A single daily dose of soybean phytosterols in ground beef decreases serum total cholesterol and LDL cholesterol in young, mildly hypercholesterolemic men\(^1\)–\(^4\)

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**ABSTRACT**

**Background:** Consumption of phytosterol-supplemented margarine lowers total plasma cholesterol (TC) and LDL-cholesterol concentrations in older middle-aged hypercholesterolemic individuals. The effects of incorporating phytosterols into lower-fat foods on the plasma lipids of young men at increased risk of developing cardiovascular disease have not been studied.

**Objective:** We tested the hypothesis that a single daily dose of soybean phytosterols added to ground beef will lower plasma TC and LDL-cholesterol concentrations in mildly hypercholesterolemic young men.

**Design:** In a triple-blind, 4-wk study, 34 male college students with elevated plasma TC (5.85 ± 0.70 mmol/L), LDL cholesterol (4.02 ± 0.60 mmol/L), and TC:HDL cholesterol (5.5 ± 1.2) were randomly assigned to the control (ground beef alone) or treatment (ground beef with 2.7 g of phytosterols) group. The phytosterol mixture was two-thirds esterified and one-third nonesterified and consisted of \(\beta\)-sitosterol (48%), campesterol (27%), and stigmasterol (21%).

**Results:** Consumption of phytosterol-supplemented ground beef lowered plasma TC and LDL-cholesterol concentrations and TC:HDL cholesterol from baseline by 9.3%, 14.6%, and 9.1%, respectively \((P < 0.001)\). The LDL particle size did not change, suggesting that the decrease was primarily of particle number. The decreases were similar in subjects with \(n = 8\) and without \(n = 9\) a family history of premature cardiovascular disease. No significant changes were found in the control group.

**Conclusion:** Phytosterol-supplemented ground beef effectively lowers plasma TC and LDL cholesterol and has the potential to become a functional food to help reduce the risk of cardiovascular disease. *Am J Clin Nutr* 2002;76:57–64.

**KEY WORDS** Lean beef, soybean phytosterols, young men, HDL cholesterol, LDL cholesterol, cardiovascular disease

**INTRODUCTION**

Atherosclerosis begins in adolescence \((1, 2)\) as fatty streak lipid deposits in the arterial wall. High concentrations of plasma total cholesterol (TC) and LDL cholesterol accelerate atherogenesis in the teenage years, with their effects amplified in young adulthood, 20–30 y before coronary artery disease becomes clinically manifest \((3)\). By 30–34 y of age, \(\approx 19\%\) of men have advanced lesions in the left anterior descending coronary arteries \((4)\). The long-term significance of the early origins of atherosclerosis is apparent from the observations of Klag et al \((5)\). They showed that 22-y-old men with plasma TC >5.40 mmol/L were 5.6 times as likely to develop coronary artery disease, 6.0 times as likely to have a heart attack, and 9.6 times as likely to die during the next 40 y than those with plasma TC <4.45 mmol/L. More recently, Stampler et al \((6)\) reported that young men with plasma TC concentrations >5.15 mmol/L had greater relative mortality risk during 16–25 y of follow-up than did men with lower plasma TC. The National Cholesterol Education Program Adult Treatment Panel recommended measuring blood cholesterol concentrations in all adults \(\geq 20\) y of age \((7)\).

Although there is controversy about using hypocholesterolemic drugs for primary prevention of atherosclerosis in young adults \((7, 8)\), dietary intervention to lower plasma TC and LDL-cholesterol concentrations remains a sensible and well-accepted preventive approach to decreasing cardiovascular disease (CVD) morbidity and mortality. Despite the well-documented rationale for medical nutrition therapy, many eligible patients with borderline \((>3.35 \text{ mmol/L})\) or high \((>4.10 \text{ mmol/L})\) LDL-cholesterol concentrations are not routinely referred for dietary treatment, and among those who are, compliance may be limited \((9)\). Indeed, cholesterol-lowering diets are usually low in total fat, saturated fat, and cholesterol and thus may not be sufficiently palatable to induce the long-term adherence necessary for health benefits \((10, 11)\).

