β-Glucan incorporated into a fruit drink effectively lowers serum LDL-cholesterol concentrations\textsuperscript{1–4}

Elke Naumann, Angelina B van Rees, Gunilla Önning, Rickard Öste, Markus Wydra, and Ronald P Mensink

ABSTRACT

Background: β-Glucan can reduce serum concentrations of total and LDL cholesterol. The mechanism of this action is not clear, however, and it is difficult to predict the cholesterol-lowering effect of a food product enriched with β-glucan.

Objectives: We examined the effects of a β-glucan–enriched fruit juice on serum lipids and lipoproteins and on markers of cholesterol absorption (serum concentrations of plant sterols) and synthesis (serum concentrations of lathosterol). In addition, we measured effects on lipid-soluble antioxidants.

Design: After a 3-wk run-in period, healthy subjects consumed daily a fruit drink providing 5 g rice starch [placebo (control) group; \( n = 22 \)] or β-glucan from oats (\( n = 25 \)) for 5 wk (parallel design). At the end of the run-in period and at the end of the intervention, blood samples were taken for analysis of lipids and lipoproteins, noncholesterol sterols, and fat-soluble antioxidants. Changes between the end of the run-in period and the end of the intervention were calculated for each subject. Differences in changes between the groups were analyzed statistically.

Results: The differences between the control and β-glucan groups in the change in serum concentrations of total and LDL cholesterol, respectively, were −4.8\% (\( P = 0.012 \)) and −7.7\% (\( P = 0.005 \)). The differences between the groups in the change in serum concentrations of lathosterol and sitosterol were −13\% (\( P = 0.023 \)) and −11\% (\( P = 0.030 \)), respectively. No significant effects were found on fat-soluble antioxidants.


KEY WORDS β-Glucan, cholesterol, lathosterol, sitosterol, campesterol, antioxidants, healthy subjects

INTRODUCTION

β-Glucan is a nonstarch polysaccharide composed of β-(1→3)–linked glucose units separated every 2–3 units by β-(1→4)–linked glucose. This soluble fiber, which is present in oats, has received much attention, because of its potential to reduce LDL cholesterol. In 1997, therefore, the Food and Drug Administration allowed the health claim that a diet high in soluble fiber from whole oats (oat bran, oatmeal, and oat flour) and low in saturated fat and cholesterol may reduce the risk of cardiovascular disease (1). Indeed, many studies showed the LDL cholesterol–lowering effects of products enriched with β-glucan (2), although some studies did not (3, 4). Differences in solubility and molecular weights of the β-glucans used may explain the contrasting findings, because those characteristics affect intestinal viscosity (5–7). A greater intestinal viscosity and the subsequently lower bile acid absorption constitute one of the proposed mechanisms by which β-glucan reduces serum cholesterol concentrations (8–10). In addition, the increased intestinal viscosity may lower cholesterol absorption (9, 10), although reduced cholesterol absorption was not found in other studies (8, 11). It has also been suggested that intestinal viscosity depends on the method of food processing or on the food matrix of the β-glucan–rich product (12). If this is true, then it is difficult to predict the cholesterol-lowering effect of a food product enriched with β-glucan. In the current study, we therefore examined the effects of a fruit drink enriched with β-glucan on the serum lipoprotein profile. To further elucidate the mechanism of action, we examined effects of the fruit drink on markers of cholesterol absorption (serum concentrations of plant sterols) and synthesis (serum concentrations of lathosterol). A greater intestinal viscosity may enlarge the intestinal unstirred-water layer (13), which suggests that β-glucan may also affect the absorption of other fat-soluble components. Therefore, plasma concentrations of lipid-soluble antioxidants were also analyzed.

