CYP7A1-rs3808607 and APOE isoform associate with LDL cholesterol lowering after plant sterol consumption in a randomized clinical trial

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ABSTRACT

Background: The benefits of plant sterols (PSs) for cholesterol lowering are hampered by large heterogeneity across individuals, potentially because of genetic polymorphisms.

Objective: We investigated the impact of candidate genetic variations on cholesterol response to PSs in a trial that recruited individuals with high or low endogenous cholesterol synthesis, estimated by lathosterol to cholesterol (L:C) ratio.

Design: Mildly hypercholesterolemic adults preselected as possessing either high endogenous cholesterol synthesis (n = 24; mean ± SEM: L:C ratio = 2.03 ± 0.39 μmol/mmol) or low endogenous cholesterol synthesis (n = 39; mean ± SEM: L:C ratio = 0.99 ± 0.28 μmol/mmol) consumed 2 g PS/d or a placebo for 28 d by using a dual-center, single-blind, randomized crossover design. Cholesterol synthesis and change in cholesterol absorption were measured with stable isotopic tracers. Candidate single-nucleotide polymorphisms and apolipoprotein E (APOE) isoform were assessed by TaqMan genotyping assay.

Results: The cholesterol fractional synthesis rate was higher (P < 0.001) in participants with high endogenous cholesterol synthesis (mean ± SEM: placebo: 9.16% ± 0.47%; PSs: 9.74% ± 0.47%) than in participants with low endogenous cholesterol synthesis (mean ± SEM: placebo: 5.72% ± 0.43%; PS: 7.10% ± 0.43%). Low-density lipoprotein (LDL) cholesterol lowering in response to PSs was associated with individuals’ genotypes. Cholesterol 7α-hydroxylase (CYP7A1-rs3808607) T/T homozygotes showed no LDL cholesterol lowering (mean ± SEM: −0.05 ± 0.07 mmol/L, P = 0.9999, n = 20), whereas the presence of the G-allele associated with LDL cholesterol response in a dose-dependent fashion (mean ± SEM G/T: −0.22 ± 0.06 mmol/L, P = 0.0006, n = 35; G/G: −0.46 ± 0.12 mmol/L, P = 0.0009, n = 8). Similarly, APOE ε3 carriers (mean ± SEM: −0.13 ± 0.05 mmol/L, P = 0.0370, n = 40) responded less than APOE ε4 carriers (mean ± SEM: −0.31 ± 0.07 mmol/L, P < 0.0001, n = 23). Moreover, genotypes CYP7A1-rs3808607 TT/APOE ε3 was associated with nonresponsiveness (mean ± SEM: +0.09 ± 0.08 mmol/L, P = 0.9999, n = 14). rs5882 in cholesterol ester transfer protein (CETP) and rs4148217 in ATP-binding cassette subfamily G member 8 (ABCG8) did not associate with LDL cholesterol lowering. Cholesterol absorption decreased as a result of PS consumption, but this decrease was not related to circulating LDL cholesterol concentrations, cholesterol synthesis phenotype, or genotypes.

Conclusion: CYP7A1-rs3808607 and APOE isoform are associated with the extent of reduction in circulating LDL cholesterol in response to PS consumption and could serve as potential predictive genetic markers to identify individuals who would derive maximum LDL cholesterol lowering with PS consumption. The trial was registered at clinicaltrials.gov as NCT01131832.

Keywords: cholesterol, gene-nutrient interactions, LDL cholesterol, nutrigenetics, plant sterols

INTRODUCTION

Many people currently consume functional food products containing plant sterols (PSs) in hopes of lowering their LDL cholesterol concentrations and preventing cardiovascular disease (CVD). It is well established that PSs have cholesterol-lowering properties, likely achieved through reduced intestinal cholesterol absorption (1). Clinical trials typically demonstrate mean LDL cholesterol lowering in the 5–15% range in response to 2–3 g PS consumption/d (2–4).

