CYP7A1-rs3808607 and *APOE* isoform associate with LDL cholesterol lowering after plant sterol consumption in a randomized clinical trial[1,2]

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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 7 alpha-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, genoset *CYP7A1-rs3808607* T/T/*APOE* ε3 was associated with nonresponsiveness (mean ± SEM: +0.09 ± 0.08 mmol/L, P = 0.9999, n = 14). *rs5882* in cholesteryl 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. *Am J Clin Nutr* 2015;102:951–7.

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

INTRODUCTION

Many people currently consume functional food products containing plant sterols (PSs)[7] 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.

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[2] Supplemental 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.

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[7] Abbreviations used: ABCG8, ATP-binding cassette subfamily G member 8; APOE, apoliprotein E; CETP, cholesteryl ester transfer protein; CVD, cardiovascular disease; CYP7A1, cholesterol 7 α-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-hydroxylase (*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*) isoform (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-^{13}C]cholesterol (Cambridge Isotope Laboratories Inc.). Fasting blood samples were then taken daily over the following 96 h to measure change in cholesterol absorption. The [^{13}C]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 ($\sim$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 $\pm$ 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. Sequence and sex and BMI (due to difference by synthesis groups and/or genotypes at baseline) were included in the model as fixed factors, and site and participant 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 χ^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 [^{13}C]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 χ^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* ε2/ε2 participants, and only 4 participants were had *APOE* ε2/ε3. Therefore, ε2/ε3 and ε3/ε3 (n = 36) participants were grouped and considered *APOE* ε3 (n = 40), whereas participants with either ε4/ε3 (n = 18) or ε4/ε4 (n = 5) were grouped and considered *APOE* ε4 (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 ± 0.04 mmol/L). However, when further stratified, HS individuals did not show LDL cholesterol lowering (−0.05 ± 0.07 mmol/L, n = 24), whereas individuals with low synthesis showed reductions (−0.29 ± 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 1**A) 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).

