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CLINICAL TRIALS

Saara Metso · Ritva Ylitalo · Matti Nikkilä
Erkki Wuolijoki · Pauli Ylitalo · Terho Lehtimäki

# The effect of long-term microcrystalline chitosan therapy on plasma lipids and glucose concentrations in subjects with increased plasma total cholesterol: a randomised placebo-controlled double-blind crossover trial in healthy men and women

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**Abstract** *Objective*: To evaluate the long-term effect of microcrystalline chitosan (MCC) on plasma lipids, especially the concentration of low-density lipoprotein (LDL) cholesterol, in subjects with a moderately increased concentration of plasma total cholesterol.
*Methods*: A total of 130 middle-aged men and women without severe disease and with a total cholesterol of 4.8–6.8 mmol/l and triglycerides below 3.0 mmol/l were randomised into two treatment groups. At the beginning of the 10-month trial, all participants received placebo 1.2 g twice daily during a 1-month run-in period. Subsequently, group 1 first received 1.2 g placebo twice daily for 3 months and then 1.2 g MCC twice daily for 3 months. Correspondingly, group 2 received 1.2 g MCC twice daily during the first and 1.2 g placebo twice daily during the second 3-month period. During the final 3-month follow-up period, both groups received MCC. Altogether, 83 participants completed the study.
*Results*: No difference was detected in the change in the LDL-cholesterol concentration between the treatments during the crossover trial ($P = 0.98$ for interaction between time period and treatment group, repeated-measures analysis of variance for crossover design). In an otherwise similar analysis, no differences were detected between the treatments in the concentrations of total cholesterol, high-density lipoprotein cholesterol, triglycerides and glucose.
*Conclusions*: Treatment with MCC had no effect on the concentrations of plasma lipids or glucose in healthy middle-aged men and women with moderately increased plasma cholesterol concentrations.

**Keywords** Microcrystalline chitosan · Plasma LDL-cholesterol · Gastrointestinal lipid absorption

S. Metso (✉)
Department of Internal Medicine,
Tampere University Hospital, P.O. Box 2000,
33521 Tampere, Finland
E-mail: saara.metso@pshp.fi
Tel.: +35-83-311611
Fax: +35-83-2474168

R. Ylitalo · P. Ylitalo
Department of Pharmacological Science, Medical School,
33014 University of Tampere, Finland

M. Nikkilä
Department of Internal Medicine,
City Hospital of Tampere, 33520 Tampere, Finland

E. Wuolijoki
Finn-Medi Research Ltd., Clinical Trial Center,
Lenkkeilijänkatu 8, 33520 Tampere, Finland

T. Lehtimäki · S. Metso · P. Ylitalo
Department of Clinical Chemistry,
Tampere University Hospital, 33521 Tampere, Finland

P. Ylitalo · T. Lehtimäki
Medical School, 33014 University of Tampere,
Finland

## Introduction

Chitosan is derived by alkaline deacetylation from chitin, an abundant polymeric product of natural biosynthesis, found especially in crustaceans [1]. Chitosan is claimed to control obesity and to lower serum cholesterol levels [2, 3, 4]. In the aqueous acidic fluid of the stomach, chitosan swells and forms a positively charged gel. The amino groups ($-NH_2$) of chitosan take on hydrogen ions ($H^+$), resulting in the formation of a positively charged tertiary amino group ($-NH_3{}^+$). Negatively charged molecules, i.e. fatty and bile acids, attach strongly to the positively charged chitosan [1]. Chitosan also interferes with normal emulsification of neutral lipids, i.e. cholesterol and other sterols, by binding them with hydrophobic bonds. This electrostatic and hydrophobic bonding causes the formation of large polymer compounds, which are weakly broken down by the digestive processes in man [1]. Microcrystalline chitosan (MCC) creates agglomerates of specific structure and size, forming an exceptionally large adsorption area [1].

