The effect of a low-fat spread with added plant sterols on vascular function markers: results of the Investigating Vascular Function Effects of Plant Sterols (INVEST) study[1–5]

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ABSTRACT

Background: Plant sterols (PSs) lower LDL cholesterol, an established risk factor for coronary artery disease (CAD). No direct evidence is available supporting a reduced risk of CAD for foods with added PSs. Endothelial dysfunction is seen as an early indicator of atherosclerotic damage.

Objectives: This study was primarily designed to investigate the effect of a low-fat spread with added PSs on brachial artery endothelial function as measured by flow-mediated dilation (FMD). Second, effects on arterial stiffness, blood pressure, serum lipids, and plasma PS concentrations were investigated. We hypothesized that PSs would not worsen FMD but would rather modestly improve FMD.

Design: This study had a double-blind, randomized, placebo-controlled, parallel design. After a 4-wk run-in period, 240 hypercholesterolemic but otherwise healthy men and women consumed 20 g/d of low-fat spread without (control) or with added PSs (3 g/d) during 12 wk. Pre- and postintervention, vascular function measurements and blood sampling were performed.

Results: In total, 232 participants completed the study period. For the primary endpoint FMD, 199 participants were included in the statistical analysis. PS intake did not affect FMD (+0.01 percentage points; 95% CI: −0.73, 0.75) compared with control. Measures of arterial stiffness (pulse wave velocity and augmentation index) and blood pressure were also not significantly changed compared with control. After PS intervention, LDL cholesterol significantly decreased on average by 0.26 mmol/L (95% CI: −0.40, −0.12) or 6.7% compared with control. Plasma sitosterol and campesterol concentrations significantly increased in the PS group up to on average 11.5 μmol/L and 13.9 μmol/L (expressed as geometric means), respectively.

Conclusions: The intake of a low-fat spread with added PSs neither improved nor worsened FMD or other vascular function markers in hypercholesterolemic men and women. As expected, serum LDL cholesterol decreased, whereas plasma PSs increased after PS intake. This study was registered at clinicaltrials.gov as NCT01803178. *Am J Clin Nutr* 2015;101:733–41.

Keywords: cholesterol, flow-mediated dilation, plant sterols, randomized controlled trial, vascular function

INTRODUCTION

Phytosterols are lipid-like compounds that occur in foods of plant origin. Comprising both plant sterols (PSs)[6] and their saturated counterparts plant stanols, phytosterols have been shown to lower LDL cholesterol concentrations through partial inhibition of intestinal cholesterol absorption. An average intake of 2 g phytosterols/d lowers LDL cholesterol by 0.31–0.34 mmol/L or 8–10% (1, 2). Elevated blood LDL cholesterol is an established risk factor in the development of atherosclerosis and coronary artery disease (CAD) (3, 4).

Direct evidence supporting an LDL cholesterol–mediated reduction in CAD risk has so far not been generated for foods with added PSs. Considering the difficulties of performing an endpoint trial with PSs (5), endothelial function is seen as a viable option to investigate effects of PSs beyond cholesterol lowering. Endothelial dysfunction is a key aspect in the initiation and progression of atherosclerosis and is partly determined by the burden of CAD risk factors, including hypercholesterolemia (6–8). Brachial artery flow-mediated dilation (FMD) is a measure of large artery endothelial function and has been shown to be associated with cardiovascular risk (9). So far, 6 studies have investigated the effect of PSs (10–12) and/or plant stanols (11–15) on FMD. Although 5 of 6 studies showed effects on FMD in the positive direction, they all failed to show significant improvements in FMD after phytosterol-enriched food intake despite significant reductions in LDL cholesterol.

Despite their established LDL cholesterol–lowering effect, there is some concern about the benefits of foods with added PSs

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[3] Supplemental Table 1 is available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[6] Abbreviations used: AE, adverse event; AIx, augmentation index; BP, blood pressure; CAD, coronary artery disease; CBP, central blood pressure; FMD, flow-mediated dilation; pp, percentage points; PS, plant sterol; PWV, pulse wave velocity.

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in the prevention of CAD risk because supplemental intake of PSs increases plasma PS concentrations (16). In patients with homozygous phytosterolemia, a rare genetic disease, the excretion of phytosterols from the body is hampered due to a loss of function of the ATP-binding cassette transporters ABCG5 and ABCG8, caused by genetic mutations. This leads to very high plasma concentrations of phytosterols in patients with this disease. These patients often (17) but not always (18) display premature atherosclerosis and CAD. In addition, elevated plasma PS concentrations have been associated with increased CAD risk in some (19, 20) but not all (21, 22) epidemiologic studies. Whether this association really exists (23) and, if so, whether this association is explained by the PSs themselves or perhaps by plasma PSs being a surrogate marker of increased cholesterol absorption (24) is still a matter of debate.

