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Increases in plasma plant sterols stabilize within four weeks of plant sterol intake and are independent of cholesterol metabolism

R.T. Ras [a,*], W.P. Koppenol [a], U. Garczarek [a], A. Otten-Hofman [a], D. Fuchs [a], F. Wagner [b], E.A. Trautwein [a]

[a] *Unilever Research and Development Vlaardingen, Vlaardingen, the Netherlands*
[b] *Charité Research Organisation, Berlin, Germany*

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Abstract *Background and aims:* Plant sterols (PS) lower plasma LDL-cholesterol through partial inhibition of intestinal cholesterol absorption. Although PS themselves are poorly absorbed, increased intakes of PS result in elevated plasma concentrations. In this paper, we report time curves of changes in plasma PS during 12 weeks of PS intake. Furthermore, the impact of cholesterol synthesis and absorption on changes in plasma PS is explored.
Methods and results: The study was a double-blind, randomized, placebo-controlled, parallel-group study with the main aim to investigate the effects of PS on vascular function (clinicaltrials.gov: NCT01803178). Hypercholesterolemic but otherwise healthy men and women (n = 240) consumed low-fat spreads without or with added PS (3 g/d) for 12 weeks after a 4-week run-in period. Blood sampling was performed at week 0, 4, 8 and 12. Basal cholesterol-standardized concentrations of lathosterol and sitosterol + campesterol were used as markers of cholesterol synthesis and absorption, respectively. In the PS group, plasma sitosterol and campesterol concentrations increased within the first 4 weeks of intervention by 69% (95%CI: 58; 82) starting at 7.2 μmol/L and by 28% (95%CI: 19; 39) starting at 11.4 μmol/L, respectively, and remained stable during the following 8 weeks. Placebo-corrected increases in plasma PS were not significantly different between high and low cholesterol synthesizers (P-values >0.05). Between high and low cholesterol absorbers, no significant differences were observed, except for the cholesterol-standardized sum of four major plasma PS (sitosterol, campesterol, brassicasterol and stigmasterol) showing larger increases in low absorbers (78.3% (95%CI: 51.7; 109.5)) compared to high absorbers (40.8% (95%CI: 19.9; 65.5)).
Conclusions: Increases in plasma PS stabilize within 4 weeks of PS intake and do not seem impacted by basal cholesterol synthesis or absorption efficiency.
This study was registered at clinicaltrials.gov (NCT01803178).

* Corresponding author. Rouyanne Ras, Unilever R&D Vlaardingen, Olivier van Noortlaan 120, P.O. Box 114, 3130 AC Vlaardingen, The Netherlands. Tel.: +31 10 460 6117; fax: +31 10 460 5993.
E-mail address: Rouyanne.Ras@unilever.com (R.T. Ras).

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Introduction

Plant sterols (PS) are lipid-like compounds that occur naturally in small amounts in plant-based foods and in higher amounts in specific enriched foods. Consumption of foods with added PS is effective in lowering total-, and especially, LDL-cholesterol concentrations [1,2]. LDL-cholesterol is the most important blood lipid risk factor for coronary heart disease (CHD) [3,4].

PS inhibit intestinal cholesterol absorption [5] through several hypothesized mechanisms such as competition with cholesterol for solubilization in dietary mixed micelles, interference with transport-mediated processes of cholesterol uptake and stimulation of cholesterol excretion via the intestine [6,7]. The inhibition of intestinal cholesterol absorption with PS intake occurs rapidly and the maximal LDL-cholesterol lowering effect for a given dose of PS is usually achieved within the first 2–3 weeks of intake. PS in contrast to cholesterol are poorly absorbed from the intestine. In fact, due to active excretion of PS by the ABCG5/G88 transporters from the enterocytes back into the intestinal lumen and from the liver into the bile, net absorption of PS is less than 5% [8]. Consequently, circulating PS concentrations in humans are relatively low, i.e. ~250 times lower compared to cholesterol [9]. Nevertheless, when intakes of PS are high, e.g. with intake of foods with added PS, plasma PS concentrations modestly increase [10]. It is not well established how rapidly plasma PS concentrations rise, and whether the increase in plasma PS will stabilize over time. Additionally, some earlier studies have suggested that part of the variation in the LDL-cholesterol-lowering efficacy of PS is explained by endogenous factors of cholesterol metabolism such as cholesterol synthesis and/or absorption efficiency [11]. Whether cholesterol metabolism would also explain some of the variation in increases in plasma PS after PS-enriched food intake requires further investigation.

