Simultaneous Intake of β-Glucan and Plant Stanol Esters Affects Lipid Metabolism in Slightly Hypercholesterolemic Subjects[1]

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Abstract

Intake of food products rich in water-soluble fiber β-glucan and products enriched with plant stanol esters lower serum cholesterol. Combining 2 functional food ingredients into one food product may achieve additional reductions of serum cholesterol. Our objective was to investigate the effects of a simultaneous intake of β-glucan plus plant stanol esters on lipid metabolism in mildly hypercholesterolemic volunteers. In a randomized, controlled, 3-period crossover study, 40 mildly hypercholesterolemic men and women received muesli in random order twice a day for 4 wk, which provided, in total, 5 g control fiber from wheat (control muesli), 5 g oat β-glucan (β-glucan muesli), or 5 g oat β-glucan plus 1.5 g plant stanols (combination muesli). β-Glucan muesli decreased serum LDL cholesterol by 5.0% compared with control muesli ($P = 0.013$). Combination muesli reduced LDL cholesterol by 9.6% compared with control muesli ($P < 0.001$), and by 4.4% compared with β-glucan muesli ($P = 0.036$). Serum HDL cholesterol and triacylglycerol concentrations did not differ after the 3 treatments. Compared with control muesli, β-glucan muesli increased bile acid synthesis ($P = 0.043$) and decreased cholesterol absorption ($P = 0.011$). Addition of plant stanols did not influence bile acid synthesis but decreased cholesterol absorption ($P < 0.001$) and raised cholesterol synthesis ($P = 0.016$) compared with control muesli, and the plant stanols decreased cholesterol absorption compared with β-glucan muesli ($P = 0.004$). The combination muesli decreased serum concentrations of sitostanol compared with control muesli ($P = 0.010$). Plasma concentrations of lipid-soluble antioxidants did not differ after the 3 treatments. β-Glucan muesli effectively lowered serum LDL cholesterol concentrations. The addition of plant stanol esters to β-glucan-enriched muesli further lowered serum LDL cholesterol, although effects were slightly less than predicted. J. Nutr. 137: 583–588, 2007.

Introduction

LDL cholesterol-lowering functional foods are gaining a prominent position in dietary guidelines. Such foods contain one or more components and therefore provide positive health effects beyond their traditional nutritional value (1). Examples of such food components with approved FDA health claims are the water-soluble fiber β-glucan from oats and plant sterol/stanol esters (2,3).

β-Glucans are water-soluble fibers, which are found in a wide variety of products such as oats, barley, and yeast (4). Regardless of their source, all β-glucans are polysaccharides composed of glucose molecules. Although the exact mechanism explaining the cholesterol-lowering effect of β-glucan is not known, the most likely explanation is that water-soluble fibers lower the (re)absorption of bile acids (5–8). As a result, hepatic conversion of cholesterol into bile acids increases, hepatic pools of free cholesterol decrease, and, to reach a new steady-state, endogenous cholesterol synthesis increases (7). Furthermore, hepatic LDL

cholesterol receptors become upregulated to re-establish hepatic cholesterol stores, which will lead to decreased serum LDL cholesterol concentrations (9). The FDA has approved the claim that consumption of soluble fiber (≥3 g/d) from whole oats, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease (2). In the most recent meta-analysis, it was estimated that 1 g of soluble fiber from oats lowered LDL cholesterol concentrations by 0.037 mmol/L (10).

Plant sterols are found in a wide variety of plant sources, such as vegetable oils, nuts, grains, seeds, wood pulp, and leaves. Typical diets commonly provide sitosterol, campesterol, and stigmasterol, along with smaller amounts of plant stanols (which are saturated plant sterols) as sitostanol. Plant sterols and stanols are comparable in chemical structure to cholesterol and decrease the absorption of dietary and endogenously derived cholesterol in the intestine (11). Like for β-glucan, endogenous cholesterol synthesis will increase (12). Additionally, receptor-mediated cholesterol uptake becomes upregulated, and this will lead to decreased serum LDL cholesterol concentrations (13). The FDA has therefore authorized the claim that foods containing at least 0.65 g per serving of plant sterol/stanol esters (daily total intake ≥1.3 g), as part of a diet low in saturated fat and cholesterol,

may reduce the risk of heart disease (3). As of January 2003, the FDA recognized that the scientific literature supports expanding the health claim to include free forms of plant sterols and stanols and to include a wider range of products, including low-fat products. The FDA further stated that the lowest effective daily intake of free sterols is 0.8 g/d (14). A recent meta-analysis concluded that daily consumption of 2.0–2.4 g plant sterols/stanols lowers LDL cholesterol concentrations by an average of 8.9% (15).