In the current large-sample Investigating Vascular Function Effects of Plant Sterols (INVEST) study, the primary aim was to better estimate the size and variability of the effect of a low-fat spread with added PSs on FMD. This would allow investigating whether consumption of PSs might negatively affect endothelial function, as suggested in an animal study that showed that plasma PS concentrations after feeding very high PS doses were correlated with impaired endothelium-dependent vasorelaxation (25), and exploring a presumed small beneficial effect of PSs on FMD, which is expected based on the available evidence (26). Second, the effect of PS intake on arterial stiffness, blood pressure (BP), serum lipids, and plasma PS concentrations was investigated.

METHODS

This study was conducted from January 2013 through August 2013 at the Charité Research Organization, in Berlin, Germany. The study was conducted in accordance with applicable laws and regulations and with the ethical principles that have their origin in the Declaration of Helsinki, Finland. The protocol, informed consent, and advertisements were approved by the ethical committee of Charité Hospital. Written informed consent was obtained from all study participants. The study was registered at clinicaltrials.gov as NCT01803178.

Study population

Individuals were recruited among inhabitants of Berlin and surroundings by using advertisements. Interested persons were invited for an information session where full details of the study protocol were shared. In total, 662 persons joined the screening procedure. During 2 screening visits, several evaluations were performed, including medical history, medication use, physical examination, electrocardiogram, height, weight, vital signs, fasted blood sampling for hematology, clinical chemistry, and blood lipids and a cotinine test. Individuals were eligible when they met the predefined selection criteria, including the following: being apparently healthy men and postmenopausal women, aged 40–65 y; having borderline-high to high LDL cholesterol at screening (130–190 mg/dL or 3.4–4.9 mmol/L); having a BMI (in kg/m^2) between 18 and 30; having no occurrence of cardiovascular disease, systemic inflammatory conditions, or diabetes mellitus; not using lipid-lowering foods, lipid-lowering drugs, or other drugs that may interfere with the study measurements; not smoking; being willing to comply with the study protocol; and having signed the informed consent.

Study design

This study was designed as a single-center, randomized, double-blind, placebo-controlled, parallel study with 240 study participants and 2 treatments: a spread with added PSs in the form of PS esters and a control spread without added PSs. The intervention period lasted 12 wk and was preceded by a run-in period of 4 wk for stabilizing blood lipid concentrations and allowing the participants to get familiarized with the study regimen. A statistician (UG) randomized the participants by using permuted block randomization with stratification for age, sex, and screening LDL cholesterol concentration. Before and after the intervention, vascular function measurements were performed and fasted blood samples were drawn for measuring serum lipids and plasma PS concentrations. Health and well-being, use of concomitant medication, and adverse events (AEs) were monitored throughout the study.

Test products and dietary and lifestyle instructions

During the run-in period, participants were provided with control spread. During the intervention period, participants were provided with low-fat spread with added PS esters or control spread. Each day, participants consumed two 10-g portions of test spread with main meals. The PS spread was produced with 22.8% PS esters. The amount of PSs expressed as free equivalents was 15% (i.e., 3 g PSs in 20 g of spread). The PS esters were sourced from BASF. In the control spread, PS esters were replaced by vegetable oil. Total fat content of both test spreads was ~40%. The proportion of SFAs, MUFAs, and PUFAs was ~25%, ~25%, and ~50% of total fat, respectively. Detailed information on the composition of the test spreads is provided in **Table 1**. The test spreads were produced at Unilever Research and Development Vlaardingen, The Netherlands. Equal amounts of flavors and colorants (β-carotene) were added to ensure that the 2 test spreads were as similar as possible with respect to taste and appearance. The participants and all staff involved in the conduct of the study were blinded for the treatments. Concentrations of PSs were measured in a random selection of the test spreads across all production batches to check correct production of the spreads; the amount of PSs in the PS spread was on average 14.4%, so 2.9 g PSs per 20 g test spread.

Study participants received detailed information on how to consume and store the test spreads. After each 4-wk period, participants returned all opened and unopened tubs to the test facility for a compliance check. Noncompliance with test product intake was defined as having consumed <90% of total spread intake and/or missing more than one intake in the 3 d preceding the study visits. Dietary intake as such was not assessed.

During the entire study period, participants were encouraged to minimize changes in their habitual diet and lifestyle. Study participants were instructed to refrain from consuming phytosterol-enriched foods or supplements or other products claiming to lower blood cholesterol. Concomitant medication that could interfere with the study outcomes (i.e., use of statins, ezetimibe, fibrates, diabetic drugs, triglyceride-reducing drugs, angiotensin II receptor blockers, and angiotensin-converting enzyme inhibitors) or antibiotics were not allowed. Strenuous exercise was not allowed for at least 48 h before the test days. Furthermore, participants were requested to refrain from taking anti-inflammatory drugs, stimulants, and/or

TABLE 1
Nutritional composition of the test spreads[1]

