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Effects of Chitosan on Plasma Lipids and Lipoproteins: A 4-Month Prospective Pilot Study

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Abstract

Chitosan can favorably modulate plasma lipids, but the available data are not conclusive. We evaluated the effect of chitosan on plasma lipids and lipoproteins in 28 patients with plasma triglyceride levels >150 mg/dL (mean age: 63 ± 12 years), not taking other lipid-lowering agents. All patients received a chitosan derived from fungal mycelium (Xantonet, Bromatech, Italy) at a fixed dose of 125 mg/d in addition to their current medications for 4 months. Polyacrylamide gel electrophoresis was used to measure low-density lipoprotein (LDL) subclasses. After treatment, total cholesterol reduced by 8%, LDL cholesterol by 2%, and triglycerides by 19%, with a concomitant 14% increase in high-density lipoprotein cholesterol. We also found a beneficial effect of chitosan on LDL subclasses, with a significant increase in LDL-2 particles (from 37 ± 8% to 47 ± 8%, $P = .0001$) and a decrease (although not significant) in atherogenic small, dense LDL. Whether these findings may affect cardiovascular risk remains to be established in future studies.

Keywords

chitosan, lipids, lipoproteins, therapy

Introduction

Chitosan is a food supplement, and its positive anions are thought to bind to negatively charged lipids from dietary fats, preventing fat digestion and reducing fat storage.[1,2] A further proposed mechanism is through inhibition of pancreatic lipase that is necessary for fat absorption, and several studies have shown reduction in weight when chitosan is given with a low-energy diet.[3] Others have investigated whether chitosan may be able to favorably modulate plasma lipids, and this is of interest since natural products tend not to have major side effects.

Indeed, chitosan is a natural product that is, after cellulose, the second more common biopolymer on the Earth.[4] Although not derived from plants, chitosan being a polysaccharide is similar to a dietary fiber that is indigestible by mammalian digestive enzymes. Chitosan is the deacetylated form of chitin, an aminopolysaccharide found in the exoskeleton of arthropods and certain fungi.[5] There are no major side effects reported with the use of chitosan, although it may cause mild stomach upset, constipation, or gas.[6] Among potential interactions, it should be highlighted that chitosan may interact with warfarin, an anticoagulant.[6]

Available data on the role of chitosan on plasma lipids are not conclusive. Several studies have shown that chitosan can reduce total cholesterol levels in patients with hypercholesterolemia,[7,8] and this has been confirmed in a meta-analysis of published randomized controlled trials.[9] In contrast, the effects of chitosan in patients with hypertriglyceridemia are largely unknown although in animal models fed with a high-fat diet chitosan was able to decrease triglycerides by 29% to 31%, with a concomitant increase in high-density lipoprotein cholesterol (HDL-C) by 8% to 11%.[10] This is consistent with the data published by Ausar et al who reported an increase in HDL-C concentrations by chitosan in patients with dyslipidemia.[7] Further, no study has so far investigated the effects of chitosan on plasma lipoproteins. This is also of potential importance, since increasing evidence suggests that the "quality" and not only the "quantity" of plasma lipids significantly influences the cardiovascular (CV) risk.[11]

The aim of the present study was to evaluate the effects of chitosan on plasma lipids and lipoproteins in patients with hypertriglyceridemia not taking other lipid-lowering agents.

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Materials and Methods

Patients and Methods

We studied 28 patients with hypertriglyceridemia (ie, plasma triglyceride levels >150 mg/dL), 13 women and 15 men, with a mean age of 63 ± 12 years who were referred to our Unit of Cardiovascular Prevention for a clinical evaluation. All patients did not use lipid-lowering agents for at least 3 months before entering the study, since they were statin-intolerant or new patients. The project design included a medical examination, anthropometric data, biochemical analyses, and echo color-Doppler examination of the carotid arteries. The procedures adopted were in agreement with the Helsinki Declaration of 1975 as revised in 1983 and were approved by the Ethics Council of the University of Palermo.