The combination of β-glucan and plant stanol esters in a food product may result in even larger reductions in LDL cholesterol concentrations also because the underlying mechanisms of these 2 ingredients may be different. In addition, a combined intake may lead to a larger product variety or food product formulations that are more easily accepted by consumers. However, a concurrent intake of these 2 food components has not been examined before. We therefore decided to investigate the effects of simultaneous intake of β-glucan and plant stanol esters on lipid metabolism in mildly hypercholesterolemic subjects.

Subjects and Methods

Subjects. Healthy men and women, aged 18–65 y, with slightly elevated serum total cholesterol concentrations, were recruited from Maastricht and the surrounding area by advertisements in local newspapers. In addition, subjects who participated in earlier studies at our department were approached. People willing to participate were given a detailed description of the study and were required to give written informed consent before they were invited for two screening visits with an interval of ≥2 d. A screening visit consisted of measurements of body weight, height, blood pressure, serum total and HDL cholesterol concentrations, serum triacylglycerol concentrations, presence of glucosuria, and hematological parameters. In addition, all subjects completed a general and a medical questionnaire. After screening, 43 men and women were selected for the study according to the following inclusion criteria: stable body weight (weight gain or loss <3 kg in the past 3 mo); Quetelet index <32 kg/m^2; systolic blood pressure <160 mm Hg, and diastolic blood pressure <95 mm Hg; mean serum total cholesterol concentrations between 5.0 mmol/L and 8.0 mmol/L; mean serum triacylglycerol concentrations <4.0 mmol/L; no presence of glucosuria, proteinuria or anemia; no use of medication or a prescribed diet known to affect lipid or glucose metabolism; no history of coronary heart disease, cancer, diabetes, kidney, liver, pancreatic disease, or malignancies <5 y ago; no abuse of drugs and/or alcohol; no pregnant or breast-feeding women; willingness to stop the consumption of vitamin supplements, fish oil capsules, or products rich in plant sterol/stanol esters 3 wk before the start of the study. Blood donation or participation in another biomedical trial was not allowed 30 d before and during the study. The Medical Ethical Committee of the University of Maastricht approved the study and all subjects gave written informed consent.

One subject withdrew in the 2nd wk of the 1st period of the study because he started blood pressure medication. All other 42 volunteers, 20 men and 22 women, completed the study. The mean age of the men (mean ± SD) was 54 ± 10 y, and the Quetelet index, 26 ± 2 kg/m^2. For the women, these values were 51 ± 12 y and 24 ± 3 kg/m^2, respectively. Fasting concentrations of serum total, LDL, HDL cholesterol, and triacylglycerols in men were 6.66 ± 0.82 mmol/L, 4.48 ± 0.77 mmol/L, 1.41 ± 0.35 mmol/L, and 1.69 ± 0.81 mmol/L, respectively. In women these values were 6.59 ± 0.89 mmol/L, 4.10 ± 0.84 mmol/L, 1.83 ± 0.42 mmol/L, and 1.45 ± 0.69 mmol/L, respectively.

Experimental design. The study had a randomized, double-blinded, controlled, multiple crossover design. Before the study started, the subjects were randomly divided into 6 groups. During the first 4 wk, one group consumed muesli with wheat fiber (control muesli), a second group consumed a muesli with β-glucan from oats (β-glucan muesli), and a third group a muesli enriched with both β-glucan and plant stanol esters (combination muesli). After a washout period of 2 wk, during which time the participants consumed their habitual diets, subjects crossed over to another type of muesli for another period of 4 wk. After this period, a second washout period of 2 wk was introduced. For the last 4 wk of the study, subjects received the type of muesli that they had not consumed during the first 2 periods. In this way, 6 treatment orders were possible and each type of muesli followed another type of muesli. Subjects consumed the products twice daily (2 sachets of 50 g), with an interval of at least 5 h.