Nutrition values[2]	PS spread	Control spread
Energy, kJ	1505.7	1492.6
Energy, kcal	365.0	361.8
Total protein, g	0.0	0.0
Total carbohydrates, g	0.0	0.0
Sugar, g	0.0	0.0
Fat total, g	39.4	40.3
SFAs, g	9.3	9.5
MUFAs, g	10.0	10.3
PUFAs, g	19.6	20.2
Total n–3 PUFAs, g	3.6	4.0
ALA, g	3.6	4.0
Total n–6 PUFAs, g	16.0	16.2
TFAs, g	0.5	0.4
Cholesterol, mg	0.7	0.9
PS ester, g	22.8	0.0
PSs,[3] g	14.4	0.0
Sodium, mg	6.9	9.0
Vitamin A, μg	610.0	610.0
Vitamin E, mg	9.8	12.9
Fiber, g	0.0	0.0
Water, g	45.6	59.6

[1]ALA, α-linolenic acid; PS, plant sterol; TFA, *trans* fatty acid.
[2]Nutrition values per 100 g of spread.
[3]The phytosterol mixture contained 70% β-sitosterol, 14% campesterol, 8% sitostanol, 3% brassicasterol, and some other phytosterols.

vasoactive substances in the 7 d before the vascular function measurements. On all test days, participants came to the research unit in a fasted state (12 h of neither food nor drinks except water) and received breakfast after all measurements were performed.

Study measurements

Endothelial function was assessed as FMD of the brachial artery, which was our primary outcome, in accordance with current guidelines (27). Measurements were performed by well-trained sonographers with participants in the supine position after a rest of at least 10 min in a quiet, temperature-controlled (22–24°C) room. Participants and sonographers were matched for the duration of the study. By using high-resolution ultrasound with a 15-MHz linear array transducer (VIVID E9; General Electric), we obtained an optimal longitudinal B-mode scan of the brachial artery ($\sim$5 cm above the elbow crease) with the probe held by a stereotactic clamp to ensure steady image recordings. After 1 min of baseline acquisition, a forearm cuff was inflated to suprasystolic pressure for 5 min and then deflated to induce reactive hyperaemia. Recordings of the brachial artery were continued for 4 min after occlusion. Brachial artery diameter was measured on acquired frames by a computerized edge detection and wall-tracking system (FMD studio; Quipu SRL). FMD was calculated as the difference between the maximum diameter after occlusion and the mean baseline diameter divided by the mean baseline diameter and expressed in percentage points (pp). All recorded scans were analyzed at a core laboratory by a single independent operator who was blind to the study's participants and phase. Directly after the FMD measurement, aortic pulse wave velocity (PWV), augmentation index (AIx), and central BP (CBP) were assessed by noninvasive oscillometry by using an Arteriograph (TensioMed). The surrogate carotid-femoral distance was measured between the sternal notch and the pubic symphysis with a tape measure. At least 2 Arteriograph measurements were performed. If the 2 PWV values differed by $>$0.5 m/s, a third measurement was performed. The PWV, AIx, and CBP values were then determined as the median of the measurements. For office BP, the nondominant arm was used, which was supported at heart level. Three BP measurements were taken with an oscillometric device at 2-min intervals, and the last 2 readings were used to calculate the mean resting systolic and diastolic BP.

After completion of the vascular function measurements, fasted blood samples were drawn from the anticubital vein by using tubes for serum or plasma. Serum lipids (LDL cholesterol, total cholesterol, HDL cholesterol, and triglycerides) were analyzed by colorimetry on a Beckman Coulter AU analyzer at Synlab, Germany. Plasma concentrations of PSs were measured by using gas chromatography–mass spectrometry with flame ionization detection at Unilever Research and Development, Vlaardingen, The Netherlands. All samples of each participant were analyzed within the same assay.

Statistical analysis

This study was powered to be able to differentiate with good certainty a potential minimal negative effect (the noninferiority margin) from that of the assumed positive effect. A true-positive effect size of +0.5 pp was assumed based on the pooled mean of 5 previous studies (10–14, 26). As the noninferiority margin, its negative counterpart of $-$0.5 pp was chosen. The necessary sample size was calculated in the familiar setting of a 2-sample *t* test with a difference of 1 pp (+0.5 pp to $-$0.5 pp), an SD of 2.5 pp, a 2-sided α of 0.05 and a power of 0.8. This would require 199 participants in total. Accounting for an overall dropout rate of 20%, 240 participants were included in the current study.

Agreement on protocol deviations and quality of FMD data points was obtained during the blind review meeting through expert consensus. For each parameter, statistical analysis was performed for the intention-to-treat population (including all available data points of all participants included in the study) and the per protocol population (including all biochemical/biological/physiologic plausible data points of all participants who correctly followed the protocol). Here, the results of the per protocol population are reported. The results of the intention-to-treat population were similar.

The primary endpoint was the change from baseline in FMD after intervention with PSs compared with control. This was estimated in an ANCOVA model with change from baseline in FMD as outcome, baseline FMD value as covariate, and treatment and FMD operator as fixed effects. Effects are reported as least squares means and 2-sided 95% CIs. All other parameters (PWV, AIx, CBP, office BP, blood lipids, and plasma PSs) were statistically analyzed in a similar way. Treatment effects on FMD were interpreted based on their 95% CIs according to the noninferiority and equivalence testing principles as outlined in the Consolidated Standards of Reporting Trials statement (28). Correlation analysis was performed to investigate the relation between changes in LDL cholesterol and changes in FMD, as well as between changes in plasma PSs and changes in FMD in the participants who received PS treatment. All analyses were performed with the statistical software package SAS version 9.4 (SAS Institute).