The primary result of this large randomized intervention study provided evidence that the regular intake of a low-fat spread with added PS (3 g/d) lowered total- and LDL-cholesterol whereas endothelial function as measured by flow-mediated dilation (FMD) was neither improved nor worsened [12]. No correlation was observed between changes in plasma PS and changes in FMD whereas a modest but significant correlation was observed between changes in LDL-cholesterol and changes in FMD [12]. In the current paper, we aimed to investigate the changes over time in plasma sitosterol and campesterol concentrations during 12 weeks of PS intake. In addition, the PS-induced changes over time in total- and LDL-cholesterol were investigated. Furthermore, we explored the impact of cholesterol metabolism, more specifically of cholesterol synthesis and absorption efficiency as determined by basal non-cholesterol sterols standardized for total cholesterol (TC), on the changes in plasma PS and serum lipids after PS intake.

Methods

Study population

Hypercholesterolemic, but otherwise healthy, men and post-menopausal women were recruited among inhabitants of Berlin and surroundings through advertisements. Individuals were selected based on the following main selection criteria: being apparently healthy; aged between 40 and 65 y; having LDL-cholesterol between 3.4 and 4.9 mmol/L; having a BMI between 18 and 30 kg/m^2; no occurrence of cardiovascular or systemic inflammatory diseases or diabetes mellitus; no use of lipid-lowering foods or drugs or other drugs that may interfere with the study outcomes; not smoking; and willing to comply with the study procedures. Informed consent was obtained from all study participants. The protocol was approved by the ethical committee of Charité Hospital and was conducted in accordance with the Helsinki declaration. This study was registered at clinicaltrials.gov (NCT01803178).

Study design and measurements

This study was designed as a double-blind, randomized, placebo-controlled, parallel-group intervention study. Following a 4-week run-in period, study participants were randomized, after stratification for age, sex and screening LDL-cholesterol, across two treatment groups: 20 g/d of low-fat (~40% fat) spread with added PS esters (3 g/d, expressed in free PS equivalents) or 20 g/d of placebo spread (comparable in fat composition, taste and appearance). Participants consumed two 10 g portion packs of spread daily. Consumption was recorded in a diary and opened and unopened portion packs were counted to determine compliance. The phytosterol mixture contained 70% sitosterol, 14% campesterol, 8% sitostanol, 3% brassicasterol, and some other phytosterols. The intervention period lasted 12 weeks. The spreads were produced by Unilever Research and Development Vlaardingen, The Netherlands. Participants were instructed to consume their typical diet and minimize changes in their diet and lifestyle habits, to refrain from foods containing PS or other ingredients claiming to lower blood cholesterol and to avoid using concomitant medication or vasoactive substances that could interfere with the study outcomes. Full details of this study were recently published [12].

Fasted blood samples were drawn at 4-week intervals (week 0, 4, 8 and 12) for measuring serum lipids by colorimetry on a Beckman Coulter AU analyzer (Synlab, Germany) and plasma non-cholesterol sterols by gas chromatography-mass spectrometry (GC-MS) with flame ionization detection (Unilever Research and Development Vlaardingen [13]). All samples of each subject were analyzed within the same assay. Lathosterol at baseline was used as a marker of cholesterol synthesis whereas the