The different types of chitosan products have been widely promoted and freely available in health stores and pharmacies. Several hundred tons of dietary chitosan products are consumed yearly in Europe and the USA. Despite the huge consumption of chitosan, there are, however, only a few short-term (1–2 months) controlled clinical trials evaluating the effects of dietary chitosan treatment on plasma cholesterol levels [2, 3, 5, 6]. Chitosan has been found to reduce the levels of plasma cholesterol without dietary intervention in patients with chronic renal disease and in obese subjects [2, 3]. There are also studies in which chitosan did not affect the levels of plasma total or low-density lipoprotein (LDL)-cholesterol without dietary control [5, 6]. No clinically significant adverse effects have been reported with chitosan compared with placebo [3, 4, 5, 6]. Since no data from long-term chitosan trials are found in the literature, the purpose of this 10-month randomised, placebo-controlled, double-blind study was to evaluate the long-term effects of MCC on plasma lipids, especially on the concentration of LDL-cholesterol in healthy, normal-weight subjects with moderately increased plasma total cholesterol concentration. We used MCC in our trial because it forms an exceptionally large adsorption area and might have a favourable lipid-binding effect compared with other chitosan preparations.

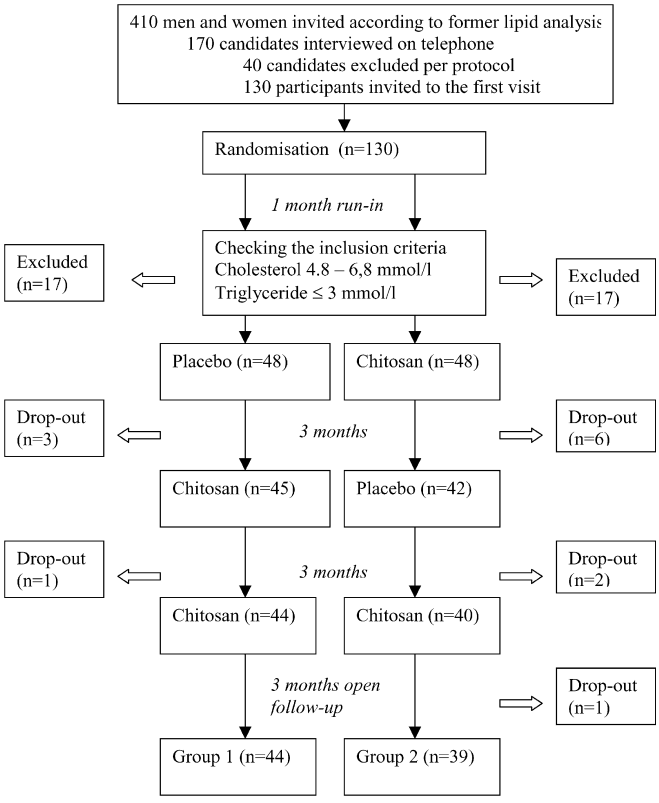


**Fig. 1** The flow and follow-up of the participants in the study

## Materials and methods

### Subjects

Invitation letters were sent to 410 middle-aged men and women chosen from the patients of Tampere Health Center and the personnel of Tampere University Hospital whose plasma total cholesterol level had been slightly increased in the context of an earlier health examination. A total of 170 candidates consented to participate. They were interviewed by telephone, after which 40 were excluded from the protocol, and 130 (54 men and 76 women) were randomised into two treatment groups. Altogether, 83 of the 130 participants (64%) completed the study. Participant flow and follow-up are shown in Fig. 1.

The exclusion criteria were: age under 18 years or over 65 years; diabetes mellitus; history of renal, adrenal or liver disease; evidence of thyroid gland dysfunction; history of coronary artery or cerebrovascular disease; malignant tumour or chronic terminal disease; use of lipid-lowering medication, functional food or continuous steroid therapy; alcoholism or addiction to narcotics; mental lability; pregnancy, lactation and childbearing potential for women not using any medically accepted birth control method; history of participation in another drug evaluation study within 1 month; a severe adverse effect which might have been caused by chitosan and allergy to crustaceans. If excluded participants had increased lipid levels or abnormal thyroid function calling for needed medication, they were guided to a health centre or an occupational health service. The inclusion criteria for entry into the double-blind treatment period were a concentration of plasma total cholesterol of 4.8–6.8 mmol/l and a concentration of plasma triglyceride of less than 3.0 mmol/l at the end of the 1-month run-in period. Participants were withdrawn from the study in the case of any intolerable adverse event, exclusion criterion, non-compliance or protocol violation.

The ethics committee of the Pirkanmaa Hospital District and the ethics committee of Tampere Heath Center approved the final protocol. The study was undertaken in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki. All participants gave their written informed consent.