Phytosterols are in the forefront of nutraceutical research on the development of food products that lower plasma cholesterol.

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concentrations. The discovery of the hypocholesterolemic effect of phytosterols (12) led to the marketing of foods such as margarine and cooking or salad oils that are high in fat and of low nutritional value. Consumption of high-fat versions of these food products can contribute 22–45% of the recommended daily fat nutritional value. Consumption of high-fat versions of these food products can contribute 22–45% of the recommended daily fat nutritional value.

The soybean-extracted phytosterol mix was obtained from the Henkel Corporation (Unilever Research Laboratories, Vlaardingen, Netherlands). The phytosterol mix contained 67% sterol esters and 33% free sterols. The sterol composition was 48% β-sitosterol, 27% campesterol, 21% stigmasterol, and 5.9% other sterols, according to a report from the Unilever Research Laboratories. The mixture also contained 4.1% fatty acids, including 61% linoleic, 23% oleic, 3.5% stearic, and 7.7% palmitic acids. The frozen phytosterols were thoroughly mixed in a blender with a small amount of boneless minced beef that was 85% lean and 15% fat by weight. This mixture was added to a large amount of boneless minced beef that was 90% lean and 10% fat by weight. After grounding, 2–5 kg of the beef blend was stuffed into color-coded plastic tubes and clipped on both ends. The raw ground beef was cooked at 177°C until the internal temperature reached 74°C. There was no visible loss due to drippings during cooking. The lipid composition of the cooked treatment and control ground beef is presented in Table 1.

The subjects were served lunch in the Human Metabolic Unit each weekday for 4 wk (March 28–April 26, 2000). Each lunch included a fixed portion of cooked ground beef (71 ± SD: 112 ± 2 g). On average, each portion for the treatment group contained 2.7 g phytosterols. The ground beef was served as a hamburger (Monday), a sloppy joe (Tuesday), spaghetti with meat sauce (Wednesday), 2 tacos (Thursday), and a cheeseburger (Friday). For weekends, the subjects received precooked frozen chili and a ground beef and rice casserole, along with heating instructions. The subjects were given color-coded cards corresponding to the colored wrapping of the hamburger, which they presented to the kitchen personnel at lunch. The subjects consumed the entire ground beef portion of each meal. Compliance was ensured by supervision of the subjects during the lunches. The subjects had ad libitum access to fresh and cooked vegetables, a variety of fruit, chips, salads, pickles, condiments, beverages, and desserts. Compliance

