of cholesterol lowering diets. For example, Jansen *et al.* [25] found that moderately overweight men (BMI $>$ 25 kg/m^2) had no significant reductions in total or LDL cholesterol when fed either an NCEP-I or high MUFA diet, relative to a high saturated fat diet. In contrast, men of normal weight (BMI $<$ 25 kg/m^2) had significant cholesterol reductions with both diets. Similar results were found by Bronsgeest-Schoute *et al.* [26], who noted that normal weight men and women experienced a significant reduction in total serum cholesterol when eggs were removed from their diet, whereas obese individuals showed no change. In a large study of hypercholesterolemic subjects ($>$6000) treated with fibrates, the degree of reduction in LDL cholesterol was inversely and significantly related to BMI at baseline [27]. Two studies have demonstrated that in women fed low cholesterol, reduced fat diets, only lean women experience significant reductions in LDL cholesterol [28,29]. Recently, in a study comparing the cholesterol-lowering effect of margarine relative to butter, initial BMI was inversely related to the degree of reduction of LDL cholesterol [30]. Initial serum cholesterol concentrations also appear to influence responsiveness. Individuals with initially normal cholesterol concentrations have been found less responsive to a cholesterol lowering diet than those with initially high cholesterol concentrations [31]. The finding that the chitosan + glucomannan supplement used in this study reduced serum cholesterol to a degree beyond that of most soluble dietary fibers, and did so in a population that was likely relatively unresponsive, suggests that this supplement is a potent cholesterol lowering agent.

Fecal excretion of bile acids and neutral sterols was determined to ascertain whether enhanced steroid excretion could be responsible for the hypocholesterolemic effect of the supplement. Excretion of bile acids and neutral sterols, measured after twenty-eight days of consumption of the supplement, tended to be increased, but the difference relative to the initial period did not achieve statistical significance ($p = 0.16$ and $p = 0.13$, respectively). This increase, however, is consistent with other studies. Gallaher *et al.* [16] found that an equal mixture of chitosan and glucomannan, fed at 7.5% of the diet, reduced cholesterol absorption and increased bile acid excretion in rats relative to a cellulose-based diet. Sugano *et al.* [32] noted an increase in cholesterol excretion in rats fed 5% chitosan, relative to cellulose. They further noted a change in the composition of the fecal sterols, with rats consuming chitosan excreting relatively more cholesterol and less coprostanol. In the present study, we found a strong trend for increased cholesterol excretion, with no change in excretion of other neutral sterols. Chitosan is known to have antimicrobial properties [33,34]. The change in the profile of fecal neutral sterols could therefore be due to a change in the type of colonic microflora or inhibition of their metabolic activities induced by the chitosan. This is also suggested by our previous study, in which we found a greater cecal pH in rats fed chitosan, with or without glucomannan, relative to a cellulose-based diet [16]. A higher cecal pH would be indicative of decreased activity of the microflora.

In this study no increase in fecal fat was detected after consumption of the chitosan + glucomannan supplement. This is in contrast to our previous study in rats, where consumption of the same supplement led to large increases in fecal fat excretion [16]. This increase could be attributed to the chitosan, as glucomannan alone did not increase fecal fat excretion. Further, others have reported that chitosan greatly reduces fat digestibility in rats [35,36] and chickens [37] when fed at 5% and 1.5% of the diet, respectively. The failure of the chitosan + glucomannan supplement to increase fat excretion in the present study may due to the dose given, which was considerably less than that used in the animal studies. The reduction in serum cholesterol and increase in fecal cholesterol excretion in the absence of an increase in fecal fat excretion indicates that these two phenomena are not linked and, therefore, must act through different mechanisms. The trend toward increased bile acid excretion coupled with the demonstrated ability of chitosan to bind bile acids, both in vitro [38,39] and ex vivo [40],

would favor bile acid binding, with subsequent micelle disruption and decreased cholesterol solubilization, as the mechanism of cholesterol lowering in the present study. However, glucomannan is a highly viscous dietary fiber and increasing intestinal contents viscosity decreases cholesterol absorption [41]. It would thus appear that a higher dietary concentration of chitosan is required to decrease fat digestibility than to achieve cholesterol lowering.

CONCLUSIONS

The present study confirms the hypocholesterolemic effect of a chitosan + glucomannan supplement reported in rats [16] and extends the finding to humans. The trend toward increased steroid excretion with supplement consumption suggests this is the primary mechanism for the effect. The failure to increase fecal fat excretion and the lack of change in body weight after 28 days of consumption of the supplement suggests that, at the dose used, this supplement would likely not be effective in accelerating weight loss.

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