**Table 1** Study design of the study. *QOL* quality of life

| Control visits | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Time (months) | −1 | 0 | 3 | 6 | 9 |
| Exclusion criteria | x | | | | |
| Inclusion criteria | | x | | | |
| Physical examination | x | x | x | x | x |
| Plasma lipids | x | x | x | x | x |
| Adverse events, QOL | | x | x | x | x |
| Treatment | | | | | |
| Placebo, P | | P | P | MCC | MCC |
| Microcrystalline chitosan, MCC | | P | MCC | P | MCC |

### Study design and randomisation

The study was a 10-month randomised double-blind, placebo-controlled crossover study designed to assess the effect of 1.2 g MCC twice daily on the concentration of plasma LDL-cholesterol in subjects with moderately increased plasma total cholesterol levels. It was conducted in Tampere, Finland, during the period from February 2001 to February 2002. The study consisted of three periods: (1) a 1-month single-blind run-in period with placebo treatment; (2) a double-blind placebo-controlled crossover intervention for 3 months with MCC and 3 months with placebo, or vice versa, for either half of the cohort; (3) after the crossover intervention period, an extra open 3-month follow-up period with MCC treatment for all participants. The study design is shown in Table 1.

Two preparations were used: capsules containing 400 mg MCC as study treatment and identical capsules containing 400 mg starch as control. The participants were requested to take three capsules twice a day before meals at 0600–0900 hours and 1700–2000 hours. No dietary changes were required; on the contrary, the participants were advised to follow their habitual diet during the study.

The pharmacist indicated the preparations as A and B and numbered treatment containers according to a randomisation list. The study physician, study nurse and participants were all blinded and saw only the numbered white plastic bottles containing capsules. The randomisation codes were not opened until the close-out meeting.

### Measures

At the first visit, the study physician conducted an initial screening assessment, which included exploration of medical history, physical examination, measurements of vital signs, weight, height, body mass index (BMI), weight (kg)/height squared ($m^2$) and blood pressure in all cases. At the second, third and fourth visits, the study nurse measured weight, height, BMI and blood pressure. At the fifth, final visit, the physician re-examined the participants with similar procedures.

Blood samples were collected five times during the study: at enrolment (visit 1), at the beginning (visit 2), in the middle (visit 3) and at the end (visit 4) of the 6-month crossover intervention period and, finally, after the open 3-month MCC treatment at visit 5 (Table 1). Alcohol consumption was prohibited for 36 h, and a minimum of 12 h fasting was required before blood samples were drawn. On the examination day, the capsules were not taken before blood sampling.

Plasma was separated by centrifugation, frozen and stored at −70°C until all samples collected during the study were analysed at the same time at the end of the study. Only the additional samples (plasma cholesterol and triglycerides and serum thyrotropin) for checking the inclusion criteria were analysed immediately at the second visit. All lipid analyses were performed in the Department of Clinical Chemistry, Tampere University Hospital, an accredited laboratory with a regular and detailed internal and external auditing program and a quality control program covering the chemical analyses used in this study. The plasma triglycerides and total and high-density lipoprotein (HDL)-cholesterol as well as plasma glucose were analysed by a Cobas Integra 700 automatic analyser with reagents and calibrators as recommended by the manufacturer (Hoffmann-La Roche Ltd., Basel, Switzerland). The concentration of LDL-cholesterol was calculated using Friedewald's formula [7]. The inter-assay coefficients of variation were 1.4% for the assessment of total cholesterol, 1.0% for the assessment of triglycerides and 3.7% for the assessment of HDL-cholesterol.

### Primary and secondary efficacy variables

The primary efficacy variable was the plasma concentration of LDL-cholesterol. In this crossover trial, efficacy was assessed on the basis of a change in the concentration of LDL-cholesterol between the second and third visits (first period) and between the third and fourth visits (second period). A clinically significant decrease in LDL-cholesterol concentration of was considered to be 7–8% (0.3–0.4 mmol/l) during one period. If the LDL-cholesterol concentration increased by more than 5% (combined physiological and analytical variability), it was counted as a failure of treatment. The secondary efficacy variables were the concentrations of plasma total and HDL-cholesterol, plasma triglycerides and blood glucose, as well as weight, BMI and systolic and diastolic blood pressure.

