Lathosterol-to-cholesterol ratio in serum predicts cholesterol-lowering response to plant sterol consumption in a dual-center, randomized, single-blind placebo-controlled trial1–3

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ABSTRACT

Background: Benefits of plant sterols (PS) for cholesterol lowering are compromised by large variability in efficacy across individuals. High fractional cholesterol synthesis measured by deuterium incorporation has been associated with nonresponse to PS consumption; however, prospective studies that show this association have yet to be conducted.

Objective: The goal was to test whether the lathosterol-to-cholesterol ratio (L:C ratio), a surrogate marker of endogenous cholesterol synthesis, serves as an a priori predictor of cholesterol lowering in response to PS consumption.

Design: Sixty-three mildly hypercholesterolemic adults who were preselected as possessing either high endogenous cholesterol synthesis [HS; n = 24; L:C = 2.03 ± 0.39 mmol/mmol (mean ± SD)] or low endogenous cholesterol synthesis (LS; n = 39; L:C = 0.99 ± 0.28 mmol/mmol) on the basis of baseline L:C consumed 2 g PS/d or a placebo for 28 d with the use of a dual-center, single-blind, randomized crossover design. Plasma lipid and noncholesterol sterol concentrations were measured at the end of each phase.

Results: PS consumption lowered total cholesterol (TC; n = 25 ± 0.05 mmol/L; P < 0.0001) and LDL cholesterol (n = 30 ± 0.04 mmol/L; P < 0.0001) overall. Specifically, LS individuals responded to PS treatment with a reduction in TC (n = 0.40 ± 0.07 mmol/L; P < 0.0001) and LDL cholesterol (n = 0.29 ± 0.05 mmol/L; P = 0.0002), whereas HS individuals failed to show cholesterol lowering (TC: n = 0.09 ± 0.09 mmol/L; P = 0.2843; LDL cholesterol: n = 0.05 ± 0.07 mmol/L; P = 0.4917). The odds of LS participants responding to PS consumption with cholesterol lowering better than the mean cholesterol lowering in all participants were 4.25 (95% CI: 1.242, 14.556; P = 0.0211) for TC and 3.36 (95% CI: 1.112, 10.161; P = 0.0317) for LDL cholesterol, which was higher than for HS participants.

Conclusions: The L:C ratio predicts the extent of reduction in circulating TC and LDL cholesterol in response to PS consumption. Cholesterol synthesis assessment may thus be a use in identifying responders and nonresponders to PS therapy. This trial was registered at clinicaltrials.gov as NCT01131832. Am J Clin Nutr 2015;101:432–9.

Keywords cholesterol lowering, interindividual variability, noncholesterol sterols, plant sterols, randomized controlled trial

INTRODUCTION

Plant sterols (PS)4 are steroid compounds that closely resemble cholesterol but have differing C24 carbon side-chain configurations and are found mostly in plants (1). PS and their saturated equivalents, plant stanols, have well established cholesterol-lowering properties, with a substantial database of clinical trials that typically demonstrate LDL-cholesterol lowering efficacy in the 5–15% range (2–4). PS have been shown to reduce cholesterol absorption, the primary mechanism through which the LDL-cholesterol reductions are achieved (5, 6).

Despite well-established lipid-lowering action across clinical trials, significant interindividual variability in LDL-cholesterol lowering in response to PS consumption exists (7–9), with some individuals showing better than average, nonresponse, or even an adverse response to PS consumption (10, 11). These individual-specific responses to PS consumption have been shown to be reproducible in individuals across repeated PS interventions (12). The strong correlations between participants’ responses to PS indicate that an endogenous determinant, such as cholesterol metabolism, may be responsible, as opposed to variations in other factors such as compliance.

The principal components of cholesterol metabolism can be estimated through the use of noncholesterol sterols, with cholesterol precursors such as desmosterol or lathosterol used as markers for endogenous cholesterol synthesis (13–16) and plant sterols such as campesterol and sitosterol used as markers of cholesterol absorption (17, 18). Noncholesterol sterol concentrations are typically expressed as ratios to cholesterol and have been validated against direct measures of cholesterol metabolism (14).