All patients gave their informed consent to participate in the study. On admission, they underwent a medical examination and were excluded from the study if they had clinical evidence of liver dysfunction or renal failure. Waist circumference, height, and weight were recorded, and body mass index (BMI) was calculated as kg/m^2. Of the patients, 9 were current smokers, 23 had type-2 diabetes, and 18 had hypertension diagnosed by the following systolic or diastolic blood pressure≥140 or ≥90 mm Hg , respectively, or previous pharmacological therapy with antihypertensive drugs. In all, 23 patients were already taking antihypertensive and/or antidiabetic drugs, and such medications were maintained at the same doses until the end of the study. Regarding antidiabetic drugs, all the 23 patients with type-2 diabetes received metformin therapy, while add-on therapy included a glucagon-like peptide 1 (GLP-1) analog in 21 patients, a sulfonylurea in 6 patients, pioglitazone in 2 patients, and insulin in 1 patient. Five patients had a previous CV event. All patients received a chitosan derived from fungal mycelium (Xantonet, Bromatech, Italy) at a fixed dose of 125 mg/d (2 tablets/d) in addition to their current medication.

The presence of the metabolic syndrome was defined by the joint American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement,[12] that is, the presence of any 3 of the following 5 risk factors: elevated waist circumference (>88 cm in women and >102 cm in men), elevated fasting triglycerides (≥150 mg/dL or on drug treatment for elevated triglycerides), reduced HDL-C (<50 mg/dL in women and <40 mg/dL in men or on drug treatment for reduced HDL-C), elevated blood pressure (systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg or on antihypertensive drug treatment in a patient with a history of hypertension), and elevated fasting glucose (≥100 mg/dL or on drug treatment for elevated glucose).

Biochemical Analyses

At baseline and after 4 months, serum parameters were measured after a 14-hour overnight fast. Total cholesterol, triglycerides, and HDL-C were measured by standard enzymatic-colorimetric methods,[13-15] while low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula.

Table 1. Effects of 4-Month Chitosan Therapy on Body Mass Index (BMI), Waist Circumference, and Plasma Lipids.

	Before Treatment	*P*	After Treatment
BMI, kg/m^2	30 ± 5	.015	29 ± 4
Waist circumference, cm	104 ± 14	.032	102 ± 12
Total cholesterol, mg/dL	192 ± 43	.019	171 ± 23
Triglycerides, mg/dL	228 ± 72	<.001	176 ± 54
HDL-cholesterol, mg/dL	39 ± 9	.016	44 ± 10
LDL-cholesterol, mg/dL	107 ± 44	NS	92 ± 20

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; NS, not significant.

Lipoprotein Subclass Analysis

Nondenaturing, linear polyacrylamide gel electrophoresis was used to separate and measure LDL subclasses in a subset of 16 randomized samples, with the Lipoprint system[16] (Quantimetrix Corporation, Redondo Beach, California). This method has been validated against gradient gel electrophoresis and nuclear magnetic resonance, and it is the only Food and Drug Administration-cleared diagnostic tool for lipoprotein subfraction testing in the United States.[17] The electrophoresed gels were scanned to determine the relative area of each lipoprotein subfraction. In detail,[18] 25 μL of the sample was mixed with 200 μL of Lipoprint loading gel and placed upon the upper part of the 3% polyacrylamide gel. After 30 minutes of photopolymerization at room temperature, electrophoresis was performed for 60 minutes with 3 mA for each gel tube. Each electrophoresis chamber involved 2 quality controls.

For quantification, scanning was performed with a digital scanner and a Mac personal computer (Apple Computer Inc). AQ3 After scanning, the electrophoretic mobility and the area under the curve were calculated. The LDL subclasses were distributed as 7 bands (LDL-1 to LDL-7, respectively): LDL-1 and LDL-2 are defined as large LDL; LDL-3 to LDL-7 are defined as small LDL.[18]

Statistical Analysis

Statistical analysis was performed using Statview 5.0 (SAS Institute Inc, Cary, North Carolina). Univariate analysis was performed using 2-tailed paired Student *t* tests, while correlation analysis was performed using the Spearman rank correlation method.

Results

In Table 1, we report the effects of the 4-month chitosan therapy on BMI, waist circumference, and plasma lipids. The BMI and waist circumference (in cm) were significantly reduced after therapy (from 30 ± 5 to 29 ± 4, $P = .015$ and from 104 ± 14 to 102 ± 12, $P = .032$, respectively). Regarding plasma lipids, total cholesterol and triglycerides were significantly lowered (from 192 ± 43 to 171 ± 23, $P = .019$ and from 228 ± 72 to 176 ± 54 mg/dL, $P < .001$, respectively),

Table 2. Effects of 4-Month Chitosan Treatment on Low-density Lipoproteins (LDL) Size and Subclasses.