### Tolerability and compliance assessments

All participants were observed and questioned regarding complaints during the treatment period, and all adverse events were recorded. Safety was assessed by quality of life (QOL) questions, the occurrence of adverse events and the results of clinical examinations. The participants were advised to follow their habitual diet during the study. Participants were asked about possible changes in diet and exercise habits at each visit. The participants were instructed to take the study capsules as prescribed and to bring their bottles with them when visiting the investigator. At each visit, the participants were asked whether they had taken the capsules according to the instructions. Furthermore, the remaining capsules were counted.

### Statistical analyses

The null hypothesis stated that the change in the plasma LDL-cholesterol concentration achieved by the MCC treatment would not differ significantly from that achieved by the placebo treatment. The null hypothesis was tested using analysis of variance for repeated measures (RANOVA), utilising a crossover design in which the independent factor was treatment group, the dependent factors were changes in the concentration of LDL-cholesterol and the repeated measure factors were the first and the second periods of the crossover intervention. According to power calculations, 83 subjects at the end of the study confirmed an 80% power to detect a difference of 0.3 mmol/l at a two-sided significance level of 0.05 in change in LDL-cholesterol concentration between the MCC and placebo treatment groups.

A RANOVA was conducted within group 1 to observe the effect of the 6-month continuous MCC treatment on LDL-cholesterol level. Within group 2, the RANOVA was used to test whether the consistency of the effect of the 3-month MCC treatment was also maintained during the 3-month placebo period. The secondary efficacy variables were tested in the same way as the primary efficacy variable. The difference in clinical characteristics between the treatment groups was tested by *t*-test for independent samples to ensure the validity of randomisation. This analysis was conducted at each visit to confirm the uniformity of the groups throughout the study. Categorical variables were compared using the $\chi^2$ test. The statistical analyses were carried out in the Laboratory of Arteriosclerosis Genetics using Statistica for Windows Version 5.0 (Statsoft Inc, Tulsa, Oklahoma, USA).

## Results

### Efficacy variables

The clinical characteristics of the participants at each visit are shown in Table 2. At the randomisation visit, the mean age of the participants was 46 years (range 32–64 years), this being similar in the two treatment groups ($P = 0.35$, *t*-test). There was no difference in the distribution of sex between the treatment groups at any visit in the study ($P = 1.0$, $P = 0.30$, $P = 0.30$, $P = 0.34$ and $P = 0.40$ at visits 1–5, correspondingly, $\chi^2$ test). Data taken at visit 1 reflected the validity of the original randomisation. All clinical characteristics were similar in the treatment groups throughout, except for the mean concentrations of total and LDL-cholesterol, which were higher in group 1 than in group 2 at the second and third visits ($P < 0.05$, *t*-test for independent samples).

The effect of MCC therapy on the concentration of LDL-cholesterol and on the other efficacy variables was compared with the effect of placebo during the

**Table 2** Clinical characteristics of the participants in different phases of the crossover study. Data is shown in mean (SD)