RESULTS

Overview of study population

A total of 150 men (62.5%) and 90 women (37.5%) were included in the study, almost all Caucasian. An overview of the participants' characteristics at baseline is provided in **Table 2**. In total, 8 participants dropped out prematurely. Eight participants violated the protocol (i.e., not being weight stable, not being fasted, or having used prohibited medication). Ultrasound scans of 24 participants were rejected because of poor quality and/or instability of the images caused by inconsistency of clear artery borders. One subject missed the last FMD visit. For FMD (i.e., the primary endpoint), 199 participants were included in the analysis (**Figure 1**). Compliance with test product intake was high (>90%).

Vascular function

Baseline FMD of the included participants (n = 199) was on average 5.1 ± 2.6 pp (i.e., CV was 0.5). Neither age nor sex significantly affected the change from baseline in FMD. The effect of PSs on FMD was +0.01 pp (95% CI: −0.73, 0.75) compared with control and not significant (**Table 3**). The 95% CI includes +0.5 pp, which was the expected small positive effect based on data from 5 published studies (10–14), and −0.5 pp, which was the predefined noninferiority margin. In relative terms, the change in FMD on PS intake was 0.1% compared with control. Descriptive statistics of the baseline artery diameter, the maximal diameter after hyperemia, and the shear rate before and after intervention are provided in **Supplemental Table 1**. Because the FMD at baseline was significantly different between the 2 treatment groups (control group: 4.7 ± 2.6 pp and PS group: 5.5 ± 2.6 pp), a simulation analysis was performed to assess the effect of PSs on FMD based on repeated (n = 100) random subsets of the population with appropriate weights to achieve balanced FMD values at baseline. This analysis did not show different estimated effects of PSs on FMD (data not shown). Measures of arterial stiffness (PWV and AIx), CBP, and office systolic BP were not significantly changed after PS intake compared with control (Table 3). Only office diastolic BP was significantly lowered by 1.4 mm Hg (95% CI: −2.7, −0.1) after PS intake compared with control.

On the basis of correlation analysis in the group that received PSs, it appeared that changes in plasma PS concentrations were not related to changes in FMD (partial correlation = −0.09; $P >$ 0.05), whereas a reduction in LDL cholesterol was modestly but significantly related to an increase in FMD (partial correlation = −0.20; $P < 0.05$) (**Figure 2**).

Blood lipid and PS concentrations

At baseline, serum total and LDL cholesterol concentrations were on average 5.77 ± 0.92 and 3.91 ± 0.60 mmol/L, respectively. Total and LDL cholesterol concentrations were significantly reduced on average by 0.26 mmol/L (4.5%) and 0.26 mmol/L (6.7%), respectively, after PS intake compared with control (**Table 4**). No significant changes were observed in HDL cholesterol (+0.6%) and triglyceride (−2.2%) concentrations compared with control. Plasma sitosterol concentrations significantly increased in the PS group from 6.7 to 11.5 μmol/L and campesterol from 11.4 to 13.9 μmol/L. These values are based on back-transformed data and represent geometric means. No obvious changes were observed in the control group. Compared with control, plasma sitosterol and campesterol concentrations increased on average by 77.9% and 32.6%, respectively. The sum of 6 major phytosterols (i.e., sitosterol, campesterol, brassicasterol, stigmasterol, sitostanol, and campestanol) was significantly increased by 41.6% compared with control (Table 4).

Adverse events

A total of 85 participants experienced one or more AEs (209 in total) during the intervention period. Overall, the incidence of AEs was mild to moderate and all not related to the study procedures. Three participants experienced a serious AE during the intervention period (thermal burn, gastroenteritis, or depression

TABLE 2
Overview of the participant characteristics at baseline[1]

Characteristic	PS group (n = 126)	Control group (n = 114)	Overall (N = 240)
Male, n (%)	77 (61.1)	73 (64.0)	150 (62.5)
Female, n (%)	49 (38.9)	41 (36.0)	90 (37.5)
Age, y	53.4 ± 6.7[2]	53.1 ± 6.9	53.2 ± 6.8
Weight, kg	78.0 ± 13.0	77.4 ± 13.1	77.7 ± 13.0
Height, cm	174.6 ± 8.6	174.8 ± 9.5	174.7 ± 9.0
BMI, kg/m^2	25.5 ± 2.8	25.2 ± 2.7	25.3 ± 2.8
Waist circumference, cm	88.9 ± 10.6	89.3 ± 10.4	89.1 ± 10.5
Hip circumference, cm	102.0 ± 6.9	101.9 ± 6.0	101.9 ± 6.5
Total cholesterol, mmol/L	5.65 ± 1.09	5.74 ± 1.01	5.69 ± 1.05
LDL cholesterol, mmol/L	3.83 ± 0.73	3.90 ± 0.63	3.86 ± 0.68
HDL cholesterol, mmol/L	1.39 ± 0.46	1.35 ± 0.41	1.37 ± 0.44
Triglycerides,[3] mmol/L	0.95 (0.74, 1.39)	1.10 (0.80, 1.47)	1.05 (0.76, 1.43)
FMD, pp	5.4 ± 2.8	4.6 ± 2.7	5.0 ± 2.8
SBP, mm Hg	122.2 ± 12.5	123.3 ± 12.1	122.7 ± 12.3
DBP, mm Hg	74.5 ± 8.0	74.6 ± 8.5	74.5 ± 8.2