	Before Treatment	*P*	After Treatment
LDL peak particle size, Å	264 ± 7	NS	265 ± 5
LDL-1, %	43 ± 18	NS	38 ± 14
LDL-2, %	37 ± 8	.0001	47 ± 8
LDL-3, %	15 ± 11	NS	13 ± 8
LDL-4, %	4 ± 3	NS	2 ± 1
LDL-5, %	1 ± 2	NS	0 ± 0
LDL-6, %	0 ± 0	NS	0 ± 0
LDL-7, %	0 ± 0	NS	0 ± 0

Abbreviation: NS, not significant.

while HDL-C significantly increased (from 39 ± 9 to 44 ± 10 mg/dL, $P = .016$). The LDL-C levels also fell, but the difference did not reach significance (from 107 ± 44 to 92 ± 20 mg/dL). In addition, fasting plasma glucose and glycated hemoglobin significantly fell after treatment (from 151 ± 70 to 134 ± 34 mg/dL, $P = .003$ and from 8.4 ± 1.2% to 7.0 ± 1.1%, $P < .001$, respectively), while the presence of the metabolic syndrome decreased from 22 patients before chitosan supplementation to 17 patients after treatment.

Table 2 shows the effects of chitosan on LDL size and subclasses. The only significant change was for LDL-2, which significantly increased after treatment (from 37 ± 8% to 47 ± 8%, $P < .001$). Other LDL subclasses, including LDL-1, LDL-3, LDL-4, and LDL-5, significantly decreased after treatment, but the difference did not reach the significance. We also report a slight but not significant increase in LDL peak particle size (from 264 ± 7 to 265 ± 5 Å). Spearman correlations between treatment changes in BMI, waist circumference, and plasma lipids and changes in LDL subclasses (see Table 3) showed that the only correlation that reached the significance was that between LDL-2 and triglycerides ($r = .456$, $P < .05$).

Discussion

This is the first study that investigated the effects of chitosan on lipoproteins. We found that after 4 months of therapy, total cholesterol reduced by 8%, LDL-C by 2%, and triglycerides by 19%, with a concomitant 14% increase in HDL-C. These findings extend previous observations on the effects of chitosan in reducing total cholesterol levels in patients with hypercholesterolemia,[5,6] as well as in increasing HDL-C concentrations in patients with dyslipidemia.[4] The most pronounced effects of chitosan in the present study were found for triglycerides (−19%), and this was suggested in animal models fed a high-fat diet, where this agent was able to decrease triglycerides by 29% to 31%.[10]

Beyond plasma lipids concentrations, several expert panels and international guidelines, such as the National Cholesterol Education Program Adult Treatment Panel III, have accepted the predominance of small, dense LDLs (sdLDLs) as an emerging CV risk factor.[19] Most studies suggest that measuring LDL particle size, sdLDL cholesterol content, and LDL particle number provides additional assessment of CV risk.[11] A European panel of experts recently provided a comprehensive consensus statement on the pathophysiology, atherogenicity, and clinical significance of LDL subclasses,[17] and the main findings are summarized in its executive statement.[20]

The LDLs comprise multiple subclasses with different size, density, physicochemical composition, metabolic behavior, and atherogenicity; based on their characteristic appearance in analytical ultracentrifugation and gradient gel electrophoresis, up to 7 distinct LDL subclasses can be defined.[17] In clinical practice, LDL phenotype B refers to patients with a predominance of sdLDL, meaning those with a predominance of LDL-3 and LDL-4 subclasses.[17] The magnitude and independence of the association of LDL size with CV risk has been tested in >100 studies, including cross-sectional and prospective epidemiologic as well as clinical intervention trials, and the vast majority of these trials demonstrate a significant association of sdLDL with increased CV risk.[21]