To improve swelling of the fibers, the muesli had to be consumed with 200 mL liquid (e.g., milk, orange juice) or with yogurt. To avoid weight gain, subjects were urged to consume the muesli instead of another food item (e.g., bread or cereals). The energy content (1700 kJ/ 100 g), total fiber content, carbohydrate and fat content, and fatty acid composition of the 3 experimental products were the same. Each sachet of the β-glucan muesli provided 2.5 g β-glucan. A sachet of the combination muesli also included 2.5 g β-glucan and 0.75 g plant stanols provided as fatty acid esters. Extra daily consumption of β-glucan was therefore 0 or 5 g, and daily consumption of plant stanols was 1.5 g (**Table 1**). Body weight without shoes or heavy clothes was recorded on d 0 and at the end of wk 3 and 4. At the end of the treatment period, subjects recorded their food intake for the previous 3 wk by completing FFQ to estimate their energy and nutrient intakes. FFQ were checked by a registered dietician in the presence of the subjects. The subjects recorded illness, medication used, menstrual phase, alcohol consumption, any deviations to the study protocol, and any side effects experienced (headache, stomach complaints, nausea, bloated feeling, flatulence, diarrhea, constipation, itching, eruptions or rashes, fatigue, and dizziness).

Other possible side effects were monitored on d 0 and at the end of wk 4 by assessing hematological variables and indices to measure kidney and liver function.

Blood sampling. The subjects fasted overnight, did not use alcohol the previous day, and did not smoke on the morning of blood sampling. Venous blood samples were taken between 0745 and 1100 on d 0 and on wk 3 and 4 of each study period. All venipunctures were generally done by the same person, in the same room, and at the same time of the day. Blood was sampled from a forearm vein using vacutainers under minimal stasis with the subject in supine position.

Blood (10 mL) was collected in a serum tube (Becton Dickinson Vacutainer Systems) for analysis of serum lipids (total serum cholesterol, HDL cholesterol, total triacylglycerols), lipoproteins (apo B and apo A-I), plant sterols and stanols (sitosterol, sitostanol, campesterol, and campestanol), a cholesterol precursor (lathosterol), and indices of kidney and liver function. Serum tubes were kept at room temperature after blood sampling. At least 1 h after venipuncture, serum was obtained by centrifuging at 2000 × g for 30 min at 4°C. Aliquots of serum were stored at –80°C. Furthermore, 10 mL of blood was collected in an EDTA tube (Becton Dickinson Vacutainer Systems) for analysis of hematological variables, lipid-soluble antioxidants, and 7α-hydroxy-4-cholesten-3-one (7α-OHC), a marker of bile acid synthesis. Hematological parameters were determined directly. Plasma was obtained immediately by centrifugation at 2000 × g for 30 min at 4°C. Plasma was then divided into aliquots, snap-frozen, and stored at –80°C.

TABLE 1 Composition of the experimental products[1]

	Control muesli	β-Glucan muesli	Combination muesli
	unit/100 g		
Energy, *kJ*	1683	1671	1672
Protein, *g*	9.60	13.40	12.40
Carbohydrates, *g*	13.90	14.20	15.10
Lipids, *g*	15.20	15.30	14.60
Total fiber, *g*	11.40	11.70	11.50
β-Glucan, *g*	0.00	5.09	5.03
Stanols, *g*	0.00	0.00	1.48[1]

[1] Plus 0.10 g sterols

Analyses. Concentrations of total cholesterol (ABX Diagnostics), HDL cholesterol (precipitation method; Roche Diagnostics), and triacylglycerol corrected for free glycerol (Sigma-Aldrich Chemie) were analyzed enzymatically in serum. Apo A-I and apo B concentrations were analyzed in serum using an immunoturbidimetric method (ABX Diagnostics). Serum LDL cholesterol concentrations were calculated using the formula of Friedewald et al. (16). All samples from one subject were analyzed in the same analytical run.

Serum plant sterols and stanols (sitosterol, sitostanol, campesterol, and campestanol) and a cholesterol precursor (lathosterol) were determined as described (12). Before analysis, serum samples of wk 3 and 4 of each period were pooled.