[1]DBP, diastolic blood pressure; FMD, flow-mediated dilation; pp, percentage points; PS, plant sterol; Q, quartile; SBP, systolic blood pressure.
[2]Mean ± SD (all such values).
[3]Triglyceride values were not normally distributed and are therefore reported as medians (Q1, Q3).

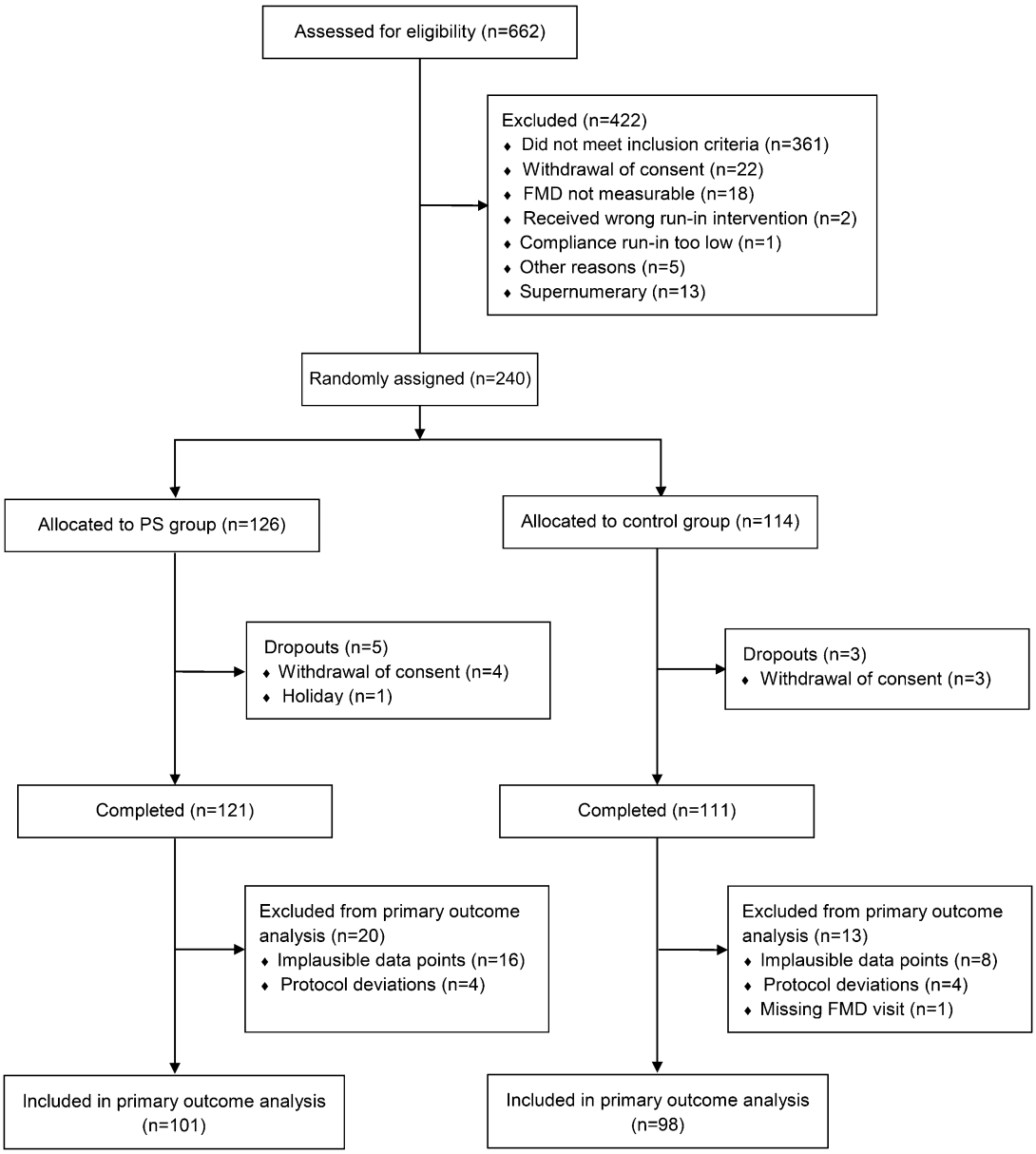

FIGURE 1 Participant flow throughout the study. Hypercholesterolemic men and women were randomly allocated across 2 different treatment groups. One group consumed a low-fat spread enriched with PSs, and one group consumed a low-fat control spread. The primary outcome was FMD. FMD, flow-mediated dilation; PS, plant sterol.

followed by weight loss). These serious AEs were not related to the test product intake, and were all resolved.