1 From the Richardson Centre for Functional Foods and Nutraceuticals (DSM, PKE, and PJH) and the Departments of Food Science and Human Nutrition Sciences (DSM, PKE, and PJH), University of Manitoba, Winnipeg, Manitoba, Canada, and the USDA, Agricultural Research Service, Beltsville Human Nutrition Research Center, Beltsville, MD (SKG and DJB).
2 Supported by the Canadian Institutes of Health Research. Placebo and plant sterol margarines were provided by Unilever Canada Inc.
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4 Abbreviations used: FID, flame ionization detector; GC, gas chromatography; HS, high endogenous cholesterol synthesis; L:C ratio, lathosterol-to-cholesterol ratio; LS, low endogenous cholesterol synthesis; NCS, noncholesterol sterol; PS, plant sterols.

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Limited studies have been completed in which the influence of cholesterol metabolism on cholesterol-lowering response to PS consumption was evaluated (9, 19–22). Thuluvu et al. (23) determined that only in individuals with low endogenous cholesterol synthesis, estimated by lathosterol-to-campesterol ratio, did plant stanols at a dose of 1 g/d reduce LDL cholesterol after 4 wk. This study was, however, limited to only 8 individuals in each group. In agreement, Rideout et al. (24) determined in a retrospective analysis of 3 clinical trials that individuals who did not exhibit a LDL-cholesterol–lowering response to PS consumption had higher basal cholesterol synthesis.

Although the relation between cholesterol absorption and synthesis is often described as reciprocal (25) to maintain cholesterol homeostasis, cholesterol synthesis is typically responsible for a much larger contribution to circulating cholesterol pools than is cholesterol absorption (26).

Therefore, we hypothesized that endogenous cholesterol synthesis modulates cholesterol lowering in response to PS consumption. Specifically, the present objective was to perform a clinical trial involving prospective recruitment of individuals with a high or low lathosterol-to-cholesterol ratio (L:C ratio), a surrogate marker of cholesterol synthesis, to determine whether LDL-cholesterol lowering due to PS is influenced by cholesterol synthesis in an a priori fashion.

METHODS

Recruitment

Mildly hypercholesterolemic, but otherwise healthy, individuals aged 30–75 y were recruited from Winnipeg, Canada, and Beltsville, MD, areas by using media and direct mail advertisements. Fasting blood samples were collected and screened for biochemical and hematologic variables. “Otherwise healthy” was defined as the absence of a known chronic or infectious disease. Inclusion criteria were fasting LDL cholesterol ≥3.0 mmol/L, age 30–75 y, and fasting glucose <6.1 mmol/L. Exclusion criteria included history of cardiovascular disease, diabetes, uncontrolled hypertension, kidney disease, or cancer; smoking; use of medications or natural health products known to affect lipid metabolism, including cholestyramine, colestipol, niacin, clofibrate, gemfibrozil, probucol, 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, methotrexate, high-dose dietary supplements, and PS use for at least the previous 2 mo; and excessive alcohol consumption (>2 servings/d) or exercise expenditure (>4000 kcal/wk). Individuals who met these criteria had their serum L:C ratio assessed by gas chromatography (GC) mass spectrometry. Individuals above the 25th and below the 75th percentiles of L:C ratio, based on the first 40 individuals screened, were enrolled into the trial; individuals falling between the 25th and 75th percentiles were not enrolled and did participate in the trial. Seventy-one participants were enrolled into the trial. Individuals who did not participate in the trial. Seventy-one participants were enrolled into the trial; individuals above the 25th criteria had their serum L:C ratio assessed by gas chromatography (GC) mass spectrometry. Individuals above the 25th criteria had their serum L:C ratio assessed by gas chromatography (GC) mass spectrometry.