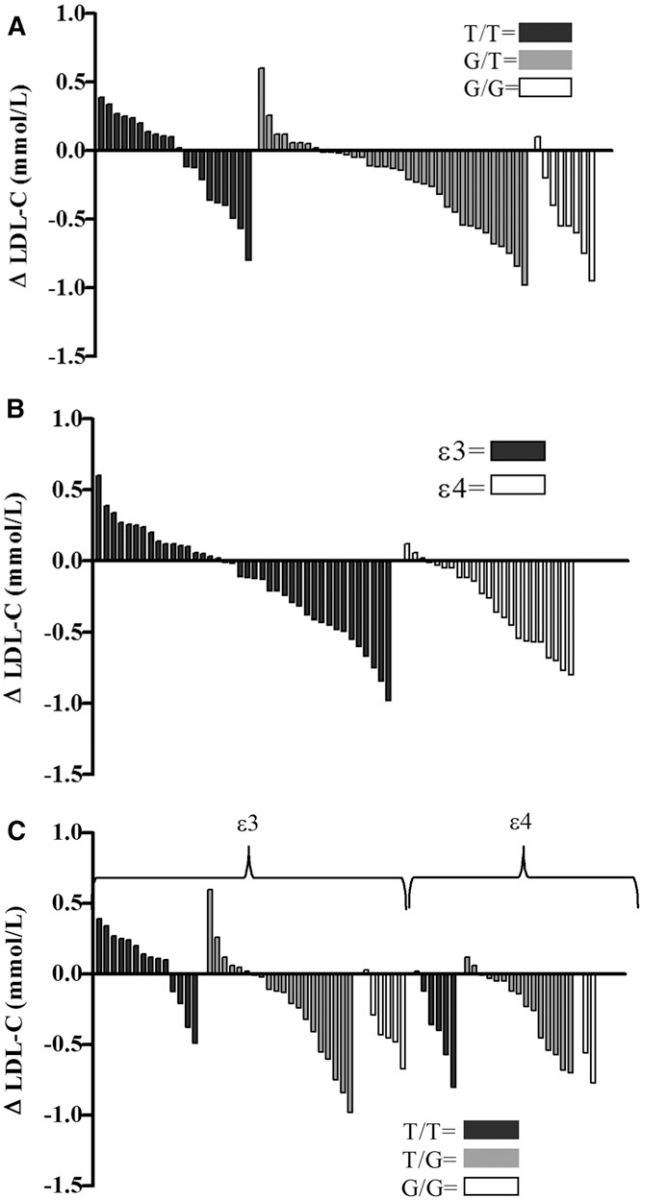

FIGURE 1 Individual changes in LDL cholesterol in response to plant sterol consumption compared with placebo stratified by *CYP7A1-rs3808607* genotype (n = 20 T/T, n = 35 T/G, n = 8 G/G) (A). Individual changes in LDL cholesterol in response to plant sterol consumption compared with placebo stratified by *APOE* isoform (n = 40 ϵ3/ ϵ3, n = 23 ϵ4/−) (B). Individual changes in LDL cholesterol in response to plant sterol consumption compared with placebo stratified by *CYP7A1-rs3808607* and *APOE* isoform genosets (n = 14 T/T ϵ3, n = 20 T/G ϵ3, n = 6 T/G ϵ3, n = 6 T/T ϵ4, n = 15 T/G ϵ4, n = 2 G/G ϵ4) (C). *APOE*, apoliprotein E; *CYP7A1*, cholesterol 7 α-hydroxylase; ΔLDL-C, change in LDL cholesterol.

Specifically, an allelic dose effect of *CYP7A1-rs3808607* was observed in LDL cholesterol response to PS consumption, where T/T homozygotes (−0.05 ± 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 ± 0.06 mmol/L, P = 0.0006, n = 35; G/G: −0.46 ± 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[1]

Gene and SNP/genotype	ΔLDL-C,[2] mmol/L	Simple effects by genotype,[3] *P*	Treatment *P*	Treatment × genotype *P*
CYP7A1 rs3808607				
T/T (20)	−0.05 ± 0.07[a]	0.9999	<0.0001	0.0146
G/T (35)	−0.22 ± 0.06[a,b]	0.0006		
G/G (8)	−0.46 ± 0.12[b]	0.0009		
APOE isoform				
ε3 (40)	−0.13 ± 0.05[c]	0.0370	0.0547	0.0466
ε4 (23)	−0.31 ± 0.07[d]	<0.0001		

[1] *n* in parentheses. *P* values are from SAS MIXED model ($n = 63$). Means 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 α-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 greater 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 genosets, 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 1**A, 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* (T/T: 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 [^{13}C] 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.

TABLE 2
APOE and *CYP7A1-rs3808607* interactions associate with LDL cholesterol lowering in response to plant sterol consumption[1]

APOE isoform	*CYP7A1 rs3808607*	*n*	ΔLDL-C,[2] mmol/L	Simple effects by genoset,[3] *P*	Treatment *P*	Treatment × genotype (*P*)
ε3	T/T	14	+0.09 ± 0.08[a]	0.9999	≤0.0001	Treatment × *CYP7A1* (0.0406)
ε3	T/G	20	−0.21 ± 0.07[a,b]	0.0246		Treatment × *APOE* (0.0163)
ε3	G/G	6	−0.38 ± 0.13[b]	0.0234		Treatment × *APOE* × *rs3808607* (0.0737)
ε4	T/T	6	−0.37 ± 0.13[b]	0.0306		All other 2-way interactive effects were not statistically significant
ε4	T/G	15	−0.24 ± 0.08[a,b]	0.0234		
ε4	G/G	2	−0.67 ± 0.22[b]	0.0216		

[1]*P* 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*, apoliprotein E; *CYP7A1*, cholesterol 7 α-hydroxylase; ΔLDL-C, change in LDL cholesterol.
[2]Values are least squares means ± SEMs.
[3]Mixed-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 7-α 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* ε4 individuals ($n = 24$) to PS consumption and the apparent ability of the ε4 isoform to override the nonresponsive T/T genotype in *CYP7A1-rs3808607*. Only within the ε3 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* ε4 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 ε4 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* ε4 isoform and ε3 isoform individuals in a clinical trial that specifically recruited ε4 and ε3 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* ε4 isoform carriers were not responders to PS consumption. Clearly, our data support the assertion that *APOE* ε4 individuals respond well to PS consumption, as do ε3 isoform carriers, except for those ε3 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* ε4 protein's preference for larger lipoproteins such as chylomicrons and VLDLs, whereas ε3 and ε2 isoforms have higher affinity for smaller lipoproteins such as HDL (29, 30). A higher proportion of APOE with the ε4 isoform should end up on chylomicrons and VLDLs relative to ε3. 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* ε4 > ε3 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; DJB: 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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