Within clinical trials, interindividual variability in LDL cholesterol lowering in response to PS consumption exists (5–7), with response ranging from better than average to nonresponse or even adverse response (8–10). Therefore, some people may be using plant sterols and deriving no benefit or may even be harmed in terms of circulating LDL cholesterol concentrations.

1Supported by Canadian Institutes of Health Research funding reference number (FRN) 83894. Placebo and plant sterol margarines were provided by Unilever Canada Inc.
2Supplemental Tables 1–4 and Supplemental Figure 1 are 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.
*To whom correspondence should be addressed. E-mail: peter.jones@umanitoba.ca.
7Abbreviations used: ABCG8, ATP-binding cassette subfamily G member 8; APOE, apolipoprotein E; CETP, cholesteryl ester transfer protein; CVD, cardiovascular disease; CYP7A1, cholesterol 7α-hydroxylase; FSR, fractional synthesis rate; HS, high endogenous cholesterol synthesis; L:C, lathosterol-to-cholesterol; LS, low endogenous cholesterol synthesis; PS, plant sterol; SNP, single-nucleotide polymorphism.

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Using a lathosterol-to-cholesterol (L:C) ratio to estimate endogenous cholesterol synthesis, we have previously shown that individuals with low endogenous cholesterol synthesis (LS) respond better to PS consumption than those with higher synthesis (11). Distinct interindividual responses to PS consumption have been shown to be reproducible in individuals across repeated PS interventions (10), in which some participants consistently lowered their LDL cholesterol when ingesting PSs, whereas others did not. These strong correlations between participants’ repeated responses to PSs indicate a potentially genetic determinant of responsiveness (8, 12, 13).

Very few single-nucleotide polymorphisms (SNPs) have been associated with variability in response to PS consumption; they include rs3808607 in cholesterol 7 α-hydroxyxylase (CYP7A1) (14), rs5882 in cholesteryl ester transfer protein ( CETP) (15), rs4148217 in ATP-binding cassette subfamily G member 8 (ABCG8) (16), and apoliprotein E (APOE) isofrom (17). To our knowledge, no studies have reported successfully replicating an SNP association with cholesterol-lowering response to PSs in a clinical trial.

Our objective was to investigate the impact of these candidate genetic variations on LDL cholesterol lowering resulting from PS consumption in a trial of individuals with high or low cholesterol synthesis (11). This trial yielded a wide range of responsiveness and possessed a crossover design, which balances the genetic diversity in the placebo and PS treatments, making it ideal for investigating the impact of genetic polymorphisms on cholesterol lowering in response to PS consumption. The trial also used stable isotopic tracers to measure cholesterol synthesis and change in cholesterol absorption resulting from PS consumption, which we report within for the first time.

METHODS

A dual-center, randomized, single-blind, crossover, placebo-controlled nutritional trial was designed with two 28-d periods with a minimum of a 4-wk washout between periods during which the participants consumed their habitual diets. During the PS period, participants consumed 2 g PS/d in margarine under supervision of a trial coordinator. This trial was conducted at the Nutrition 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. Full trial design details have been reported previously (11). Individuals with high endogenous cholesterol synthesis (HS) or LS were selectively recruited into this trial by using the L:C ratio as a surrogate marker of cholesterol synthesis.

Participants

Mildly hypercholesterolemic individuals (42 female, 29 male) aged 30–75 y were recruited from Winnipeg, Manitoba, Canada, and Beltsville, Maryland, as has been reported previously (11). The trial was conducted according to the principles expressed in the Declaration of Helsinki. Trial procedures were approved by the University of Manitoba’s Biomedical Research Ethics Board (protocol no. B2007-073). All participants provided written informed consent. Trial procedures were approved by the University of Manitoba’s Biomedical Research Ethics Board (protocol no. B2007-073) and MedStar Health Research Institute’s institutional review board (protocol no. 2010–409). The trial was registered at clinicaltrials.gov as NCT01131832.