SUBJECTS AND METHODS

Subjects

Volunteers were recruited through announcements in local newspapers or from among subjects who had participated in

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earlier studies at our department. Ninety-six subjects were invited for 2 screening visits if they met our first inclusion criteria. These criteria were age from 18 to 70 y, body mass index (BMI; in kg/m²) of 20–32, stable body weight (weight gain or loss ≤ 5% in the previous 3 mo), no intention to change the physical activity pattern during the study, no use of medication or a prescribed diet known to affect lipid metabolism, no current pregnancy or breastfeeding, no participation in another biochemical trial at the same time, willingness to stop the consumption of vitamin supplements or products enriched with plant stanol or sterol esters 3 wk before the start of the study, and no holiday planned in the weeks of blood sampling. During the screening visits, blood pressure, body weight, and height were measured and a urine sample was analyzed for the presence of glucose. Blood samples were taken from fasting subjects for lipid and lipoprotein analysis at intervals of ≥3 d. All subjects completed a medical questionnaire. Forty-eight subjects were included in the study. They had mean serum concentrations of ≤ 8.0 mmol total cholesterol/L and ≤ 4.0 mmol triacylglycerol/L; no indications for treatment for hyperlipidemia according to the Dutch Cholesterol Consensus (14); diastolic and systolic blood pressure ≤ 95 mm Hg and ≤ 160 mm Hg, respectively; no presence of glucosuria; no clinical condition known to affect lipid metabolism; no drug or alcohol abuse; and no history of coronary artery disease, decompensatio cordis (class III or IV), cardiomyopathy or kidney, liver, or pancreatic disease or malignancy <5 y ago. At the start of the trial, one man decided for personal reasons not to participate. Thus, 47 subjects (18 men and 29 women) began the study.

The women were ± SD 49 ± 16 y old and had a BMI of 23 ± 3. Their mean serum concentrations of total, LDL, and HDL cholesterol and triacylglycerol were 6.71 ± 0.77, 4.33 ± 0.68, 1.63 ± 0.40, and 1.66 ± 0.64 mmol/L, respectively. Eight women smoked, 9 used oral contraceptives, and 12 were menopausal. The men were aged 56 ± 9 y and had a BMI of 26 ± 2. Their mean serum concentrations of total, LDL, and HDL cholesterol and triacylglycerol were 7.25 ± 0.61, 4.97 ± 0.53, 1.53 ± 0.23, and 1.62 ± 0.49 mmol/L, respectively. Two of the men were smokers.

All subjects gave written informed consent before entering the study. The study protocol was approved by the Ethics Committee of Maastricht University.

Study design

The study was a placebo-controlled, double-blind parallel design. During the first 3 wk of the study (run-in period), subjects consumed 2 packages (250 mL each) of a fruit drink, which provided 5 g rice starch (placebo) per day. Then, the volunteers were randomly allocated to 1 of 2 treatment groups. For the next 5 wk of the study, one group continued to consume daily 500 mL of the placebo fruit drink (n = 22), and the other group received daily the fruit drink enriched with 5 g β-glucan from oats (n = 25). Subjects were required to consume the beverages during 2 of the 3 main meals (breakfast, lunch, and dinner) and to record in their diaries the daily time of consumption and any signs of illness or physical discomfort.

β-Glucan preparations were manufactured by CEBAB AB (Lund, Sweden). The fruit drinks, with an apple or pear taste, were produced by Döhler GmbH (Darmstadt, Germany; Table 1). The beverages were sent to Maastricht at regular intervals during the study. Subjects visited the department at least every other week to receive a supply of experimental beverages or for blood sampling. Subjects received exactly the amount of fruit drink that they were to consume between 2 visits at the department.

Food intake was measured at the end of the run-in and intervention periods by using a food-frequency questionnaire. Energy and nutrient intakes were calculated by using the 2001 Dutch Food Composition Table (15). The subjects were asked not to change their usual diets, level of physical exercise, smoking habits, or use of alcohol during the study.