Trial design

A dual-center, randomized, single-blind, 2-period, crossover placebo-controlled clinical trial was conducted at the Nutritional Research Unit of the Richardson Center for Functional Foods and Nutraceuticals, University of Manitoba, and the Food Components and Health Laboratory, at the USDA Beltsville Human Nutrition Research Center. The trial consisted of two 4-wk periods separated by a minimum 4-wk washout period during which participants consumed their habitual diets. Participants were randomly assigned by a trial coordinator to 1 of the 2 possible experimental treatment sequences by coin flip after enrollment into the trial. During the two 4-wk periods, participants attended the research site (Nutritional Research Unit or Food Components and Health Laboratory) and consumed 1 meal/d under supervision for a minimum of 4–5 d/wk and without supervision off-site for 2–3 d/wk. Participants were blinded to the treatment because meals appeared identical and contained either 25 g of margarine with 2 g-free PS/d (treatment), as esters, or without PS (placebo) depending on the period. (Placebo and PS margarines were provided by Unilever Canada Inc.) The participants’ other meals were not provided nor controlled; however, participants were asked to refrain from consuming foods and natural health products that contained PS. Participants were also asked to refrain from drinking >2 alcoholic or caffeinated beverages/d and advised to maintain their typical diet and physical activity levels and were asked to report any changes in diet or physical activity and symptoms or changes in health and medications throughout the trial.

Blood sampling and serum lipid analysis

On days 1, 2, 24, 25, 26, 27, and 28 of each phase, 12-h fasted serum and plasma samples were collected. Within 1 h of blood collection, serum, plasma, buffy coat, and erythrocyte fractions were separated into aliquots, and immediately stored at −80°C until further analysis.

Serum total cholesterol (TC), HDL cholesterol, triglycerides, and glucose concentrations were determined by automated enzymatic methods on a Vitros-350 chemistry analyzer (Ortho-Clinical Diagnostics). LDL-cholesterol concentrations were calculated by the Friedewald equation (27). Noncholesterol sterols (NCSs) and cholesterol were determined by using a GC equipped with a flame ionization detector (FID; Agilent Technologies), as described (28), with some modifications. An internal standard, 5-α-cholestane, was added to 500 μL of serum samples that were then saponified with methanolic potassium hydroxide. Sterols were extracted twice from the mixture with petroleum ether, evaporated to dryness under nitrogen gas, and resuspended in 400 μL hexane and trimethylsilyl derivatized with the addition of 100 μL hexamethyldisilazane + trimethylchlorosilane + pyridine (3:1:9; Supelco). NCSs and cholesterol were determined by GC-FID. The injector temperature was set to 280°C, and the detector temperature was 300°C. The column temperature was initially set to 130°C for 2 min, followed by an increase to 270°C at 30°C/min and held for 10 min, then increased to 290°C at 10°C/min and held for 9 min, then finally increased to 320°C at 40°C/min and held for 5 min. The carrier gas (helium) flow rate was 1.0 mL/min with the inlet splitter set.
at 22.9:1. Cholestanol, desmosterol, lathosterol, campasterol, and sitosterol were separated and expressed as ratios in $\mu$mol/mmol of cholesterol, which were measured in each sample by GC-FID. Individual sterols and stanols were identified by using authenticated standards (Sigma-Aldrich Canada).

**Statistical analysis**

We performed a power calculation using PROC POWER ($\alpha = 0.05$ and $\beta = 0.80$; SAS Institute) with the use of the average reduction in LDL cholesterol ($-0.34 \text{mmol/L}$) and variance (SD = $\pm 1.05 \text{mmol/L}$) due to PS consumption from Demonty et al. (2). From this power calculation, we determined that $\geq 17$ subjects were needed. However, a goal of 100 participants, 50 HS and 50 LS, was originally planned to investigate contributors to variability in response. Enrollment was stopped at 71 participants due to the difficulty with recruitment and because both HS ($n = 27$) and LS ($n = 44$) groups exceeded the required size for the main outcome. By using a crossover design, endpoints of the treatment and control phases were compared. The statistical analyses were performed by using SAS 9.2. Results are expressed as estimated least-squares means ± SEMs for all values. Differences in baseline characteristics based on synthesis group (HS or LS) were analyzed by SAS GLM with sex as a fixed factor. Effects of treatment were analyzed by the SAS MIXED procedure. Sequence, sex, and BMI (due to differences by synthesis group at baseline) were included in the model as fixed factors; site and participant were included as random factors, with participants repeated by period. Synthesis and treatment by synthesis were also included as fixed factors when investigating the impact of high or low baseline L:C ratios. Significant treatment by synthesis effects were examined by the SAS SLICE function, with Bonferroni correction for the number of slices. Treatment effect sizes by synthesis, from significant 1-factor treatment by synthesis interactions, were compared by $t$ test or ANOVA by using differences in mixed-model least-squares means summary statistics for the treatment effect slices and Tukey-Kramer adjustment for multiple comparisons. The OR of responding to PS consumption with cholesterol lowering was calculated for LS vs. HS participants by using SAS LOGISTIC. Participants were classified as “responders” if they had a reduction in TC or LDL cholesterol after PS consumption compared with placebo that was greater than the mean cholesterol lowering of all ($n = 63$) participants. Significance was set at $P < 0.05$ for all analyses.