Blood sampling and analysis

Overnight fasting blood samples were collected on days 1 and 2 and on days 24–28 of each trial period. Blood sampling, lipid analysis, and noncholesterol sterol analysis protocols have been reported previously (11). On day 24 of each trial period, a fasting baseline blood sample (0 h) was collected before administration of a 75-mg oral dose of [3,4,13C]cholesterol (Cambridge Isotope Laboratories Inc.). Fasting blood samples were then taken daily over the following 96 h to measure change in cholesterol absorption. The [13C]cholesterol was dissolved in 5 g warmed margarine and spread on a half of a small bun for the participants to consume (5, 18). Endogenous cholesterol synthesis was measured by deuterium incorporation and reported as cholesterol fraction synthesis rate (FSR) (19–21). Twenty-four hours before the end of each experimental period, participants were given an oral dose of deuterium oxide ( ~30 mL, 0.7 g estimated body water/kg) before breakfast as a tracer for measuring cholesterol FSRs according to previously established procedures (18, 22, 23).

Genomic DNA was extracted from white blood cells by using a column-based DNA extraction kit (DNeasy Blood and Tissue Kit; QIAGEN Sciences) according to the manufacturer’s instructions. The concentration and integrity of the genomic DNA were assessed by microvolume spectrophotometer (NanoDrop 2000; Thermo Fisher Scientific). DNA samples were genotyped by TaqMan SNP genotyping assays [CYP7A1-rs3808607, assay identification (ID) C2749212120; CETP-rs5882, assay ID C79005710; ABCG8-rs4148217, assay ID C37506110; APOE-rs7412, assay ID C2749212120; APOE-rs429358, assay ID C308479320; Life Technologies] on a StepOnePlus Real-Time PCR System (Applied Biosystems; Life Technologies).

Statistical analysis

Using a crossover design, endpoints of the treatment and placebo periods were compared. Statistical analyses were performed with SAS version 9.4 (SAS Institute). Results are expressed as estimated least squares means ± SEMs for all values. Differences in baseline characteristics based on genotype were analyzed by the SAS GLM procedure with sex as a fixed factor. Effects of treatment were analyzed by the SAS MIXED procedure. Sex and BMI (due to difference by baseline L:C ratios) were included as a random factor, with participant repeated by period. Synthesis and treatment by synthesis were also included as fixed factors when the impact of high or low baseline L:C ratios were investigated. Genotype and treatment by genotype were included as fixed factors when the impact of genotype was investigated. Statistically significant treatment-by-synthesis or treatment-by-genotype effects were examined by the SAS SLICE function, with Bonferroni correction for the number of slices. Treatment effect sizes by synthesis or by genotype, from statistically significant treatment-by-synthesis or treatment-by-genotype interactions, were compared by t test or ANOVA by using the difference in mixed-model
least squares means summary statistics for the treatment effect slices, with Tukey-Kramer adjustment for multiple comparisons. Pearson $\chi^2$ tests were used to test the distribution of genotypes between HS and LS groups. Statistical significance was set at $P < 0.05$ for all analyses, with adjustments as above. The power calculation for the trial has been reported previously (11).

**RESULTS**

**Baseline characteristics**
Sixty-three individuals ($n = 24$ HS, $n = 39$ LS) completed the 2-period crossover trial design and were genotyped for candidate SNPs. Baseline characteristics of the trial population ($n = 63$) who completed the trial have been published previously (11). All participants ($n = 63$) were successfully genotyped for each SNP of interest. Not all participants agreed to participate in, did not completely consume the required tracer for, or did not make all the required blood sampling days for the isotopic tracer measurements. Therefore, cholesterol FSR ($n = 56$) from deuterium incorporation and change in cholesterol absorption ($n = 53$) using $[13C]$cholesterol were not obtained for all participants. Participants reported no change in physical activity, and no differences were observed in body weight during the trial.