| | Visit 1 ($n=130$) | Visit 2 ($n=96$) | Visit 3 ($n=87$) | Visit 4 ($n=84$) | Visit 5 ($n=83$) |
|---|---|---|---|---|---|
| Sex (male/female) | | | | | |
| Group 1 | 27/38 | 22/26 | 21/24 | 21/23 | 21/23 |
| Group 2 | 27/38 | 17/31 | 15/27 | 15/25 | 15/24 |
| Weight, kg | | | | | |
| Group 1 | 78.6 (13.9) | 78.3 (12.7) | 77.5 (12.9) | 77.4 (12.8) | 78.1 (12.3) |
| Group 2 | 75.5 (12.5) | 75.6 (12.3) | 74.0 (12.2) | 74.0 (12.7) | 74.1 (13.0) |
| BMI, $kg/m^2$ | | | | | |
| Group 1 | 26.9 (3.7) | 26.9 (3.7) | 26.7 (3.6) | 26.6 (3.7) | 26.7 (3.6) |
| Group 2 | 25.8 (3.3) | 25.9 (3.3) | 25.3 (3.2) | 25.2 (3.4) | 25.1 (3.4) |
| Systolic blood pressure, mmHg | | | | | |
| Group 1 | 134 (20) | 134 (17) | 126 (13) | 130 (12) | 133 (16) |
| Group 2 | 134 (17) | 135 (12) | 128 (18) | 130 (17) | 134 (19) |
| Diastolic blood pressure, mmHg | | | | | |
| Group 1 | 84 (11) | 86 (10) | 82 (10) | 84 (11) | 86 (11) |
| Group 2 | 85 (10) | 86 (8.8) | 81 (10) | 82 (10) | 84 (8) |
| Plasma total cholesterol, mmol/l | | | | | |
| Group 1 | 6.0 (0.8) | 6.2 (0.5)* | 5.8 (0.6)* | 5.7 (0.6) | 5.7 (0.6) |
| Group 2 | 5.8 (0.7) | 5.8 (0.6) | 5.6 (0.6) | 5.6 (0.6) | 5.5 (0.7) |
| Plasma LDL-cholesterol, mmol/l | | | | | |
| Group 1 | 3.8 (0.8) | 4.0 (0.6)* | 3.7 (0.6)* | 3.6 (0.6) | 3.6 (0.7) * |
| Group 2 | 3.7 (0.8) | 3.6 (0.6) | 3.4 (0.6) | 3.4 (0.7) | 3.3 (0.7) |
| Plasma HDL-cholesterol, mmol/l | | | | | |
| Group 1 | 1.6 (0.5) | 1.5 (0.3) | 1.5 (0.4) | 1.5 (0.3) | 1.5 (0.3) |
| Group 2 | 1.5 (0.4) | 1.5 (0.3) | 1.6 (0.4) | 1.5 (0.3) | 1.6 (0.4) |
| Plasma triglycerides, mmol/l | | | | | |
| Group 1 | 1.5 (1.0) | 1.3 (0.6) | 1.3 (0.6) | 1.4 (0.8) | 1.4 (0.9) |
| Group 2 | 1.3 (0.8) | 1.3 (0.6) | 1.2 (0.7) | 1.3 (0.6) | 1.4 (0.7) |
| Glucose, mmol/l | | | | | |
| Group 1 | 5.2 (0.6) | 4.9 (0.5) | 4.6 (0.6) | 4.6 (0.4) | 4.7 (0.5) |
| Group 2 | 5.1 (0.5) | 4.8 (0.6) | 4.5 (0.5) | 4.5 (0.4) | 4.6 (0.5) |

*Statistically significant difference between the group 1 and 2 ($P<0.05$, $t$-test for independent samples)

crossover trial, as shown in Table 3. Group 1 received placebo during the first period and MCC during the second. Group 2 received MCC during the first and placebo during the second. The change in the concentration of plasma LDL-cholesterol during MCC treatment did not differ statistically significantly from that during placebo treatment ($P=0.98$ for the interaction between the treatment groups and the time period, RANOVA for a crossover design). Neither were there any differences in the secondary efficacy variables. The concentrations of total cholesterol, LDL-cholesterol and glucose, as well as weight and blood pressure, decreased more during the first than the second period in both groups, i.e. independently of the MCC and placebo treatments.

The concentrations of mean LDL-cholesterol in group 1 and group 2 at visits 1–5 are shown in Table 2. The 6-month continuous treatment with MCC in group 1 did not significantly reduce the mean concentration of LDL-cholesterol (a decrease of 0.1 mmol/l, 2%, $P=0.46$, RANOVA). However, the mean concentration of LDL-cholesterol fell by 0.3 mmol/l (8%) during the intermittent MCC treatment, i.e. when the participants received MCC during the first 3-month period, placebo during the second 3-month period and MCC during the third 3-month period ($P=0.001$, RANOVA).

## Tolerability

Of the 130 randomised participants, 83 completed the study. The reasons for premature withdrawal included lipid levels not fulfilling the inclusion criteria ($n=21$), subclinical hypothyreosis ($n=5$), adverse events ($n=7$), changes in state of health ($n=2$), loss to follow-up ($n=11$) and noncompliance ($n=1$). Withdrawals ($n=47$) were equally frequent in both treatment groups ($n=21$ in group 1 and $n=26$ in group 2, $P=0.62$, $\chi^2$ test). The flow and follow-up of the participants are shown in Fig. 1.