Serum samples were also analyzed to determine parameters of liver function (alanine transaminase, aspartate aminotransferase, γ-glutamyl transpeptidase (GGT), alkaline phosphatase, and total bilirubin), kidney function (creatinine), and inflammation (C-reactive protein). These measurements were carried out on a Beckman Coulter Synchron LX20 PRO Clinical System (Beckman Coulter).

Plasma samples were analyzed on a Coulter Ac·T diff for following hematological variables: number of white blood cells, percentage of lymphocytes, monocytes, and granulocytes, number of red blood cells, hemoglobin, hematocrit, mean corpuscular volume, and number of platelets.

Before analyzing lipid-soluble antioxidants, plasma samples taken at wk 3 and 4 of each period were pooled. Concentrations of tocopherols (α-tocopherol, δ-tocopherol, and $\beta + \gamma$-tocopherol), carotenoids (α-carotene, β-carotene, lycopene, lutein/zeaxanthin, β-cryptoxanthin, and phytofluene), and retinol were determined simultaneously according to a modification of the assay of Hess et al. (17), as described by Oostenbrug et al. (18). All samples from one subject were analyzed in the same analytical run.

Before analyzing 7α-OHC, plasma samples of wk 3 and 4 of each period were pooled. 7α-OHC was determined by HPLC after modification of the method described by Gälman et al. (19). All analyses were done in duplicate. Plasma (0.5 mL) was diluted with 2.5 mL of saline and 25 μL of the internal standard 7β-hydroxy-4-cholesten-3-one (5 ng/μL) dissolved in H_2O was added. EC-C8 Isolute SPE columns (500 mg, 3 mL, Sopachem NV) were prewashed with 2 × 2 mL pure methanol and with 2 × 2 mL of water. Columns were then mounted on a Vac Elut SPS 24 (Analytichem International), to which a removable custom-made stainless steel water-jacketed block heater with 12 positions was added. The column heater was connected to a water bath (model K6, MGW Lauda) set on 65.3°C to obtain a column temperature of 64°C. To facilitate passage through the columns, the samples were first heated to 64°C for 20 min. Subsequently, the supernatant, obtained after centrifugation at 2000 × *g* for 5 min at 21°C, was sonicated in water for 15 min and incubated at 37°C for 15 min prior to loading onto the preheated columns. A stream of nitrogen was used occasionally to facilitate passage through columns. The eluted product was completely evaporated at 60°C under a stream of nitrogen and dissolved in 110 μL acetonitrile; 75 μL of this was injected into the HPLC system. All samples from one subject

were analyzed within one run. 7α-OHC was quantified from the heights of the respective peaks using the known amount of internal standard and the response factor.

Statistical analyses. The statistical power needed to detect a true difference of 5% in serum LDL concentrations between the treatments, with $\alpha = 0.05$, was 80%. Before the statistical analyses were carried out, serum lipid and lipoprotein concentrations from wk 3 and 4 of each period were averaged for each subject. The data were analyzed by a 3-way (subject number, period, and diet) ANOVA using the general linear model procedure. Differences in effects on the variables of interest were examined with diet and period as fixed factors and subject number as a random factor. When a significant diet effect was found, the 3 treatments were compared pairwise using a Tukey post hoc test for multiple comparisons. Values are presented as means ± SD. Differences were considered significant at $P \leq 0.05$. Statistical analyses were performed using SPSS 11.0 (version 11.0.3) for Macintosh OS X (version 10.3.9).

Results

Side effects. Side effects were monitored by assessing hematological variables and indices of kidney and liver function. Throughout the study, all variables remained within the normal range for all subjects and no treatment effects were present. Compared with other subjects, however, GGT values were consistently increased for 2 subjects during the study (values during the control period were 100 and 180 U/L; 90 and 132 U/L during the β-glucan period; and 93 and 105 U/L during the combination period). Results of these 2 subjects were therefore excluded from further statistical analyses. Except for the effects of the treatments on the bile acid synthesis marker, this did not change the conclusions.