### Table 1

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Control beef (n = 8)</th>
<th>Treatment beef (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triacylglycerol (% by wt)</td>
<td>10.9 ± 0.91</td>
<td>11.1 ± 1.91</td>
</tr>
<tr>
<td>Fatty acids (% of total fat) ±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:0</td>
<td>0.01 ± 0.002</td>
<td>0.02 ± 0.003</td>
</tr>
<tr>
<td>10:0</td>
<td>0.05 ± 0.001</td>
<td>0.06 ± 0.003</td>
</tr>
<tr>
<td>12:0</td>
<td>0.07 ± 0.001</td>
<td>0.08 ± 0.004</td>
</tr>
<tr>
<td>14:0</td>
<td>2.86 ± 0.022</td>
<td>2.59 ± 0.167</td>
</tr>
<tr>
<td>14:1</td>
<td>0.70 ± 0.013</td>
<td>0.53 ± 0.028</td>
</tr>
<tr>
<td>15:0</td>
<td>0.53 ± 0.009</td>
<td>0.46 ± 0.023</td>
</tr>
<tr>
<td>16:0</td>
<td>25.8 ± 0.16</td>
<td>24.0 ± 0.365</td>
</tr>
<tr>
<td>16:1</td>
<td>3.57 ± 0.119</td>
<td>1.09 ± 0.082</td>
</tr>
<tr>
<td>17:0</td>
<td>1.34 ± 0.02</td>
<td>1.09 ± 0.019</td>
</tr>
<tr>
<td>17:1</td>
<td>0.8 ± 0.009</td>
<td>0.60 ± 0.011</td>
</tr>
<tr>
<td>18:0</td>
<td>18.4 ± 0.265</td>
<td>16.5 ± 0.35</td>
</tr>
<tr>
<td>18:1</td>
<td>18.1 ± 0.181</td>
<td>16.5 ± 0.614</td>
</tr>
<tr>
<td>18:2</td>
<td>2.4 ± 0.144</td>
<td>7.8 ± 0.362</td>
</tr>
<tr>
<td>18:3</td>
<td>0.45 ± 0.028</td>
<td>0.61 ± 0.065</td>
</tr>
<tr>
<td>20:0</td>
<td>0.54 ± 0.033</td>
<td>ND</td>
</tr>
<tr>
<td>20:4</td>
<td>0.46 ± 0.09</td>
<td>0.70 ± 0.194</td>
</tr>
<tr>
<td>Total sterols (% by wt)</td>
<td>ND</td>
<td>2.4 ± 0.41</td>
</tr>
<tr>
<td>Cholesterol (% by wt)</td>
<td>0.0857 ± 0.0057</td>
<td>0.0815 ± 0.0111</td>
</tr>
<tr>
<td>β-Sitosterol (% by wt)²</td>
<td>ND</td>
<td>1.35</td>
</tr>
<tr>
<td>Campesterol (% by wt)²</td>
<td>ND</td>
<td>0.75</td>
</tr>
<tr>
<td>Stigmasterol (% by wt)²</td>
<td>ND</td>
<td>0.58</td>
</tr>
</tbody>
</table>

1 ± SD, ND, not detectable.
2 Calculated from information provided by the Henkel Corporation (Unilever Research Laboratories, Vlaardingen, Netherlands; see Methods).

### Subjects and Methods

#### Subjects

The Iowa State University Human Subjects Review Committee approved the study protocol. The subjects were recruited from the undergraduate and graduate student body at Iowa State University with newspaper advertisements and posted flyers. One hundred eighty-one men volunteered to participate in the study and were screened for blood lipid concentrations and health-related behavioral characteristics. Thirty-six white men met the selection criteria, which included a plasma TC concentration >5.10 mmol/L and a LDL-cholesterol concentration >3.35 mmol/L. Each subject agreed to consume lunch Monday through Friday in the Human Metabolic Unit for 28 d. The exclusion criteria were a medical history of diabetes mellitus or coronary artery disease, smoking or using alcohol or drugs, and consuming any type of medication affecting blood lipids.

The subjects were randomly assigned to either a treatment (phytosterol-supplemented ground beef; n = 18) or control (ground beef only; n = 18) group. Thirty-four subjects completed the study. One subject dropped out for personal reasons, and one subject was excluded for failing to show up for lunch. The subjects gave written, informed consent before the study. Each subject received a $250 stipend on completion of the study. Six subjects who were enrolled in a university meal plan were also reimbursed for missed meals.

#### Study design

We used a randomized triple-blind design to evaluate the subjects’ plasma lipid and lipoprotein responses to the consumption of control and treatment ground beef lunches. ConAgra Foods, Inc provided the control and treatment ground beef packaged in 2 differently colored wrappers. Only scientists at ConAgra Foods, Inc knew the color code: neither the investigators nor the subjects knew which color corresponded to which type of ground beef. The color code was changed to a number code (group 1 and group 2) by the investigators; the scientists at ConAgra Foods, Inc did not know which data belonged to which group. The codes were broken simultaneously after the plasma lipoprotein data were analyzed and summarized. Sensory tests conducted in January 2000 with 31 men of a similar age showed that the subjects could not tell the difference between the types of ground beef.
for weekend meal consumption was assessed on the last day of the study by the subjects’ anonymous responses to the question, “How many weekend meals did you not eat?” Each subject was asked to provide only their group identification, not their individual identification. Overall weekend compliance was 94%, with no significant difference between the treatment and control groups. All subjects were instructed to maintain their habitual dietary and physical activity patterns; however, they were requested not to consume red meat other than that provided in the experimental lunch and to limit egg consumption to 2–3 eggs/wk.