**RESULTS**

**Baseline characteristics**

Sixty-three individuals ($n = 24$ HS, $n = 39$ LS) completed the 2-period trial and were included in all analyses (Figure 1). One participant withdrew without explanation, 3 participants were asked to leave the trial due to inability to make minimum visits to the research center for meals, and 4 participants were excluded before sample analysis: 1 due to admitted consumption of PS products throughout the trial, 1 due to illness and subsequent medication, and 2 due to large changes in physical activity during the trial. Baseline characteristics of the participants who completed the trial are presented in Table 1. Participants in the HS group, by design, possessed higher serum L:C ratios ($P < 0.0001$) than did LS participants. Although there were more than twice as many women in the LS group ($n = 27$ women) compared with the HS group ($n = 12$ women), the group sizes were different and the distribution of sexes between the HS and LS groups was not significant ($P = 0.1821$), when analyzed by a 2-tailed Fisher’s exact test. HS participants also had higher body weight ($P = 0.007$), BMI ($P = 0.0016$), and triglyceride concentrations ($P = 0.0042$) and had lower HDL cholesterol.

**FIGURE 1** Trial flowchart. HS, high endogenous cholesterol synthesis; L/C, lathosterol-to-cholesterol; LS, low endogenous cholesterol synthesis.
Effects of PS consumption on serum lipids and NCSs

The consumption of 2 g of PS/d for 28 d reduced TC in all participants (−0.25 ± 0.05 mmol/L; \( P < 0.0001 \)) compared with the placebo (Table 2). LDL-cholesterol concentration was reduced by PS consumption across all participants (−0.17 ± 0.04 mmol/L; \( P < 0.0002 \)) compared with the placebo. However, significant heterogeneity in the extent of lowering of both TC and LDL cholesterol was seen in the trial population. HDL-cholesterol concentration decreased by PS consumption across all participants (0.04 ± 0.02 mmol/L; \( P < 0.0414 \)) compared with the placebo. Serum campesterol (\( P < 0.0001 \)) and sitosterol (\( P < 0.0001 \))-to-cholesterol ratios increased across all the participants after PS consumption compared with the placebo, indicating compliance to the diets. Across all trial participants, lathosterol (\( P = 0.5395 \)) and desmosterol (\( P = 0.9197 \))-to-cholesterol ratios did not change after PS consumption compared with placebo. Trends toward a reduction in triglyceride concentrations (\( P = 0.0861 \)) and cholesterol-to-cholesterol ratio (\( P = 0.0651 \)) were seen after PS consumption compared with placebo.