**Genotype distributions**
The distribution of each of the SNPs CYP7A1-rs3808607, CETP-rs5882, and ABCG8-rs4148217 and the APOE isoform between HS and LS participants is shown in Supplemental Table 1. CYP7A1-rs3808607 was found to be unequally distributed between HS and LS participants (Pearson $\chi^2$, $P = 0.011$), with proportionally more G-alleles in the LS group and more T-alleles in the HS group. For ABCG8-rs4148217, only 5 participants were homozygous for the minor C-allele, and therefore we grouped C/C and C/A carriers together in subsequent analyses. There were no APOE $\varepsilon$2/2 participants, and only 4 participants were had APOE $\varepsilon$2/3. Therefore, $\varepsilon$2/3 and $\varepsilon$3/3 ($n = 36$) participants were grouped and considered APOE $\varepsilon$3 ($n = 40$), whereas participants with either $\varepsilon$4/3 ($n = 18$) or $\varepsilon$4/4 ($n = 5$) were grouped and considered APOE $\varepsilon$4 ($n = 23$).

**Serum lipids**
The impact of consuming 2.0 g PS/d for 28 d on LDL cholesterol has been reported previously (11). Briefly, across all participants, LDL cholesterol was reduced after PS consumption compared with placebo ($−0.17 \pm 0.04$ mmol/L). However, when further stratified, HS individuals did not show LDL cholesterol lowering ($−0.05 \pm 0.07$ mmol/L, $n = 24$), whereas individuals with low synthesis showed reductions ($−0.29 \pm 0.05$ mmol/L, $n = 39$) (11). When the genotypes of interest were associated individually with LDL cholesterol response to PS consumption, without inclusion of synthesis groups in the model, CYP7A1-rs3808607 genotypes ($P$-interaction = 0.0146) (Figure 1A) and APOE isoforms ($P$-interaction = 0.0447) (Figure 1B) associated with LDL cholesterol lowering in response to PS consumption. CETP-rs5882 ($P$-interaction = 0.6007) and ABCG8-rs4148217 ($P$-interaction = 0.1730) did not associate with LDL cholesterol lowering (data not shown).

**Specifically,** an allelic dose effect of CYP7A1-rs3808607 was observed in LDL cholesterol response to PS consumption, where T/T homozygotes ($−0.05 \pm 0.07$, $P = 0.9999$, $n = 20$) failed to show a reduction in LDL cholesterol concentrations after PS consumption, whereas G/T and G/G carriers achieved reductions in LDL cholesterol dependent on the G-allele presence (G/T: $−0.22 \pm 0.06$ mmol/L, $P = 0.0006$, $n = 35$; G/G: $−0.46 \pm 0.12$ mmol/L, $P = 0.0009$, $n = 8$) (Table 1). The CYP7A1-rs3808607 genotype may be responsible for the association of cholesterol synthesis phenotype with cholesterol lowering previously reported...
TABLE 1
Single-nucleotide polymorphisms associated with LDL cholesterol lowering in response to plant sterol consumption

<table>
<thead>
<tr>
<th>Gene and SNP genotype</th>
<th>ΔLDL-C, mmol/L</th>
<th>Simple effects by genotype</th>
<th>Treatment P</th>
<th>Treatment × genotype P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP7A1 rs3808607</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T (20)</td>
<td>−0.05 ± 0.07†</td>
<td>0.9999</td>
<td>&lt;0.0001</td>
<td>0.0146</td>
</tr>
<tr>
<td>G/T (35)</td>
<td>−0.22 ± 0.06**</td>
<td>0.0006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G (8)</td>
<td>−0.46 ± 0.12‡</td>
<td>0.0009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APOE isoform</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε3 (40)</td>
<td>−0.13 ± 0.05§</td>
<td>0.0370</td>
<td>0.0547</td>
<td>0.0466</td>
</tr>
<tr>
<td>ε4 (23)</td>
<td>−0.31 ± 0.07**</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 n in parentheses. P values are from SAS MIXED model (n = 63). Meas not sharing a common superscript letter are statistically significantly different based on t test or ANOVA (P < 0.05) with Tukey-Kramer adjustment. APOE, apolipoprotein E; CYP7A1, cholesterol 7 a-hydroxylase; SNP, single-nucleotide polymorphism; ΔLDL-C, change in LDL cholesterol.