Methods

At weeks 0, 2, 3, 7, and 8, subjects were weighed after an overnight fast, while they were wearing light indoor clothing and no shoes, and fasting blood samples were taken. At each occasion, a 10-mL serum tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) was used. To obtain serum, the tube was left for 1 h after venipuncture at room temperature. Serum was prepared by centrifuging the tube at 2000 × g for 30 min at 4 °C. Serum samples were stored at −20 °C until the end of the study. In all serum samples, total cholesterol (ABX Diagnostics, Montpellier, France), HDL cholesterol (precipitation method; Roche Diagnostics Corporation, Indianapolis, IN), and triacylglycerol with correction for free glycerol (Sigma Aldrich Chemie, Steinheim, Germany) were analyzed enzymatically. Serum LDL-cholesterol concentrations were calculated by using the formula of Friedewald et al (16). Serum concentrations of plant sterols were analyzed as described elsewhere (17). At weeks 2, 3, 7, and 8, blood was also sampled in a 10-mL EDTA-coated tube (Becton Dickinson). To obtain plasma for measurements of concentrations of lipid-soluble antioxidants (ie, α-carotene, β-carotene, lycopene, lutein, canthaxanthin, β-cryptoxanthin, α-tocopherol, β+γ- tocopherol, δ-tocopherol, and phytofluene) and retinol, the EDTA-coated tube was centrifuged at 2000 × g for 30 min at 4 °C. Plasma concentrations of retinol and these lipid-soluble antioxidants were analyzed as described elsewhere (17). Before analyses, plasma samples from weeks 2 and 3 and from weeks 7 and 8 were pooled. At weeks 0, 3, and 8, hematologic variables (ie, the number of white blood cells, red blood cells, and platelets) were analyzed in blood from the EDTA-coated tubes on a Coulter Counter (MD series; Beckman Coulter, Inc, Miami, FL), and variables of liver function (eg, alanine aminotransferase, aspartate aminotransferase, bilirubin, γ-glutamyltransferase, and alkaline phosphatase), kidney function (creatinine), and inflammation (C-reactive protein) were analyzed with the use of a Beckman Synchron CX7 System (Beckman Instruments, Inc, Palo Alto, CA).
Statistical analysis

Before the start of the study, it was calculated that the power needed to detect a difference of 0.30 mmol/L between the treatment groups was 80%. For lipids and lipoproteins, results of the serum samples taken at the end of the run-in period (weeks 2 and 3) and at the end of the intervention period (weeks 7 and 8) were averaged before statistical analysis. For each subject, responses to the fruit drinks were calculated as the change between values at the end of the run-in period and those at the end of the intervention period. Differences between groups in serum concentrations of lipids and lipoproteins, plant sterols and stanols, and body weight and nutrient intake were evaluated by analysis of variance. Differences between groups in variables that were not normally distributed (eg, lipid-soluble antioxidant concentrations, hematologic variables, and variables of liver function, kidney function, and inflammation) were analyzed by using the Mann-Whitney test.

Statistical analyses were performed by using SPSS for MACINTOSH software (version 11.0; SPSS, Chicago, IL). Values are presented as mean (±SD), and nonnormally distributed values are presented as median (range). *P < 0.05 was considered significant.

RESULTS

Dietary intakes, fruit drink consumption, body weight, and safety variables

During the run-in period, the mean daily intakes for all subjects were 9.3 ± 2.4 MJ of energy; 35 ± 5% of energy as total fat, 12 ± 2% of energy as saturated fatty acids, 16 ± 2% of energy as protein, and 46 ± 6% of energy as carbohydrates; 192 ± 71 mg of cholesterol; and 25 ± 8 g of dietary fiber. There were no significant differences between the groups in either period, except in β-glucan intake (data not shown).

Inspection of the diaries showed no serious deviations from the protocol, except by one subject in the β-glucan group, who did not consume 4 drinks during the run-in period (week 3) or 8 drinks during the intervention period (weeks 4 and 5). Exclusion of this subject did not change the outcomes of the study.

Changes in body weight were 0.2 ± 1.1 kg for the control group and 0.5 ± 1.0 kg for the β-glucan group (*P = 0.363). There were no significant differences between the groups in the number of white blood cells, red blood cells, or platelets or in serum concentrations of alanine aminotransferase, aspartate aminotransferase, bilirubin, γ-glutamyltransferase, alkaline phosphatase, creatinine, and C-reactive protein (data not shown).