### TABLE 2
Changes in lipids and noncholesterol sterols after plant sterol consumption

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<thead>
<tr>
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<th>( \Delta ) Least-squares mean (treatment − placebo)</th>
<th>( P )</th>
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<tr>
<td></td>
<td>All participants (( n = 63 ))</td>
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<tr>
<td></td>
<td>HS participants (( n = 24 ))</td>
<td>LS participants (( n = 39 ))</td>
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<tr>
<td>TC, mmol/L</td>
<td>(-0.25 ± 0.05)</td>
<td>(-0.09 ± 0.09)</td>
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<tr>
<td>LDL cholesterol, mmol/L</td>
<td>(-0.17 ± 0.04)</td>
<td>(-0.05 ± 0.07)</td>
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<td>HDL cholesterol, mmol/L</td>
<td>(-0.04 ± 0.02)</td>
<td>(-0.04 ± 0.03)</td>
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<tr>
<td>Triglycerides, mmol/L</td>
<td>(-0.10 ± 0.06)</td>
<td>(-0.02 ± 0.09)</td>
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<tr>
<td>Desmosterol, ( \mu )mol/mmol cholesterol</td>
<td>(-0.002 ± 0.02)</td>
<td>(-0.01 ± 0.03)</td>
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<tr>
<td>Lathosterol, ( \mu )mol/mmol cholesterol</td>
<td>(+0.03 ± 0.04)</td>
<td>(-0.01 ± 0.06)</td>
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<tr>
<td>Campesterol, ( \mu )mol/mmol cholesterol</td>
<td>(+1.23 ± 0.07)</td>
<td>(+0.98 ± 0.12)</td>
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<tr>
<td>Sitosterol, ( \mu )mol/mmol cholesterol</td>
<td>(+0.39 ± 0.03)</td>
<td>(+0.34 ± 0.05)</td>
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<tr>
<td>Cholesterol, ( \mu )mol/mmol cholesterol</td>
<td>(-0.03 ± 0.02)</td>
<td>(-0.04 ± 0.03)</td>
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\(^1\) All values are differences in estimated least-squares means ± SEMs. \( P \) values were derived by using an SAS MIXED model. HS, high endogenous cholesterol synthesis; LS, low endogenous cholesterol synthesis; N/A, not applicable; TC, total cholesterol.

\(^2\) Simple effects of treatment tested for synthesis groups by using SAS SLICE function when treatment \( \times \) synthesis interaction was significant. Values with different symbols had significantly different treatment effect sizes (HS vs. LS), derived by \( t \) test (\( P < 0.05 \)).
Influence of endogenous cholesterol synthesis on the response of lipids and NCSs to PS consumption

When stratified by endogenous cholesterol synthesis phenotype, PS consumption reduced TC and LDL cholesterol for LS participants (−0.40 ± 0.07 mmol/L, P < 0.0001, and −0.29 ± 0.05 mmol/L, P < 0.0001, for TC and LDL cholesterol, respectively), whereas no changes were observed in TC or LDL cholesterol in HS participants (−0.09 ± 0.09 mmol/L, P = 0.2843, and −0.05 ± 0.07 mmol/L, P = 0.4917, for TC and LDL cholesterol, respectively) compared with placebo (Table 2, Figure 2). Endogenous cholesterol synthesis phenotype had an interactive effect with PS consumption in relation to the campesterol-to-cholesterol ratio. The campesterol-to-cholesterol ratio increased in both HS and LS participants (+0.98 ± 0.12, P < 0.0001, and +1.49 ± 0.09, P < 0.0001, for HS and LS groups, respectively), but LS participants had higher campesterol-to-cholesterol ratios after PS consumption than did HS participants (4.15 ± 0.19 vs. 3.10 ± 0.23; P < 0.05).

Correlations between baseline characteristics, NCSs, and response of lipids to PS consumption

Correlations between baseline characteristics and changes in TC and LDL cholesterol after PS consumption are shown in Table 3. Baseline L:C ratio, which was the final determining inclusion criterion in this trial, correlated positively with baseline body weight (r = 0.481, P < 0.001), BMI (r = 0.512, P < 0.001), and triglyceride concentrations (r = 0.359, P < 0.01) and negatively with TC (r = −0.250, P < 0.05), HDL cholesterol (r = −0.475, P < 0.01), campesterol (r = −0.288, P < 0.05), sitosterol (r = −0.409, P < 0.01), and cholesterol (r = −0.483, P < 0.001). Changes in TC after PS consumption compared with placebo positively correlated with baseline L:C ratio (r = 0.284, P < 0.05), body weight (r = 0.241, P < 0.05), and BMI (r = 0.294, P < 0.05) and negatively correlated with HDL cholesterol (r = −0.280, P < 0.05).

Responsiveness of TC and LDL cholesterol to PS consumption according to endogenous synthesis

The odds of LS participants responding to PS consumption were 4.25 (95% CI: 1.242, 14.556; P = 0.0211) for TC and 3.36 (95% CI: 1.112, 10.161; P = 0.0317) for LDL cholesterol, which were higher than for HS participants. Responsiveness was defined as a reduction in cholesterol equal or greater than the overall unadjusted mean cholesterol reduction for all 63 participants (≥0.28 mmol/L for TC and ≥0.21 mmol/L for LDL cholesterol).