2 Values are least squares means ± SEMs.

3 Mixed-model simple effects of treatment sliced by genotype by using SAS SLICE function when treatment and treatment by genotype were statistically significant (Bonferroni correction for number of slices).

(11), with more CYP7A1-rs3808607-T/T homozygotes in the HS group and the G-allele more common in the low synthesizers group (Supplemental Table 1). When synthesis phenotype and CYP7A1-rs3808607 genotype were combined in the same mixed model, both the synthesis (P-interaction = 0.1819) and CYP7A1-rs3808607 (P-interaction = 0.0530) and the 3-way treatment by synthesis by CYP7A1-rs3808607 interaction (P = 0.4075, data not shown) did not associate with LDL cholesterol lowering, indicating a potentially similar physiologic mechanism.

In response to PS consumption, participants with the APOE ε4 isoform had higher LDL cholesterol lowering (−0.31 ± 0.07, P < 0.0001, n = 23) than did APOE ε3 participants (−0.13 ± 0.05, P = 0.0370, n = 40) (Table 1). In contrast to observations for SNP CYP7A1-rs3808607, the effects of the APOE isoforms were independent of the endogenous cholesterol synthesis, indicating a fundamentally different physiologic mechanism. This is demonstrated by the fact that the APOE isoform (P-interaction = 0.0343) and synthesis phenotype (P-interaction = 0.0194) both continued to associate with LDL cholesterol lowering when combined in the same mixed model, with no 3-way interactive effect (P = 0.3907, data not shown).

The combination of CYP7A1-rs3808607 (P-interaction = 0.0406) and APOE isoform (P-interaction = 0.0163 for interaction) formed genotypes, which were associated with LDL cholesterol lowering by PSs (3-way interactive effect, P = 0.0737) (Figure 1C). In particular, the participants with genoset CYP7A1-rs3808607 T/T/APOE ε3 (±0.09 ± 0.08 mmol/L, P = 0.9999, n = 14) were the only genoset that did not benefit from LDL cholesterol lowering after PS consumption (Table 2).

All the patterns of responsiveness reported for LDL cholesterol were also seen for total cholesterol concentrations (data not shown). Baseline participant characteristics stratified by CYP7A1-rs3808607 and APOE isoform can be seen in Supplemental Table 2 and Supplemental Table 3, respectively. CYP7A1-rs3808607 T/T participants had a higher L:C ratio (P = 0.0196), body weight (P = 0.0147), and BMI (P = 0.0453) than T/G but not G/G participants. However, the increased BMI associated with CYP7A1-rs3808607 T/T, as well as BMI overall, was likely not involved in the association between CYP7A1-rs3808607 and LDL cholesterol lowering after PS consumption (Supplemental Figure 1A, B).

Stable isotopic assessment of cholesterol metabolism

Cholesterol FSR, measured by deuterium incorporation, was increased (P = 0.0001) by PS consumption (8.42% ± 0.31%) compared with placebo (7.44% ± 0.31%). Sex had an overall effect on FSR (P = 0.0308), with females (8.56% ± 0.36%) having higher cholesterol FSR than males (7.29% ± 0.43%). HS participants (placebo: 9.16% ± 0.47%, PS: 9.74% ± 0.47%) had higher FSR during the placebo (P = 0.0001) and PS periods (P = 0.0008) than LS participants (placebo: 5.72% ± 0.43%, PS: 7.10% ± 0.43%). Synthesis phenotype (P = 0.0365) influenced the percent change in cholesterol FSR after PS consumption compared with placebo. This effect was driven by the LS group (+25.6% ± 9.6%, P = 0.0097), whereas the HS participants (+9.3% ± 9.9%, P = 0.3505) did not increase cholesterol FSR after PS consumption. Cholesterol FSR was not associated with CYP7A1-rs3808607 (TT: 7.89% ± 0.72%, G/T: 7.70% ± 0.57%, G/G: 6.60% ± 0.99%, P = 0.2177) or APOE isoform (ε3: 7.41% ± 0.55%, ε4: 7.85% ± 0.69%, P = 0.5541), with no genotype-by-treatment interactions (P = 0.7721). The percentage change in cholesterol FSR after PS consumption compared with placebo also did not associate with any of the genotypes (data not shown). Change in cholesterol FSR did correlate with cholesterol FSR values from the placebo period (r = −0.5217, P < 0.0001, n = 56), as would be expected given the influence of synthesis phenotype on change in cholesterol FSR reported above.