Serum lipids and lipoproteins

Mean changes in serum concentrations of lipids and lipoproteins are shown in Table 2. Compared with the changes in the control group, the changes in serum concentrations of total and LDL cholesterol in the β-glucan group were −0.30 mmol/L or −4.8% (95% CI for the difference: −0.07, −0.52 mmol/L) for total cholesterol and −0.31 mmol/L or −7.7% for LDL cholesterol (95% CI for the difference: −0.10, −0.52 mmol/L). No significant differences between the groups were observed in changes in serum concentrations of HDL cholesterol (95% CI for the difference: 0.02, −0.08 mmol/L) or triacylglycerol (95% CI for the difference: 0.32, −0.24 mmol/L) or in the ratio of total to HDL cholesterol (95% CI for the difference: 0.03, −0.41). Except for total: HDL cholesterol (*P = 0.033), variables at the end of the run-in period did not differ significantly between the groups.

Serum lathosterol and plant sterols

The change in serum concentrations of lathosterol was −0.20 μmol/mmol cholesterol in the β-glucan group when compared with that in the control group, as shown in Table 3. The difference between the groups in changes in serum campesterol concentrations was not statistically significant (*P = 0.099). Compared with the change in the control group, the change in serum concentrations of sitosterol in the β-glucan group was −0.11 μmol/mmol cholesterol. Variables at the end of the run-in period did not differ significantly between the groups.

Lipid-soluble antioxidants

No significant differences between the groups were observed in absolute changes in lipid-soluble antioxidants, although plasma concentrations of lycopene and total hydrocarbon carotenoids (the sum of α-carotene, β-carotene, and lycopene) tended be lower in the β-glucan group than in the control group. No differences in LDL cholesterol–standardized plasma concentrations of antioxidants were observed between the groups.
al (12) reported that method of processing of the oat products. Indeed, Kerckhoffs et lowering effect of analysis (2). It has been suggested, however, that the cholesterol-intake from oats, as calculated by Brown et al in their meta-

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Mean serum concentrations of lathosterol, campesterol, and sitosterol</th>
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<tbody>
<tr>
<td></td>
<td>Control group (n = 22)</td>
</tr>
<tr>
<td>Lathosterol</td>
<td>Run-in period 1.84 ± 0.53</td>
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<tr>
<td></td>
<td>Test period 1.75 ± 0.60</td>
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<tr>
<td></td>
<td>Change −0.09 ± 0.29</td>
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<td></td>
<td>Change (%) −5 ± 7</td>
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<tr>
<td>Campesterol</td>
<td>Run-in period 3.52 ± 1.35</td>
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<tr>
<td></td>
<td>Test period 3.45 ± 1.18</td>
</tr>
<tr>
<td></td>
<td>Change −0.07 ± 0.36</td>
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<tr>
<td></td>
<td>Change (%) 1 ± 16</td>
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<tr>
<td>Sitosterol</td>
<td>Run-in period 1.29 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>Test period 1.36 ± 0.51</td>
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<tr>
<td></td>
<td>Change 0.07 ± 0.17</td>
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<td></td>
<td>Change (%) 9 ± 16</td>
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1 All values are x ± SD. All values are μmol/mmol cholesterol unless indicated otherwise. ² P values for diet effects were calculated by using ANOVA with group as the fixed factor.

DISCUSSION

In the current study, serum concentrations of total cholesterol decreased significantly by 0.060 mmol/L and those of LDL cholesterol by 0.062 mmol/L, when expressed per gram of β-glucan intake. These decreases are approximately twice those of 0.037 and 0.032 mmol/L, respectively, for each gram of soluble fiber intake from oats, as calculated by Brown et al in their meta-analysis (2). It has been suggested, however, that the cholesterol-lowering effect of β-glucan depends on the food matrix or the method of processing of the oat products. Indeed, Kerckhoffs et al (12) reported that β-glucan incorporated into bread and cookies lowered serum LDL cholesterol nonsignificantly (by 0.020 mmol/L) for each gram of β-glucan. When the same source of β-glucan was incorporated into a fruit juice, the decrease was 0.052 mmol/L. In addition, incorporation of β-glucan into oat milk lowered serum concentrations of LDL cholesterol by 0.063 mmol/L for each gram of β-glucan (18). These results are in good agreement with the results of the current study, and they do suggest that the efficacy of β-glucan preparations increases when they are incorporated into liquid products. However, Beer et al (5) did not find a cholesterol-lowering effect of a β-glucan–enriched instant whip. This lack of effect was explained by the low solubility and moderate molecular weight (1000 kDa) of the β-glucan used. A high molecular weight may be associated with an increased intestinal viscosity, which may decrease the absorption of bile acids and cholesterol (19). Recently, it was shown, however, that, in healthy volunteers, both high- and low-molecular-weight β-glucan (797 or 217 kDa, respectively) reduced serum concentrations of total and LDL cholesterol equally in relation to baseline (20). Similar findings were observed in hamsters who consumed β-glucan with a molecular weight of either 175 or 1000 kDa (21). In the current study, we used β-glucan with a mean molecular weight of only 80 kDa and still found a reduction in serum cholesterol concentrations. These results suggest that molecular weight alone cannot predict the cholesterol-lowering effects of β-glucan. Another reason that Beer et al (5) did not find cholesterol-lowering effects of β-glucan may be their subjects’ low baseline concentrations of cholesterol. In fact, Ripsin et al (22) reported a more pronounced decrease in serum concentrations of total cholesterol when baseline concentrations were increased. On the other hand, Brown et al (2) found no evidence that initial serum concentrations of total cholesterol were related to changes in total cholesterol. However, LDL-cholesterol concentrations decreased significantly more at higher baseline concentrations of LDL cholesterol.