Dietary intake and body weight. As calculated from the returned sachets, the daily consumption of β-glucan during the β-glucan period was 4.80 ± 0.30 g. During the combination period, the daily intake of β-glucan and plant stanols was 4.80 ± 0.26 g and 1.44 ± 0.08 g, respectively.

The daily energy intake and the nutrient composition of the diets did not differ among the 3 treatments, except for polyunsaturated fatty acids ($P = 0.045$ for diet effect). However, between-diet comparisons were not significant (**Table 2**).

Body weights did not differ after the control (73.8 ± 11.1 kg), β-glucan (73.9 ± 11.0 kg), and combination (73.8 ± 11.1 kg) diet periods. Weight changes during each diet period were significant but consistent and were 0.42 ± 0.80 kg during the control period ($P = 0.001$), 0.37 ± 0.93 kg during the β-glucan

TABLE 2 Nutrient intakes during the 3 diet periods according to the food-frequency questionnaires[1]

	Control muesli	β-Glucan muesli	Combination muesli	*P* for diet effect[2]
Energy, *MJ/d*	8.1 ± 2.4	8.3 ± 2.5	8.0 ± 2.4	0.256
Energy, *kcal/d*	1929 ± 5820	1980 ± 6030	1919 ± 5810	0.255
Protein, *% of energy*	17.8 ± 3.00	18.0 ± 3.20	18.0 ± 3.40	0.815
Fat, *% of energy*	34.0 ± 5.70	34.0 ± 5.50	33.7 ± 5.70	0.737
Saturated fatty acids	12.5 ± 2.80	12.2 ± 2.40	12.4 ± 2.50	0.450
Monounsaturated fatty acids	11.1 ± 2.60	11.1 ± 2.70	11.0 ± 2.60	0.990
Polyunsaturated fatty acids	6.5 ± 6.6	6.8 ± 1.7	6.4 ± 1.6	0.045
Carbohydrates, *% of energy*	45.7 ± 5.80	45.1 ± 5.30	45.8 ± 6.10	0.425
Alcohol, *% of energy*	2.7 ± 2.6	3.0 ± 2.8	2.8 ± 2.9	0.506
Cholesterol, *mg/MJ*	23.3 ± 6.10	22.2 ± 5.30	22.1 ± 6.50	0.139
Dietary fiber, *g/MJ*	2.8 ± 0.8	2.7 ± 0.7	2.7 ± 0.8	0.457

[1] Values are means ± SD; *n* = 40 (19 men and 21 women). Paired comparisons did not reveal significant differences between any 2 diets.
[2] Calculated using a general linear model with subject number as a random factor and diet and period as fixed factors.

period ($P = 0.013$), and 0.25 ± 0.68 kg during the combination period ($P = 0.027$).

Serum lipids and lipoproteins. The β-glucan muesli decreased serum total cholesterol concentrations by 0.23 mmol/L (3.4%) compared with control muesli ($P = 0.015$; 95% CI for the difference between the dietary periods: −0.36, −0.10 mmol/L) (**Table 3**). The combination muesli reduced total cholesterol concentrations by 0.48 mmol/L (7.1%) compared with control muesli ($P < 0.001$; 95% CI: −0.68, −0.30 mmol/L), and by 0.25 mmol/L (3.6%) compared with β-glucan muesli ($P = 0.008$; 95% CI: −0.43, −0.07 mmol/L).

After β-glucan consumption, serum LDL cholesterol concentrations were 0.21 mmol/L (5.0%) lower than after the control period ($P = 0.013$; 95% CI: −0.31, −0.11 mmol/L). The combination muesli effectively lowered LDL cholesterol concentrations by 0.39 mmol/L (9.6%) compared with control diet ($P < 0.001$; 95% CI: −0.56, −0.23 mmol/L), and by 0.18 mmol/L (4.4%) compared with β-glucan intake only ($P = 0.036$; 95% CI: −0.35, −0.01 mmol/L).

Serum HDL cholesterol and triacylglycerol concentrations did not differ after the 3 diet treatments. Consumption of β-glucan significantly decreased the ratio of total to HDL cholesterol by 0.09 (1.7%) compared with control muesli ($P = 0.016$; 95% CI: −0.25, 0.07). Consumption of β-glucan plus plant stanol esters reduced the ratio of total to HDL cholesterol by 0.27 (5.5%) compared with control muesli ($P < 0.001$; 95% CI: −0.41, −0.13), and by 0.18 (3.4%) compared with consumption of β-glucan only ($P = 0.004$; 95% CI: −0.32, −0.04).