Cholesterol absorption, measured by the change in [13C] cholesterol absorption, was reduced (−41.10% ± 2.08%, P < 0.0001) in all participants after PS consumption compared with placebo. No difference in change in cholesterol absorption was seen between synthesis groups (−36.05% ± 5.03% and −41.20% ± 4.27% for HS and LS groups, respectively, P = 0.2332) or between genotypes. The change in LDL cholesterol after PS consumption compared with placebo did not correlate with change in cholesterol absorption, cholesterol FSR from the placebo period, or change in cholesterol FSR after PS consumption compared with placebo (Supplemental Table 4). Change in cholesterol absorption did correlate with change in cholesterol FSR (r = −0.33, P = 0.0203, n = 49), suggesting that as cholesterol absorption was decreased by PS consumption, a reciprocal increase in cholesterol FSR occurred.
improving cholesterol absorption, which would enhance the gene expression and subsequently increases bile acid compared with the T-allele, the G-allele at homeostasis (24). De Castro-Oros et al. (14) concluded that cholesterol in the body and has a large influence on cholesterol involved in the rate-limiting step of the classic bile acid synthesis, which codes for cholesterol 7-

Table 2: APOE and CYP7A1-rs3808607 interactions associate with LDL cholesterol lowering in response to plant sterol consumption

<table>
<thead>
<tr>
<th>APOE Isoform</th>
<th>CYP7A1-rs3808607</th>
<th>n</th>
<th>ΔLDL-C, mmol/L</th>
<th>Simple effects by genotype, ³P</th>
<th>Treatment P</th>
<th>Treatment × genotype (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>e3</td>
<td>T/T</td>
<td>14</td>
<td>-0.09 ± 0.08⁴</td>
<td>0.9999</td>
<td>≤0.0001</td>
<td>Treatment × CYP7A1 (0.0406)</td>
</tr>
<tr>
<td>e3</td>
<td>T/G</td>
<td>20</td>
<td>-0.21 ± 0.07⁴b</td>
<td>0.0246</td>
<td>Treatment × APOE (0.0163)</td>
<td></td>
</tr>
<tr>
<td>e3</td>
<td>G/G</td>
<td>6</td>
<td>-0.38 ± 0.13b</td>
<td>0.0234</td>
<td>Treatment × APOE × rs3808607 (0.0737)</td>
<td></td>
</tr>
<tr>
<td>e4</td>
<td>T/T</td>
<td>6</td>
<td>-0.37 ± 0.13b</td>
<td>0.0306</td>
<td>All other 2-way interactive effects were not statistically significant</td>
<td></td>
</tr>
<tr>
<td>e4</td>
<td>T/G</td>
<td>15</td>
<td>-0.24 ± 0.08b</td>
<td>0.0234</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e4</td>
<td>G/G</td>
<td>2</td>
<td>-0.67 ± 0.22b</td>
<td>0.0216</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1P values from SAS MIXED model (n = 63). Means not sharing a common superscript letter are statistically significantly different tested by ANOVA (P < 0.05) with Tukey-Kramer adjustment. APOE, apolipoprotein E; CYP7A1, cholesterol 7 α-hydroxylase; ΔLDL-C, change in LDL cholesterol.
2Values are least squares means ± SEMs.
3Mixed-model simple effects of treatment sliced by genoset by using SAS SLICE function when treatment and treatment by genoset were statistically significant (Bonferroni correction for 6 slices).