DISCUSSION

The presented data show that the L:C ratio can be used to predict an individual’s response to PS consumption a priori. LS participants were >3 times as likely to respond to PS consumption with cholesterol lowering compared with HS participants. This observation supports the hypothesis that an individual’s response in circulating cholesterol biomarkers to PS intervention is determined by his or her degree of endogenous cholesterol synthesis.

Our findings expand on those of Rideout et al. (24), who showed that nonresponse of LDL cholesterol to PS consumption in mildly hypercholesterolemic individuals was associated with high basal fractional cholesterol synthesis, and Carr et al. (20), who demonstrated a strong negative correlation between baseline lathosterol concentrations and percentage of reductions in LDL cholesterol in adults consuming PS. However, our data provide new information indicating that additional modifying factors remain to be determined, as seen by the heterogeneity of each individual’s LDL-cholesterol lowering as a response to PS intervention. Although the overwhelming majority of individuals who showed the LS pattern strongly responded to PS consumption with cholesterol lowering, a few LS individuals did not (Figure 2). Moreover, some of the HS participants showed a strong LDL-lowering response to PS, whereas others demonstrated increased LDL-cholesterol concentrations. Therefore, although low endogenous cholesterol synthesis seems to be a good predictor of the degree of LDL-cholesterol lowering subsequent to PS intervention, high endogenous cholesterol synthesis does not rule out PS effectiveness in all individuals (Figure 2).

At baseline, HS participants had higher body weight, BMI, and triglyceride concentrations and lower HDL-cholesterol concentrations than did LS participants. This constellation of traits is reminiscent of a metabolic syndrome phenotype (29). Metabolic syndrome itself (30, 31), and individual traits of metabolic syndrome such as elevated visceral fat (32), low HDL cholesterol (33), and insulin resistance (34), have all been linked to elevated whole-body cholesterol synthesis and depressed cholesterol absorption. A proposed mechanism for the link between metabolic syndrome with elevated cholesterol synthesis is insulin resistance, where elevated insulin concentrations drive cholesterol synthesis via activation of sterol regulatory element binding transcription factor 2 (35). Our data would suggest that many individuals with high cholesterol synthesis, which may be seen in metabolic syndrome, may benefit less from PS consumption. This might explain why the effects of PS consumption for individuals with metabolic syndrome, which, on average, shows cholesterol lowering, have been inconsistent (11), with some trials failing to show benefits (36, 37). Our recruitment criteria required individuals with fasting glucose values <6.1 mmol/L in an attempt to reduce the confounding of glucose dysregulation on our study outcomes but did not specifically exclude individuals who might meet one of the various definitions of metabolic syndrome (29). Our data suggest that the metabolic characteristic most likely to

![FIGURE 2](https://academic.oup.com/ajcn/article-abstract/101/3/432/4569395/436-MACKAY-ET-AL) Individual changes in LDL cholesterol in response to plant sterol consumption compared with control stratified by endogenous cholesterol synthesis phenotype. HS, high endogenous cholesterol synthesis; LDL-C, LDL cholesterol; LS, low endogenous cholesterol synthesis.
be modulating the response to PS consumption is endogenous cholesterol synthesis, building on the findings of Rideout et al. (24) and others (20, 22, 23). Because insulin resistance is associated with elevated cholesterol synthesis, the use of PS products by individuals with insulin resistance requires re-evaluation.

In this trial population, PS consumption led to a small (−0.04 ± 0.02 mmol/L) reduction in HDL cholesterol, which was seen in both HS and LS participants. Although small, given the known cardioprotective benefits of HDL cholesterol (38), reductions in HDL cholesterol are not desirable and in this circumstance would be even less beneficial for HS individuals who did not receive the TC and LDL-cholesterol lowering due to PS consumption. Generally, PS consumption is not associated with HDL-cholesterol lowering (39); however, some evidence suggests that the effect of PS may be dependent on baseline HDL cholesterol, with lower baseline HDL-cholesterol concentrations increasing and higher concentrations decreasing with PS consumption (40).