Several mechanisms have been suggested to explain the enhanced atherogenicity of sdLDL. These particles are taken up more easily by arterial tissue than larger counterparts, suggesting greater transendothelial transport.[22] In addition, sdLDL have also decreased receptor-mediated uptake and increased proteoglycan binding; further, sialic acid plays a role in the in vitro association of LDL with the polyanionic proteoglycans, and the sialic acid content of LDL particles in patients with a predominance of sdLDL is reduced.[23] It has also been shown that oxidative susceptibility increases and antioxidant concentrations decrease with lowering LDL size.[24]

The present study shows for the first time a beneficial effect of chitosan on LDL subclasses, with a significant increase in less atherogenic LDL particles (eg, LDL-2 particles) and a concomitant decrease (although not significant) in atherogenic sdLDL. It should be highlighted that correlation analysis revealed that the reduction in LDL-2 was significantly correlated with the decrease in plasma triglyceride levels. Therefore, the triglyceride reduction induced by chitosan seems to have a direct impact on the modulation of LDL subclasses, toward less atherogenic particles.

Beyond the effects on plasma lipids and lipoproteins, in the present study we have found a beneficial effect of chitosan on weight loss, as reported previously.[25] We cannot exclude a potential action of this weight loss on the plasma lipid variables measured, including triglycerides, HDL-C, and LDL subfractions, through amelioration of insulin resistance.[16,26] Further, glycemic indices improved, and fewer patients had the metabolic syndrome after treatment; this is consistent with previous findings on the potential effects of chitosan on adipokines and/or nonalcoholic fatty liver disease (NAFLD), as discussed below.

Chitosan has been previously shown to beneficially affect insulin resistance (assessed by homeostasis model assessment resistance index, plasma glucose, and GLP-1 levels) and fatty liver (assessed by hepatic triglycerides and cholesterol content) in animal studies[27-29]; both insulin resistance and NAFLD are

Table 3. Spearman Correlations Between Treatment-Related Changes (Δ) in Body Mass Index (BMI), Waist Circumference, and Plasma Lipids and Δ in Low-density Lipoproteins (LDL) Subclasses.[a]

	ΔLDL-1	ΔLDL-2	ΔLDL-3	ΔLDL-4	ΔLDL-5	ΔLDL-6	ΔLDL-7
ΔBMI	−.123	.112	.441	.177	–	–	–
ΔWaist circumference	−.113	.034	.198	.335	–	–	–
ΔTotal cholesterol	−.156	.275	.137	.-253	–	–	–
ΔTriglycerides	−.398	**.456**	.434	.115	–	–	–
ΔHDL-cholesterol	.191	.086	−.312	.121	–	–	–
ΔLDL-cholesterol	−.001	.155	.093	.324	–	–	–

Abbreviation: HDL, high-density lipoprotein.
[a]Bold value indicate the relationships that reached statistical significance ($P < .05$).

associated with increased vascular risk.[30,31] Furthermore, plasma adiponectin was increased, whereas plasma inflammatory markers such as necrosis factor-α and interleukin 6 were reduced following chitosan treatment.[27,29] With regard to leptin, chitosan has been reported to decrease circulating leptin levels in animals with diet-induced obesity[25] and nonalcoholic steatohepatitis,[29,32] both of which arecharacterized by hyperleptinemia.[33] Such adipokines are associated with increased vascular risk,[34,35] and several drugs may affect their levels.[36,37] Therefore, the clinical implications of chitosan effects on these biochemical markers remain to be evaluated in future studies. Yet, in our study, liver tests did not improve after chitosan treatment (data not shown).

Potential limitations of our study include the relatively small number of patients as well as the use of cardiometabolic medications. Indeed, we cannot exclude the fact that the use of antidiabetic agents did not influence the results of this study; GLP-1 analogswere widely used in the diabetic patientsincluded in this study, and increasing evidence suggests nonglycemic effects of these agents, including significant effects on weight loss as well as plasma lipids and lipoproteins.[38,39]

In conclusion, in this pilot study we report significant beneficial effects of chitosan on plasma lipids and lipoproteins after 4 months of treatment. Whether these findings may affect CV risk remains to be established in the future studies.

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Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: This study has been conducted independently. MR and MC have received research funds, given talks and participated in conferences sponsored by Bromatech (Italy), but this company had no role in the design of the study and had no influence on the interpretation of the data or writing the manuscript.

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