The combination muesli lowered serum apo B concentrations by 0.09 g/L (6.7%) compared with the control muesli ($P < 0.001$; 95% CI: −0.12, −0.05 g/L), and by 0.06 g/L (4.1%) compared with the β-glucan muesli ($P = 0.003$; 95% CI: −0.10, −0.02 g/L). Apo B concentrations did not differ after the control and β-glucan periods and apo A-I concentrations did not differ after any of the 3 treatments.

Markers of cholesterol absorption, cholesterol synthesis, and bile acid synthesis. β-Glucan consumption increased cholesterol-standardized serum lathosterol concentrations by 0.20 μmol/mmol (17.6%) compared with control muesli ($P = 0.050$; 95% CI: 0.01, 0.40 μmol/mmol), indicating an elevated cholesterol synthesis (**Table 4**). The combination muesli increased cholesterol-standardized serum lathosterol concentrations by 0.26 μmol/mmol (19.1%) compared with control muesli ($P = 0.016$; 95% CI: 0.09, 0.42 μmol/mmol). Relative to β-glucan muesli, the combination muesli did not alter cholesterol-standardized serum lathosterol concentrations.

Compared with the control period, cholesterol-standardized serum campesterol concentrations decreased by 0.36 μmol/mmol (7.1%) after β-glucan consumption ($P = 0.011$; 95% CI: −0.61, −0.12 μmol/mmol), indicating decreased intestinal cholesterol absorption. The combination muesli decreased cholesterol-standardized campesterol concentrations by 0.77 μmol/mmol (20.7%) compared with control muesli ($P < 0.001$; 95% CI: −1.05, −0.47 μmol/mmol), and by 0.41 μmol/mmol (11.1%) compared with β-glucan muesli ($P = 0.004$; 95% CI: −0.64, −0.17 μmol/mmol). Changes in sitosterol concentrations paralleled those of campesterol, except that the concentration after subjects consumed β-glucan muesli did not differ from that after consuming control muesli.

The combination muesli decreased cholesterol-standardized serum sitostanol concentrations by 0.7 μmol/mmol $\times$ 100 (21.3%) compared with control muesli ($P = 0.010$; 95% CI: −1.1, −0.3 μmol/mmol $\times$ 100). Compared with β-glucan muesli, the combination muesli tended to decrease the cholesterol-standardized serum sitostanol concentrations ($P = 0.066$). The concentration did not differ after the control and β-glucan periods. Campestanol concentrations did not differ after the 3 treatments.

Compared with control muesli, muesli enriched with β-glucan effectively increased cholesterol-standardized plasma 7α-OHC concentrations by 0.70 μg/mmol (24.4%) ($P = 0.043$; 95% CI: 0.16, 1.24 μg/mmol). The cholesterol-standardized plasma 7α-OHC concentration did not differ after the control and combination muesli periods or after the β-glucan and combination muesli periods.

Lipid-soluble antioxidants. After correction for LDL cholesterol concentrations, plasma total tocopherol, oxygenated carotenoid, and hydrocarbon carotenoid concentrations did not differ after the 3 treatment periods. Plasma retinol concentrations also were not affected (data not shown).

Discussion

In this study, consumption of muesli enriched with oat β-glucan significantly decreased serum LDL cholesterol concentrations. Subjects consumed twice daily 2.4 g of β-glucan, whereas the observed decrease in LDL cholesterol was 0.21 mmol/L. This is in agreement with the predicted reduction in LDL cholesterol of 0.18 mmol/L based on the meta-analysis by Brown et al. (10). Results between studies on the effects of oat β-glucan on LDL cholesterol concentrations are, however, variable. This variability may be due to several factors, such as fiber intake, baseline serum cholesterol concentrations, mode of administration, food matrix, and solubility or molecular weight of the fiber.