The cholestanol-to-cholesterol ratio represents a surrogate measure of cholesterol absorption, which can be used during PS feeding, unlike sitosterol or campesterol (41). No association was observed in comparing this ratio with TC or LDL-cholesterol lowering after PS consumption, which is in agreement with our hypothesis that cholesterol synthesis, as opposed to cholesterol absorption, is a stronger determinant of PS responsiveness. Similarly, Houweling et al. (22) conducted a trial to access the impact of baseline plasma sitosterol and campesterol concentrations, both surrogate markers for cholesterol absorption, on response to PS in 82 mildly hypercholesterolemic men using a crossover format. Individuals with high or low plasma combined sitosterol and campesterol concentrations at baseline failed to show any difference in the magnitude of cholesterol lowering after 4 wk of PS consumption.

PS consumption caused an elevation in campesterol-to-cholesterol ratio in both HS and LS groups. However, a greater elevation in campesterol-to-cholesterol ratio was seen in the LS group than in the HS group. This interactive effect between endogenous cholesterol synthesis and PS consumption was not seen in the sitosterol-to-cholesterol ratio because LS participants already had higher sitosterol-to-cholesterol ratios than did HS participants at baseline. Typically, campesterol and sitosterol are the only markers used for compliance and are not used as cholesterol absorption surrogates in PS studies, because PS formulations always contain high amounts of both campesterol and sitosterol (41, 42).

Potential limitations of this trial include the unequal number of participants in the HS and LS groups, as well as the higher proportion of women in the LS group. However, both the HS and LS groups exceeded the number of participants required according to the a priori power analysis to detect an effect of PS consumption, and sex was included as a fixed factor in all applicable statistical analyses. This trial was designed to specifically interrogate the individuals with high or low L:C ratios and individuals between these L:C ratio extremes were not enrolled. However, participants with mean endogenous cholesterol synthesis would be expected to show a range of responsiveness somewhere between the HS and LS groups seen in our trial. Within the LS group, and even more so in the HS group, there was a wide range of responsiveness seen for TC and LDL cholesterol in response to PS consumption. This range of responsiveness suggests that additional factors that modify response to PS consumption, beyond endogenous cholesterol synthesis, remain to be determined. These factors likely have a genetic underpinning, because some studies have shown associations between single nucleotide polymorphisms and responsiveness to PS consumption (43–45). Therefore, investigation of genetic polymorphism in genes in cholesterol metabolism pathways should be undertaken to see if any polymorphisms are associated with the degree of lipid responsiveness to PS consumption.

In summary, an individual’s endogenous cholesterol synthesis, as determined by the circulating L:C ratio, could be a biomarker predicting an individual’s response of cholesterol biomarkers to PS intervention. Our data shed light on the results reported in previous trials, in which large interindividual variation hampered the ability to decisively determine whether and to what degree PS consumption contributes to decreased TC and LDL cholesterol and therefore reduced cardiovascular disease risk (2). These findings can be used in the context of personalized nutritional recommendations to help predict response to PS consumption.
consumption and thereby maximize efficacy in reducing cardiovascular disease risk.

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The authors’ responsibilities were as follows—DSM: was the principal manuscript author and was involved in the development of the overall research plan, conducted the majority of the sample analysis, and performed the statistical analysis; SKG: was involved in conducting the human clinical trial at the USDA Beltsville site and contributed to the preparation of the manuscript; PKE: contributed to the development of the overall research plan and contributed to the preparation of the manuscript; DBJ: was the lead investigator for the human clinical trial at the USDA Beltsville site and revised the final manuscript; and PJHJ was the principal investigator on the research program, contributed to the development of overall research plan, and contributed to the preparation of the manuscript. DSM, SKG, PKE, and DBJ had no conflicts of interest to declare. PJHJ reported receiving grants from Danone, Enzymotec, and Unilever, which all have plant sterol-containing products. PJHJ is Past President of the Danone Institute of Canada and serves as President of Nutritional Fundamentals for Health, Inc., which markets plant sterols among other nutraceuticals. Unilever Canada Inc. played no role in the design, implementation, or analysis of the trial or in the interpretation of the data.

REFERENCES