Discussion

Considerable genetic heterogeneity in the response of LDL cholesterol to PS consumption was demonstrated within our trial. The present work shows for the first time, to our knowledge, the dual association of APOE isoform and CYP7A1-rs3808607 with the response of LDL cholesterol after PS consumption (Figure 1A–C). These secondary findings build on our previous results, which have shown that the response of total and LDL cholesterol to PS consumption is influenced by endogenous cholesterol synthesis, which was the primary objective of this trial (11).

The CYP7A1-rs3808607 association with LDL cholesterol lowering and PS consumption demonstrated an allelic dose effect, from a nonresponse in the T/T carriers to an increasing response with each G-allele. CYP7A1-rs3808607 may be a major contributing factor to the association between the HS and LS phenotypes (11) and the LDL cholesterol response to PS consumption. This observation is supported by unequal distribution of CYP7A1-rs3808607 across the synthesis phenotypes, with more T-allele in the HS phenotype group and more G-allele in the LS phenotype group.

Our results expand on findings by De Castro-Oros et al. (14), by showing an association between CYP7A1-rs3808607 and LDL cholesterol lowering and by replicating the association of CYP7A1-rs3808607 with total cholesterol. Our association of CYP7A1-rs3808607 with both total and LDL cholesterol showed an allelic dose effect, whereas in the De Castro-Oros et al. trial, all minor allele carriers were compared with homozygous major allele carriers. In our participants, BMI was higher in CYP7A1-rs3808607 T/T carriers than in T/G carriers. However, as seen in Supplemental Figure 1A, B, BMI was not as strongly associated with response as the CYP7A1-rs3808607 genotype.

CYP7A1-rs3808607 is in the promoter region of the gene, which codes for cholesterol 7α hydroxylase, the enzyme involved in the rate-limiting step of the classic bile acid synthesis pathway. Bile acid synthesis is the major metabolic fate of cholesterol in the body and has a large influence on cholesterol homeostasis (24). De Castro-Oros et al. (14) concluded that compared with the T-allele, the G-allele at CYP7A1-rs3808607 enhances gene expression and subsequently increases bile acid synthesis. This increased synthesis expands the bile acid pool, improving cholesterol absorption, which would enhance the cholesterol-lowering properties of PS consumption in G-allele carriers.

The influence of the APOE isoform was evident in the response of LDL cholesterol concentrations to PS consumption, and this influence was independent of the synthesis phenotype. Of interest was the elevated LDL cholesterol–lowering response of the APOE e4 individuals (n = 24) to PS consumption and the apparent ability of the e4 isoform to override the nonresponsive T/T genotype in CYP7A1-rs3808607. Only within the e3 individuals is the association with the CYP7A1-rs3808607 genotype and LDL cholesterol response seen (Figure 1). Unlike what could be expected from Figure 1, no 3-way interaction (P = 0.0737) of the APOE isoform, CYP7A1-rs3808607, and PS consumption was seen with LDL cholesterol reduction. It is important to note that the trial size (n = 63) could have limited the ability to detect such higher order interactive effects.

The association between APOE e4 isoform with enhanced PS-induced cholesterol lowering has been proposed by Miettinen and Vanhanen (25) and Vanhanen et al. (26), who both demonstrated enhanced cholesterol lowering in e4 individuals after different plant stanol consumption regimens. However, Geelen et al. (27) did not see a difference in cholesterol lowering after PS consumption between APOE e4 isoform and e3 isoform individuals in a clinical trial that specifically recruited e4 and e3 participants. Similarly, Lottenberg et al. (15) and Plat and Mensink (28) also did not see an association between APOE isoform and cholesterol lowering after PS consumption. Sanchez-Muniz et al. (17) suggested that APOE e4 isoform carriers were not responders to PS consumption. Clearly, our data support the assertion that APOE e4 individuals respond well to PS consumption, as do e3 isoform carriers, except for those e3 carriers who were also T/T carriers for CYP7A1-rs3808607.