DISCUSSION

The present study showed that the regular intake of a low-fat spread with added PSs over 12 wk neither improved nor worsened FMD in hypercholesterolemic but otherwise healthy men and women. Measures of arterial stiffness and BP were also not affected. The PS intake led to a significant reduction in total and LDL cholesterol concentrations, although the effect on LDL cholesterol was smaller (~7%) than anticipated (~12%) for the dose of PSs provided (3 g/d) (1, 2). Plasma PS concentrations were significantly increased with PS intake as expected based on previous investigation (16).

So far, only a few studies have been performed that investigated the effect of phytosterols on FMD. Three of these studies were performed in hypercholesterolemic adults who consumed spreads enriched with PSs or plant stanols at doses of ~2 g/d (11–13). In these studies, FMD was not significantly changed; placebo-corrected FMD effect sizes ranged between 0.37 and 1.02 pp for PSs and between 0.12 and 0.91 pp for plant stanols. LDL cholesterol was significantly reduced in these studies by 9–16%. Two other studies were performed in prepubescent familial hypercholesterolemic children who were provided with spreads or yogurts enriched with ~2 g/d PSs (10) or plant stanols (14). These studies also found no significant changes in FMD (effects ranged between 0.05 and 0.50 pp), despite significant reductions of 9–14% in LDL cholesterol. In a study with patients with type 1 diabetes (15), plant stanol

TABLE 3
Vascular function and blood pressure in hypercholesterolemic men and women who consumed a low-fat spread enriched with plant sterols or a low-fat control spread[1]

Outcome/treatment	*n*	Baseline, mean ± SD	End of intervention, mean ± SD	Absolute change[2]	95% CI
FMD, pp					
Control	98	4.70[3] ± 2.61	4.73 ± 2.60	−0.27	−0.80, 0.25
PSs	101	5.53[3] ± 2.56	5.10 ± 3.05	−0.27	−0.79, 0.26
Δ	199			0.01	−0.73, 0.75
PWV, m/s					
Control	97	8.35 ± 1.86	8.12 ± 1.68	−0.18	−0.35, 0.00
PSs	109	8.08 ± 1.38	7.81 ± 1.08	−0.32*	−0.49, −0.15
Δ	206			−0.14	−0.38, 0.10
AIx, %					
Control	99	30.65 ± 16.62	29.46 ± 16.01	−1.11	−2.55, 0.33
PSs	109	29.48 ± 13.92	27.44 ± 14.01	−2.11*	−3.49, −0.74
Δ	208			−1.01	−3.00, 0.99
Central SBP, mm Hg					
Control	98	125.8 ± 17.9	120.6 ± 17.7	−5.3*	−7.1, −3.4
PSs	109	126.7 ± 17.6	119.4 ± 15.1	−7.2*	−8.9, −5.4
Δ	207			−1.9	−4.4, 0.6
Central DBP, mm Hg					
Control	98	80.0 ± 9.6	77.0 ± 10.3	−3.2*	−4.4, −1.9
PSs	109	81.3 ± 9.5	77.0 ± 8.9	−4.2*	−5.3, −3.0
Δ	207			−1.0	−2.7, 0.7
Office SBP, mm Hg					
Control	107	123.5 ± 12.3	119.4 ± 13.5	−3.9*	−5.3, −2.6
PSs	117	122.1 ± 12.8	116.9 ± 10.9	−5.4*	−6.7, −4.1
Δ	224			−1.5	−3.4, 0.4
Office DBP, mm Hg					
Control	107	74.6 ± 8.6	72.6 ± 8.8	−2.1*	−3.0, −1.2
PSs	117	74.5 ± 8.1	71.1 ± 7.8	−3.5*	−4.3, −2.6
Δ	224			−1.4*	−2.7, −0.1

[1]*Significant at $P < 0.05$. AIx, augmentation index; DBP, diastolic blood pressure; FMD, flow-mediated dilation; pp, percentage points; PS, plant sterol; PWV, pulse wave velocity; SBP, systolic blood pressure.

[2]Absolute changes from baseline are expressed as least squares means and 95% CIs after correction for baseline (and FMD operator in case of FMD results). Treatment effects were estimated in an ANCOVA model.

[3]FMD at baseline was significantly different between the 2 treatment groups.

intake showed a tendency for a worsening in FMD (−2.47 pp); this effect, however, was not significant and mainly driven by an improvement in FMD in the control group.