TABLE 3 Serum lipid and lipoprotein concentrations in healthy men and women who consumed each of the 3 muesli diets daily for 4 wk[1]

	Control muesli	β-Glucan muesli	Combination muesli	P for diet effects[2]
Total cholesterol, *mmol/L*	6.49 ± 1.03	6.26 ± 0.96^a	$6.01 \pm 0.92^{a,b}$	<0.001
LDL cholesterol, *mmol/L*	4.31 ± 0.96	4.09 ± 0.91^a	$3.89 \pm 0.90^{a,b}$	<0.001
HDL cholesterol, *mmol/L*	1.46 ± 0.46	1.45 ± 0.49	1.45 ± 0.52	0.768
Triacylglycerols, *mmol/L*	1.52 ± 0.84	1.66 ± 1.36	1.56 ± 0.94	0.277
Total:HDL cholesterol	4.83 ± 1.59	4.74 ± 1.55^a	$4.57 \pm 1.51^{a,b}$	<0.001
Apolipoprotein B, *g/L*	1.24 ± 0.27	1.21 ± 0.27	$1.15 \pm 0.25^{a,b}$	<0.001
Apolipoprotein A-I, *g/L*	1.62 ± 0.31	1.60 ± 0.34	1.57 ± 0.32	0.065

[1] Values are means $\pm$ SD; $n = 40$ (19 men and 21 women). Means in a row without a common letter differ, $P \le 0.05$; [a] vs. control; [b] vs. β-glucan.

[2] Calculated using a general linear model with subject number as a random factor and diet and period as fixed factors.

TABLE 4 Serum markers of cholesterol synthesis, cholesterol absorption, and bile acid synthesis in healthy men and women[1]

	Control muesli	β-Glucan muesli	Combination muesli	P for diet effects[2]
	μmol/mmol total cholesterol × 100			
Lathosterol	195 ± 60	215 ± 60[a]	221 ± 57[a]	0.010
Campesterol	318 ± 145	282 ± 129[a]	241 ± 114[a,b]	<0.001
Sitosterol	123 ± 59.8	113 ± 54.8	90.7 ± 43.9[a,b]	<0.001
Campestanol	8.26 ± 7.19	6.72 ± 4.77	6.69 ± 6.36	0.164
Sitostanol	2.26 ± 1.19	2.10 ± 1.21	1.62 ± 0.94[a]	0.008
	μg/mmol total cholesterol			
7α-OHC[3]	3.57 ± 2.22	4.28 ± 3.00[a]	4.18 ± 3.03	0.031

[1] Values are means ± SD; n = 40 (19 men and 21 women), except for analyses of sterols and stanols (n = 39; 19 men and 20 women; 1 missing value). Means in a row without a common letter differ, P ≤ 0.05; [a] vs. control; [b] vs. β-glucan.

[2] Calculated using a general linear model with subject number as a random factor and diet and period as fixed factors.

[3] To convert μg to mmol, divide by 400.64.