Why β-glucan can lower serum LDL-cholesterol concentrations is not clear. One of the proposed mechanisms is that β-glucan binds bile acids or increases intestinal viscosity, which results in a decreased reabsorption of bile acids and increased fecal bile acid excretion (8–10). As a consequence, bile acid synthesis and excretion into the intestine are increased (23). At the same time, hepatic cholesterol synthesis will also increase (8) because of a higher need for cholesterol in the liver for bile acid production (8). Indeed, in a study with ileostomy subjects (8), a strong positive correlation was found between changes in lathosterol concentrations and bile acid excretion. Thus, the increased cholesterol synthesis after β-glucan consumption in the current study may be due to increased bile acid excretion. However, higher cholesterol synthesis can also be due to lower cholesterol absorption. We measured cholesterol-standardized campesterol and sitosterol concentrations as markers of cholesterol absorption. Concentrations of both markers decreased, which suggested less cholesterol absorption, although only the effect on sitosterol was statistically significant. Our findings agree with the increased excretion of plant sterols and the reduced cholesterol absorption found in ileostomy subjects after consumption of β-glucan (9). On the other hand, Lia et al (8) did not find changes in cholesterol absorption in ileostomy subjects after consumption of β-glucan, but they mentioned that this finding should be interpreted with caution because of a low statistical power. In addition, Uusitupa et al (11) did not find any significant change in serum concentrations of campesterol or sitosterol during consumption of 10 g β-glucan/d for 8 wk. However, serum concentrations of total cholesterol also did not change. On the basis of these findings, we conclude that not only increased bile acid synthesis but also decreased cholesterol absorption contributes to the cholesterol-lowering effect of β-glucan.

Because β-glucan may enlarge the intestinal unstirred-water layer (13), the absorption of lipid-soluble antioxidants may also be affected. However, we did not observe any change in absolute or LDL cholesterol–standardized concentrations of lipid-soluble antioxidants, although absolute plasma concentrations of lycopene and total hydrocarbon carotenoids (α-carotene, β-carotene, and lycopene) tended to decrease in the β-glucan group. Information from other studies of the effects of β-glucan on lipid-soluble antioxidants is limited. Kerckhoffs et al (24) reported decreases in absolute plasma concentrations of tocopherols and hydrocarbon carotenoids. However, LDL cholesterol–standardized plasma concentrations were not affected by β-glucan, which is agreement with the findings of the current study.

To summarize, results of the current study indicate that our β-glucan preparation lowers serum concentrations of total and LDL cholesterol when incorporated into a fruit drink. Not only an increase in bile acid synthesis (8, 10, 23) but also reduced cholesterol absorption contributes to the cholesterol-lowering effect of β-glucan without affecting plasma concentrations of lipid-soluble antioxidants.
We thank all the volunteers for their cooperation and interest, and FJJ Cox and H Aydeniz for their analytic assistance. RPM and GO were responsible for the study design. EN and ABR were responsible for writing the manuscript. No author had any financial or personal conflict of interest.

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