No serious adverse events were reported during the study. Seven participants discontinued the study because of adverse effects. After the run-in period with placebo treatment, 29% (28/96) of the participants reported an adverse event. During the first period of the crossover trial, 26% (23/87) reported that they had experienced one or more adverse events. The corresponding values were 39% (33/84) and 19% (16/83) during the second period and the open follow-up period with MCC treatment, respectively. The reported adverse events included constipation, flatulence, increased defecation frequency, swelling, different kinds of pain, rash, heart palpitation and insomnia. There was no difference in the number of reported adverse events between the treatment groups at any phase of the study ($P=0.36$ at visit 2, $P=0.96$ at visit

**Table 3** Changes in the clinical characteristics during the crossover trial with microcrystalline chitosan (MCC) and placebo treatment. Participants in the group 1 received placebo during the first and MCC during the second period. Participants in the group 2 received MCC during the first and placebo during the second period

| Characteristic | Mean change (SD) | | |
|---|---|---|---|
| | Period 1 | Period 2 | Statistical probability ($P$) |
| Weight, kg | | | 0.82 for interaction |
| Group 1 | −0.9 (2.0) | −0.02 (2.0) | 0.02 for time* |
| Group 2 | −0.9 (1.8) | −0.14 (1.3) | 0.85 for group |
| BMI, kg/m$^2$ | | | 0.78 for interaction |
| Group 1 | −0.3 (0.8) | ±0.0 (0.6) | 0.01 for time* |
| Group2 | −0.3 (0.6) | ±0.0 (0.4) | 0.86 for group |
| Systolic blood pressure, mmHg | | | 0.38 for interaction |
| Group 1 | −9 (13) | −3 (12) | <0.001 for time* |
| Group 2 | −7 (13) | ±0 (11) | 0.85 for group |
| Diastolic blood pressure, mmHg | | | 0.74 for interaction |
| Group 1 | −4 (8) | +2 (8) | <0.001 for time* |
| Group 2 | −5 (10) | +2 (7) | 0.43 for group |
| Plasma total cholesterol, mmol/l | | | 0.83 for interaction |
| Group 1 | −0.3 (0.6) | −0.1 (0.5) | 0.03 for time* |
| Group 2 | −0.20 (0.5) | ±0.0 (0.6) | 0.02 for group* |
| Plasma LDL-cholesterol, mmol/l | | | 0.98 for interaction |
| Group 1 | −0.3 (0.6) | −0.1 (0.5) | 0.02 for time* |
| Group 2 | −0.2 (0.6) | ±0.0 (0.5±0) | 0.08 for group |
| Plasma HDL-cholesterol, mmol/l | | | 0.38 for interaction |
| Group 1 | ±0 (0.2) | −0.1 (0.2) | 0.01 for time* |
| Group 2 | +0.1 (0.1) | −0.1 (0.1) | 0.046 for group* |
| Plasma triglycerides, mmol/l | | | 0.92 for interaction |
| Group 1 | −0.1 (0.4) | +0.1 (0.6) | 0.09 for time |
| Group 2 | −0.1 (0.6) | +0.1 (0.4) | 0.81 for group |
| Blood glucose, mmol/l | | | 0.47 for interaction |
| Group 1 | −0.2 (0.6) | −0.1 (0.4) | 0.01 for time* |
| Group 2 | −0.3 (0.5) | ±0.0 (0.5) | 0.77 for group |

*$P < 0.05$, repeated measures analysis of variance

3, $P = 0.44$ at visit 4, and $P = 0.82$ at visit 5, $\chi^2$ test). The QOL was inquired after at each visit. At the first visit, 5 of 130 (6%) of the participants reported that their QOL was excellent, 31 of 130 (24%) very good, 81 of 130 (62%) good, 12 of 130 (9%) fairly good, and 1 of 130 (1%) poor. There was no statistically significant difference in QOL between the treatment groups at any phase of the trial ($P = 0.31$ at visit 1, $P = 0.49$ at visit 2, $P = 0.47$ at visit 3, $P = 0.74$ at visit 4, and $P = 0.36$ at visit 5, $\chi^2$ test).

### Compliance

Four participants returned more than 500 capsules during the whole study ($n = 3$ in group 1 and $n = 1$ in group 2, $P = 0.31$, $\chi^2$ test). At each visit, the participants were asked whether they had taken capsules according to the instructions. At the second visit, 44 of 48 (92%) participants in group 1 and 46 of 48 (96%) participants in group 2 had done so ($P = 0.40$, $\chi^2$ test). At the third, fourth and fifth visits, the proportions of compliant participants in group 1 and group 2 were 34/45 versus 34/42 ($P = 0.52$), 31/44 versus 30/40 (P = 0.64), and 38/44 versus 33/39 ($P = 0.95$), respectively.

