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The effect of high-fiber rye bread enriched with nonesterified plant sterols on major serum lipids and apolipoproteins in normocholesterolemic individuals☆,☆☆

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Abstract *Background and aims:* Plant sterols are naturally occurring cholesterol-lowering compounds which are industrially incorporated in various foods. A novel food carrier is rye bread, the intake of which can be monitored in trials utilizing newly defined plasma biomarkers. Our aim was to determine the effects of plant sterols incorporated into high-fiber rye bread on serum total and LDL cholesterol, apoB/apoA1 and total cholesterol/HDL cholesterol ratios and lipophilic (pro)vitamins in healthy free-living normocholesterolemic individuals.
Methods and results: In this double-blind, dietary intervention trial the subjects ($n = 68$) were randomized to receive a rye bread (9.3 g/d fiber) with added plant sterols (2 g/d) (active) or without (control). In the second phase of the study the amount of rye bread was doubled providing 18.6 g/d fiber and in the active group 4 g/d plant sterols. Compliance was monitored utilizing 3-day food diaries and a novel rye fiber-derived biomarker in plasma. Intake of rye bread enriched with 2 g/d of plant sterols during two weeks reduced significantly serum total and LDL cholesterol, apoB/apoA1 and total cholesterol/HDL cholesterol ratios by 5.1%, 8.1%, 8.3% and 7.2%, respectively, compared to controls. Correspondingly, the following two-week treatment with 4 g/d of plant sterols resulted in 6.5%, 10.4%, 5.5% and 3.7% difference compared

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to controls, being most pronounced for LDL (0.33 mmol/L). The treatments did not affect lipophilic (pro)vitamin levels.
Conclusion: Rye bread enriched with 2—4 g/d of nonesterified plant sterols beneficially modifies cardiovascular lipid risk factors in normocholesterolemic subjects compared to controls.

Introduction

Plant sterols (PS) occur naturally in vegetable oils, nuts, seeds and grains and their chemical structure resembles that of cholesterol [1]. PS possess serum cholesterol-lowering properties equal to plant stanols [2,3] and among food industry they are widely used ingredients in functional foods either in nonesterified (less lipophilic) or esterified (more lipophilic) form. The matrix of the food vehicle influences which form of PS will be more soluble. The industrial incorporation of PS into various foods easily multiplies the intake of these substances [4] but no adverse effects have been associated in humans with high doses [5—7], except in the rare cases of sitosterolemia [8]. In contrast, animal studies have indicated adverse effects on colon cancer and red blood cell abnormalities [9]. The main mechanism underlying the hypocholesterolemic effect of PS is that they compete with dietary and biliary cholesterol for the uptake into the micelles [10] from which sterols are transferred into enterocytes by a specific carrier protein [4]. Thereafter ATP-binding cassette (ABC) transporter proteins selectively pump PS back into the intestinal lumen [4] and only 0.5—2% is absorbed [11]. Moreover, an increase in LDL receptor mRNA expression has been reported [9,12], suggesting increased clearance of LDL. According to recent systematic meta-analysis, PS or plant stanol containing products decrease low density lipoprotein cholesterol (LDL-C) concentration on the average by 0.31 mmol/L compared to controls [13]. Furthermore, the cholesterol-lowering efficacy has been reported to be greater in subjects with high baseline cholesterol, and being influenced by the food carrier [13—16]. Whole grain cereals rich in fiber reportedly possess cardiovascular health effects [17—19] including serum LDL-C and total cholesterol (TC) lowering properties [20]. Low-fat foods have already been introduced as carriers for nonesterified PS [21] and if high-fiber cereals turn out to be efficacious vehicles as well, their enrichment with PS could provide improved cardiovascular protection. Rye fiber, and wheat fiber are almost exclusive sources of alkylresorcinols [22,23] whose plasma metabolites (21) can be utilized for monitoring dietary treatment compliance.

Elevated serum LDL-C concentration is a well established risk factor for cardiovascular disease and thus an important target in drug and dietary treatment trials [24,25] but also normocholesterolemic individuals are likely to purchase functional foods for reducing LDL-C or TC. However, particularly in subjects with relatively low LDL-C concentration, the measurement of serum apolipoprotein B/apolipoprotein A1 (apoB/apoA1) ratio (balance between pro-atherogenic and anti-atherogenic lipoproteins) provides more predictive power than conventional lipid risk factors [26—28].

In the present study we explored the influence of two doses of high-fiber rye bread enriched with nonesterified PS (2 g/d or 4 g/d) on serum lipid risk factors, the apoB/apoA1 ratio and lipophilic (pro)vitamins (α- and β-carotenes and α- and γ-tocopherols) in normocholesterolemic subjects and evaluated treatment compliance by monitoring plasma alkylresorcinol metabolite concentrations.

Methods

Subjects

Sixty-eight healthy free-living volunteers were recruited for this randomized, double-blind, placebo-controlled dietary intervention trial. The volunteers were given written and oral information about the study in advance and they completed an eligibility questionnaire and attended a screening blood test. As exclusion criteria we specified low hemoglobin values (below 125 g/L for females, and 135 g/L for males), total cholesterol >6.5 mmol/L or the use of antibiotics within the past 3 months. Volunteers with severe diseases or taking drugs that affect serum lipids or gastrointestinal function were excluded. The use of vitamins and fish oil preparations was forbidden within one month before and during the study. The participants were instructed to maintain medication, body weight and physical activity constant throughout the study. All the participants signed informed consent. This study was approved by the Ethics Committee at the Helsinki University Central Hospital, Helsinki, Finland.

The study participants were randomly assigned to either active (rye bread enriched with PS) or control (rye bread without PS) treatment groups. These two treatment groups were stratified according to total cholesterol concentration, and gender. Of the 68 study participants three dropped out due to personal or schedule reasons. In addition, one participant failed to return the food diary after the trial and reported not having ingested the rye bread at all

Table 1 Basic characteristics of the participants (mean ± SD) in the active and control groups.

	Active group ($n = 32$)	Control group ($n = 31$)
Gender (M/F)	8/24	8/23
Age (y)	34.6 ± 11.7	37.1 ± 12.4
Weight (kg)	67.8 ± 13.5	69.8 ± 13.8
Length (cm)	167.4 ± 8.7	169.6 ± 8.3
BMI (kg/m^2)	24.0 ± 3.3	24.1 ± 3.2
Waist circumference (cm)	81.2 ± 10.9	82.6 ± 10.8
Serum cholesterol (mmol/L)	5.06 ± 0.77	5.08 ± 0.94
LDL cholesterol (mmol/L)	3.07 ± 0.75	3.11 ± 0.75
HDL cholesterol (mmol/L)	1.63 ± 0.48	1.63 ± 0.45
VLDL cholesterol (mmol/L)	0.31 ± 0.22	0.25 ± 0.17
Serum triglycerides (mmol/L)	1.17 ± 0.53	1.08 ± 0.48

None of the differences in the basic characteristics between the groups were statistically significant.