In the present study, we observed no change in FMD after PS intake compared with control, whereas we anticipated a modest improvement of 0.5 pp based on the combined evidence from FMD studies with phytosterols (26). It could be speculated that the LDL cholesterol–lowering effect observed in our study (−7%) was not sufficient to mediate an improvement in endothelial function. It could also be speculated that changes in LDL

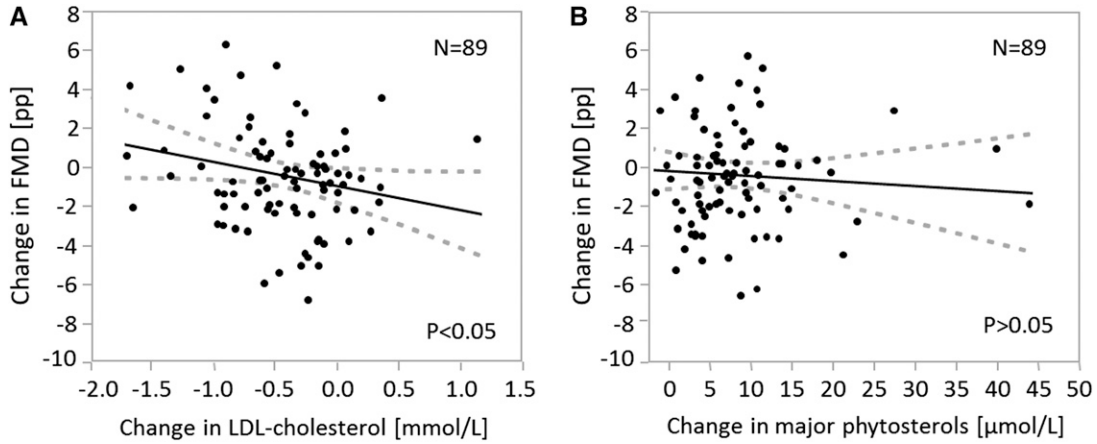

FIGURE 2 Correlation between changes in LDL cholesterol and plasma phytosterols and changes in FMD. The participants in the plant sterol group were included for this correlation analysis. FMD, flow-mediated dilation; pp, percentage points.

TABLE 4
Serum lipid and plasma plant sterol concentrations in hypercholesterolemic men and women who consumed a low-fat spread enriched with plant sterols or a low-fat control spread[1]

Outcome/treatment	*n*	Baseline[2]	End of intervention[2]	Absolute change[3]	Relative change, %
Total cholesterol, mmol/L					
Control	105	5.80 ± 0.91	5.45 ± 1.01	−0.34* (−0.48, −0.20)	−5.8
PSs	113	5.75 ± 0.93	5.16 ± 0.90	−0.60* (−0.73, −0.46)	−10.4
Δ	218			−0.26* (−0.46, −0.07)	−4.5
LDL cholesterol, mmol/L					
Control	105	3.94 ± 0.59	3.71 ± 0.69	−0.22* (−0.32, −0.12)	−5.5
PSs	113	3.89 ± 0.62	3.42 ± 0.64	−0.48* (−0.58, −0.38)	−12.3
Δ	218			−0.26* (−0.40, −0.12)	−6.7
HDL cholesterol, mmol/L					
Control	105	1.36 ± 0.40	1.29 ± 0.39	−0.07* (−0.11, −0.03)	−5.0
PSs	113	1.42 ± 0.45	1.35 ± 0.43	−0.06* (−0.10, −0.02)	−4.3
Δ	218			0.01 (−0.04, 0.06)	0.6
log(triglycerides),[4] mmol/L					
Control	105	0.11 ± 0.50	0.08 ± 0.49	−0.02 (−0.08, 0.04)	−2.1
PSs	113	0.04 ± 0.43	0.00 ± 0.47	−0.04 (−0.10, 0.02)	−4.2
Δ	218			−0.02 (−0.11, 0.07)	−2.2
log(sum of major phytosterols),[4,5] μmol/L					
Control	104	2.91 ± 0.37	2.90 ± 0.38	−0.04 (−0.08, 0.01)	−3.5
PSs	109	3.07 ± 0.37	3.37 ± 0.36	0.31* (0.27, 0.36)	36.6
Δ	213			0.35* (0.28, 0.41)	41.6
log(sitosterol),[4] μmol/L					
Control	107	1.74 ± 0.38	1.74 ± 0.39	−0.02 (−0.07, 0.03)	−1.8
PSs	114	1.90 ± 0.40	2.44 ± 0.41	0.56* (0.51, 0.61)	74.7
Δ	221			0.58* (0.51, 0.65)	77.9
log(campesterol),[4] μmol/L					
Control	107	2.24 ± 0.41	2.20 ± 0.44	−0.06* (−0.11, −0.02)	−6.0
PSs	115	2.43 ± 0.42	2.63 ± 0.38	0.22* (0.18, 0.26)	24.7
Δ	222			0.28* (0.22, 0.35)	32.6

[1]*Significant at $P < 0.05$. PS, plant sterol.
[2]All values are means ± SDs.
[3]Absolute changes from baseline are expressed as least squares means and 95% CIs after correction for baseline. Treatment effects were estimated in an ANCOVA model.
[4]Serum triglyceride and plasma PS values were not normally distributed and were log-transformed to allow statistical analysis. Relative changes from baseline in serum triglyceride and plasma PSs are expressed on the original scale.
[5]The major phytosterols included sitosterol, campesterol, brassicasterol, stigmasterol, sitostanol, and campestanol.