## Discussion

The effect of MCC on the concentration of plasma LDL-cholesterol did not differ from that of placebo in this crossover design. The change in the concentration of LDL-cholesterol was not a clinically significant decrease during the 3-month MCC therapy period in either of the groups studied or during the 6-month MCC therapy in group 1. Previous controlled studies have found the concentrations of total or LDL-cholesterol to be reduced with chitosan, but these studies were conducted in patients with chronic disease or obesity [2, 3]. Furthermore, most of those controlled studies were short-term trials of up to 1–2 months [3, 4, 5, 6]. This current study was the first to investigate the effect of long-term chitosan treatment on the concentration of plasma LDL-cholesterol in healthy and normal-weight subjects with moderately increased plasma total cholesterol levels.

We selected the design without any dietary restrictions because, in our pilot trial, MCC seemed to reduce LDL-cholesterol without any dietary interventions [3]. We also wanted to test MCC in the normal clinical situation, where patients have already been encouraged to follow a cholesterol-lowering diet, but they still have moderately increased plasma total cholesterol concentration. Furthermore, the design with strict dietary restrictions would have been vulnerable to selection bias, i.e. the participants strictly following the dietary recommendations would also consume the study product according to the instructions, while those forgetting the diet would also forget the study product. Chitosan is commonly advertised as a fat binder, which can reduce energy intake without dietary interventions. However, the present findings constitute evidence that chitosan does not cause sustained reduction in the concentration of plasma LDL-cholesterol in healthy participants with moderately increased plasma cholesterol without dietary intervention. Dietary changes, i.e. restriction of total fat, saturated fat and cholesterol intake, as well as an increase in polyunsaturated fat intake, should always be recommended in the management of dyslipidaemia [8]. If medication is required, it should be given primarily together with a lipid-lowering diet and not as a substitute for the diet.

Here, the mean weight decreased during the first treatment period more than during the second in both groups. Participants in group 2 lost weight slightly more than those in group 1. The study commenced during the winter season, in February, and the crossover period was conducted during the spring and the summer, from March to September. The observed decrease in the total and LDL-cholesterol levels seen in both groups between visits 2 and 3 and the difference developing between the

treatment groups during the spring and the summer (and vanishing in the autumn and the winter) may, thus, have resulted from changes in diet and exercise habits, as commonly found to occur in countries with marked seasonal alteration [9]. However, there were no differences in eating or exercise habits between the treatment groups when reviewed by the subjects themselves in the interview at every visit. Nor were any seasonal changes in eating or exercise habits during the study reported in either of the treatment groups. One may, thus, suggest that the change in the concentration of plasma LDL-cholesterol might have been due to factors other than seasonal dietary alteration. Indeed, the variability in the response of LDL-cholesterol to diet is substantial and partly related to apolipoprotein E (apoE) genotype [8]. In the near future, we will examine whether apoE genotype has any effect on observed changes in total and LDL-cholesterol levels.

The LDL-cholesterol concentration decreased slightly with the intermittent MCC treatment, namely in group 2 by 0.3 mmol/l (8%), during the 9-month trial, when the participants first received MCC, then placebo, and then again MCC for a 3-month period each. MCC inhibits lipid absorption in the gastrointestinal tract. Hepatocytes, whose cholesterol uptake is reduced, might compensate this favourable effect of continuous MCC treatment by upregulation of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG CoA)-reductase enzyme activity, which leads to increased cholesterol synthesis in the liver [10]. Intermittent treatment with MCC, which would not be long enough to make possible long-term upregulation of cholesterol synthesis, might thus be more effective in reducing plasma LDL-cholesterol levels than the continuous MCC treatment.

Theoretically, MCC might provide a cholesterol-lowering effect additive to that achieved with statin treatment only. Statins inhibit the HMG CoA-reductase and the cholesterol synthesis in the liver, which leads to upregulation of LDL-receptors on the hepatocytes and to increased removal of LDL from plasma into the liver [10]. MCC might interfere with the enterohepatic circulation of the bile acids, leading, thereby, to stimulation of bile acid formation in the liver. In consequence of the increased requirement for cholesterol in the liver, the LDL-receptors on the hepatocytes would be upregulated, and the beneficial effect of statins might be potentiated by MCC.

In conclusion, treatment with MCC had no effect on the concentrations of plasma lipids or glucose in healthy middle-aged men and women with moderately increased plasma cholesterol concentrations.

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