A potential mechanism for the enhanced LDL cholesterol response to PS consumption may be the APOE e4 protein’s preference for larger lipoproteins such as chylomicrons and VLDLs, whereas e3 and e2 isoforms have higher affinity for smaller lipoproteins such as HDL (29, 30). A higher proportion of APOE with the e4 isoform should end up on chylomicrons and VLDLs relative to e3. Because the clearance of chylomicron remnants, which contain dietary- or biliary-derived cholesterol, is APOE dependent and primarily via the LDL receptor, we propose that the hepatic delivery of cholesterol from the cholesterol absorption is faster for APOE e4 > e3 isoforms. This observation is supported by the fact that chylomicron remnant...
clearance is faster for APOE ε4 > ε3 (31), and chylomicron remnants are the primary hepatic delivery vehicle of absorbed cholesterol.

No difference in the change in cholesterol absorption after PS consumption was observed between APOE ε4 and ε3 participants in our trial. If hepatic cholesterol concentrations are tightly linked to absorbed cholesterol in APOE ε4 individuals, then the effect of lowered cholesterol absorption would be greatest in APOE ε4 individuals, leading to an increased hepatic cholesterol uptake and enhanced LDL cholesterol lowering. However, a limitation of the isotopic method used in this trial was that it could only measure percent change in cholesterol absorption from placebo compared with the PS period and not the actual cholesterol absorption in each period (32).

The results of our isotopic assessment of cholesterol synthesis (FSR) reinforced the design of this trial, which used the L:C ratio to recruit individuals with high or low cholesterol synthesis (11). The HS participants had higher cholesterol FSR than did LS participants, in both the placebo and PS periods, with LS participants experiencing a larger increase in cholesterol FSR after PS consumption than did HS participants. These findings reinforce previous work that suggested that cholesterol synthesis may be a determinant of responsiveness to PS consumption (22, 33). It appears that individuals who endogenously synthesize more of their cholesterol are less affected by PS consumption in terms of cholesterol metabolism and circulating cholesterol concentrations.

One limitation of our trial was size (n = 63), which could be considered small for investigation of genetic associations. However, a specific goal of this trial was to look for nutrient-by-gene interactive effects, which can be seen only in trials that carefully phenotype responsiveness to a given nutrient. Using this size trial, we were able to replicate and expand on an association for CYP7A1-rs3808607 with total cholesterol lowering in response to PS consumption (14). This association was first reported in an equally small population, and replication is critical in this type of nutrigenetic research to strengthen the credibility of other reports. Replication of the additional current findings in future studies will be critical to confirming the associations we have reported.

In summary, our data represent a first step in evaluating the use of common genetic variations to predict an individual’s response to PS intervention. Our results demonstrate that the response of plasma lipids to PS consumption has high interindividual variability, which is influenced by CYP7A1- and APOE polymorphisms. These genetic variants could be used in the future to identify individuals who will benefit the most from PS intervention in terms of LDL cholesterol and thereby positively modify their risk profiles in both primary and secondary prevention of CVD. The use of PS consumption, in the context of personalized nutritional recommendations, based on predicted response would greatly increase its efficacy in reducing CVD risk factors.

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The authors’ responsibilities were as follows—DSM, PKE, and PJHJ: developed the overall research plan; DSM: was the principal manuscript author, conducted the majority of the sample analysis, and performed the statistical analysis; PKE, SKG, and PJHJ: contributed to the preparation of the manuscript; SKG: was involved in conducting the human clinical trial at the USDA Beltsville site; 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. PJHJ has reported receiving grants from Danone, Enzymotec, and Unilever, which all have PS-containing products. PJHJ also serves as president of Nutritional Fundamentals for Health Inc., which markets PS among other nutraceuticals. DSM, PKE, SKG, and DJB have no conflicts of interest to declare. Unilever Canada Inc. played no role in the design, implementation, or analysis of the trial or in interpretation of the data.

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