cholesterol do not per definition result in changes in endothelial function. Studies with ezetimibe monotherapy have overall not shown clear correlations between changes in LDL cholesterol and changes in FMD (29–31). Furthermore, in a community study with 5000 individuals, classic risk factors, including dyslipidemia, explained only 15.4% of the variation in FMD (32). On the other hand, in patients who underwent LDL apheresis and thereby acutely reduced their LDL cholesterol by 76.5%, endothelium-dependent vasodilation was significantly improved (33). Furthermore, in familial hypercholesterolemia, a disease associated with lifelong elevations in plasma cholesterol concentrations (~3 mmol/L higher compared with healthy controls) due to inherited mutations in LDL receptor genes, FMD is clearly impaired (3–4 pp lower compared with healthy controls) (10, 34). In an attempt to explore the relation between changes in LDL cholesterol and changes in endothelial function on PS intervention, we plotted the individual changes in LDL cholesterol against those in FMD of the participants who received the PS spread and observed a small but significant correlation (partial correlation = −0.20; $P < 0.05$; Figure 2). Because the placebo-controlled effect of PSs on FMD was zero, it could be hypothesized that a certain minimal reduction in LDL cholesterol (>0.26 mmol/L) is required to improve endothelial function. This theory requires further investigation.

The observation that the PS-induced change in FMD was 0.01 pp demonstrates that endothelial function was not impaired. Also, there was no correlation found between changes in plasma PSs and changes in FMD. This suggests that an increase in plasma PSs is unlikely to counteract beneficial vascular effects of PSs, which are expected based on their LDL cholesterol–lowering properties. Indeed, plant stanols, which are known to lower plasma PS concentrations, do not affect FMD (11–14) differently than PSs do (10, 11, 12), whereas they are equally effective in lowering LDL cholesterol (35). In an animal study with normal, wild-type mice, it was speculated that elevated plasma PS concentrations after PS feeding could be atherogenic because these concentrations were correlated with impaired endothelial vasorelaxation in situ (25). However, in this mouse model, cholesterol concentrations were unaffected, suggesting that this model was probably not appropriate for studying the effects of PSs. Also, the dose of PSs used in these mice was ~100 times higher than the amount of PSs that is recommended for lowering

LDL cholesterol in humans (2 g/d). Furthermore, it was recently demonstrated that, in hamsters fed a high-cholesterol diet, endothelial function was improved after intake of sitosterol and stigmasterol compared with control and compared with their oxidized counterparts (36).

Our study has several strengths that give support to the conclusions drawn, such as the straightforward and rigid design of the study, inclusion of one of the largest numbers of participants, and the high compliance with test product intake. The latter was reflected in a clear increase in the plasma PS concentrations after PS intervention. Also, vascular ultrasound was performed by well-trained sonographers according to current guidelines (27). FMD was assessed by a single, blinded, independent, and experienced technician with computer-assisted analysis, using edge detection and wall-tracking software, which has been demonstrated to be very reproducible (37). The CV in FMD at baseline was comparable with those reported in other FMD studies (i.e., ~0.5) (37, 38). Furthermore, the PS-induced FMD effect based on FMD assessment by the sonographers at the time of the ultrasound acquisition was comparable with the FMD effect based on centrally assessed FMD by a single, blinded central reader (data not shown).

Some limitations of the study need to be mentioned as well. First, although we assumed that the study participants would have suboptimal FMD because of their elevated prestudy LDL cholesterol concentrations, we cannot exclude that the participants were too healthy (e.g., all nonsmokers, no type 2 diabetes) to allow improvement in endothelial function on treatment. Follow-up studies should preferably select participants with impaired FMD at baseline. Second, LDL cholesterol was significantly changed from baseline (by ~5%) in the control group. Also, central and office BP were significantly changed from baseline. Although the participants were instructed not to change their diet and lifestyle during the study, we cannot rule out that seasonal influences or increased awareness of having elevated blood LDL cholesterol, for example, led to unintended changes in their typical habits, which may have influenced the outcomes of this study. Third, despite stratification for age, sex, and LDL cholesterol, FMD at baseline was significantly different between the 2 intervention groups. The performed ANCOVA analysis with baseline as covariate was one planned safeguard against differences in baseline FMD. Because the difference was larger than expected, we performed simulation analysis with equally balanced baseline FMD. This analysis showed that upfront balancing for baseline FMD would not have changed the outcomes of our study. Last, although changes in endothelial function can occur quickly on intervention [e.g., fat loads can affect FMD within a few hours (39)], it cannot be ruled out that the duration of the current study (3 mo) was insufficient to induce small effects on the vascular system, particularly when assuming that such an effect would be mediated through LDL cholesterol reduction and considering that the observed LDL cholesterol–lowering effect was only rather modest (~7% for a dose of 3 g PS/d).

In summary, endothelial function as measured by brachial artery FMD was neither improved nor worsened with PS intake. The LDL cholesterol–lowering effect of PS intake was confirmed, although the effect was lower than anticipated for the dose of PS tested (3 g/d). LDL cholesterol is an established risk factor in the development of atherosclerosis leading to CAD. Whether reductions in LDL cholesterol due to PS intake would reduce CAD risk via improvements of vascular function requires further investigation. Future studies should investigate vascular effects of PS after prolonged intakes of PS with enhanced LDL cholesterol lowering, preferably in participants with compromised vascular function.

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