The combined effects of β-glucan and plant stanol esters on LDL cholesterol concentrations have not been systematically examined. We found that addition of plant stanol esters to the β-glucan-enriched muesli significantly lowered LDL cholesterol concentrations by 4.4%. This effect was smaller than the estimated mean change of −8.5% for daily intakes of 1.5–1.9 g of sterols or stanols (15). Thus, our findings suggest that the water-soluble fiber β-glucan reduces the efficacy of plant stanols. Several studies have, however, concluded that effects of plant sterols and stanols do not change when subjects consumed a recommended diet (15,20), which is usually accompanied by a decrease in fat and cholesterol intake. When fats are replaced by complex carbohydrates, an increase in fiber intake is expected as well. The majority of these studies (21–26) did not document changes in the intake of water-soluble dietary fibers. Yoshida et al. (27), however, recently examined the effects of 1.8 g/d free plant sterols with or without 10 g/d glucomannan, a water-soluble fiber derived from konjac root, in individuals with and without type II diabetes. In the nondiabetic group, LDL cholesterol concentrations decreased after plant sterol, glucomannan, and the combined intakes by 5.4, 13.8, and 21.7%, respectively. Results were less pronounced in the diabetic patients. In agreement with our results, the effect of the plant sterols was less than the estimated mean change of −8.5%. These findings did not suggest that glucomannan attenuated the LDL-cholesterol lowering effects of the free plant sterols, although the effects of plant sterols were less than predicted. Nevertheless, a combination therapy including 2.6 g/d plant sterols and 10 g/d psyllium also decreased LDL cholesterol beyond expectations (10,15). According to the literature, daily consumption of >2.5 g sterols or stanols lowers LDL cholesterol concentrations by a mean of 11.3%, which is already higher than the 10% decrease in LDL cholesterol found after the combination therapy. Separate contributions of individual components were, however, not evaluated (28). Also, other recent studies with similar intakes of plant sterols and stanols reported effects less than the estimated mean change of −8.5% (29–32). Moreover, Clifton et al. (33) reported that plant sterols (1.6 g/d) incorporated in milk, yogurt, bread, and cereals decreased LDL cholesterol concentrations by 15.9, 8.6, 6.5, and 5.4%, respectively. Their results suggest that the food matrix influences the cholesterol-lowering effectiveness of plant sterols. Altogether, the estimated mean percentage reduction of 8.5% in LDL cholesterol at daily intakes of 1.5–2.1 g of plant sterols or stanols may be overrated (15). Nevertheless, the 4.4% change in LDL cholesterol is somewhat less than that found in other studies at comparable intakes, and an effect of β-glucan or the food matrix on the efficacy of plant stanols can therefore not be excluded. In support, β-glucan consumption decreased serum cholesterol-standardized stanol concentrations, although the effect on campestanol was not significant (P = 0.164). Adding plant stanol esters to the β-glucan-enriched muesli did not change serum cholesterol-standardized stanol concentrations. Consumption of plant stanols normally leads to an increase in their serum concentrations (34). Thus, these findings suggest that β-glucan interferes with the absorption of plant stanols. Plant stanols reduce intestinal cholesterol absorption through competition for micellar incorporation (11). However, recent findings indicate that stanols also enter the intestinal cells and promote cholesterol efflux back into the intestinal lumen (11,35). We speculate that a higher intestinal viscosity lowered the transport of plant stanols into the enterocytes. As a result, not only serum concentrations, but also the cholesterol-lowering efficacy of plant stanols, may have been decreased. Alternatively, an increase in intestinal viscosity may affect lipid emulsification by increasing the droplet size of emulsions thereby decreasing the emulsion interface area. This may reduce the rate of lipolysis (36) and possibly also the hydrolysis of plant stanol esters. The active free plant stanols may then be released later in the intestinal tract, affecting their efficacy and absorption.

Relative to the control muesli, the β-glucan muesli significantly increased total cholesterol-standardized 7α-OHC concentration, indicating increased bile acid synthesis. Additionally, cholesterol-standardized campesterol decreased, suggesting reduced cholesterol absorption. Cholesterol-standardized lathosterol concentrations, which are positively associated with endogenous cholesterol synthesis, increased. Overall, these findings are in line with the proposed mechanism by which water-soluble fibers decrease serum cholesterol concentrations (5–7,34). Compared with β-glucan, the combination muesli did not further affect bile acid synthesis. As expected, plant stanol esters significantly lowered cholesterol absorption. These results also correspond to the suggested working mechanism of plant stanols.

Because water-soluble fibers can change the physical characteristics of the contents of the small intestine, they may disturb the absorption of lipid-soluble antioxidants. β-Glucan consumption did, however, not change LDL cholesterol-standardized lipid-soluble antioxidant concentrations, which agrees with the results of previous studies (34,37). In addition, we found no effects of a daily intake of 1.5 g plant stanols on LDL cholesterol-standardized lipid-soluble antioxidant concentrations. Indeed, a meta-analysis showed that lipid-soluble antioxidants are not affected by stanols after correction for the decrease in cholesterol, with the exception of β-carotene (15). However, effects on

β-carotene concentrations are mainly seen at daily intakes of >2 g of plant stanols (15,35).

In summary, our results indicate that muesli rich in β-glucan effectively lowers serum LDL cholesterol concentrations. The addition of plant stanol esters to the β-glucan-enriched muesli further lowered serum LDL cholesterol, although less than predicted (15). Whether this is indeed true and due to the effect of β-glucan or the food matrix needs further investigation.

Acknowledgments

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