Nutrient intake, health history, and body weight and height

A trained graduate research assistant using interviewer-administered questionnaires obtained health information at screening and during the first week of the study. This information included personal medical history, current medications, family history of CVD, presence of high blood lipid concentrations or diabetes mellitus, weekly alcohol consumption, and previous tobacco use. Baseline nutrient and energy intakes were estimated by a semiquantitative food-frequency questionnaire (13). Physical activity during the previous 7 d was assessed with the use of the Five-City Project physical activity recall, which allowed calculation of daily energy expenditure (total and per kilogram body weight; 14).

Height was measured twice and averaged. Weight was measured at baseline and on the days of the blood draws at weeks 2 and 4. The subjects wore light clothing and removed their shoes for the weight and height measurements. Body mass index (in kg/m²) was calculated for each subject at baseline and at weeks 2 and 4.

Plasma lipid measurements

Blood samples were obtained at baseline and weeks 2 and 4 after the subjects had fasted overnight. Each sample was drawn into a red-gray mottled-top evacuated tube containing serum separator tube gel and clot activator (yellow separator) and into two 7-mL purple-top EDTA evacuated tubes (all obtained from Quest Diagnostics, Ceterboro, NJ). The samples were centrifuged at 1380 × g 4°C for 15 min within 30–60 min after phlebotomy. The plasma samples were stored at 4°C before cholesterol and lipoprotein cholesterol and comprehensive metabolic panel measurements and at −80°C before lipoprotein subclass and plasma sterol measurements. Sample analyses included lipid and comprehensive metabolic panels performed at the regional facility of Quest Diagnostics (Des Moines, IA), a certified clinical laboratory. Plasma HDL cholesterol was measured after precipitation of apolipoprotein B with dextran sulfate and magnesium chloride. The intraassay CV was <5%. The concentration of LDL cholesterol was calculated according to the Friedewald equation (15).

Separation and quantification of lipoprotein subclasses were performed with the use of nondenaturing polyacrylamide gradient gel electrophoresis, as described elsewhere (16). Use of a composite gradient gel allowed simultaneous separation of LDL and HDL particles (17). Cholesterol in gels was stained with Sudan Black B (Sigma Chemical Co, St Louis) and scanned by an LKB-Ultrascan XL laser densitometer (Pharmacia-LKB Biotechnology, Piscataway, NJ) with GelScan XL software (version 2.1). Absorbance profiles, representing the size distributions of lipoprotein constituents, were converted to ASCII files with the Gelcon program (Pharmacia-LKB Biotechnology) and analyzed with software developed at the Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX (17). We measured 4 traits representing size distributions of lipoproteins: large and small LDL and large and small HDL. Large LDL was defined as the proportion of LDL absorbance (21–29 nm) on particles ≥25.5 nm and small LDL as the proportion on particles <25.5 nm. Similarly, large and small HDL were defined as the proportion of HDL absorbance (7.2–20 nm) on particles between 8.8 and 12.0 nm and particles <8.8 nm, respectively (ie, separation into HDL₂ and HDL₃). In addition, we measured LDL peak diameter. All samples were run in duplicate on different gels, and the values analyzed represent the average values. For a subset of the samples at 4 wk, the number of LDL particles was determined by nuclear magnetic resonance (NMR; LIPOMED, Raleigh, NC) as described elsewhere (18).

Neutral sterol quantitation

Neutral sterols were extracted from the human plasma samples by the Folch extraction method (19) and subjected to gas chromatography analysis in the Hewlett Packard 6890 gas chromatograph (Palo Alto, CA). Samples were analyzed as trimethylsilyl ether derivatives of the neutral sterols. Peaks were identified by comparison with known standards (Steraloids, Inc, Newport, RI) and quantified with the use of an internal standard, 5α-cholestanol.

Statistical analyses

The effects of the consumption of the treatment ground beef on concentrations of plasma lipids, neutral sterols and metabolic values, lipoprotein particle sizes and distributions, body weight, and BMI were analyzed by repeated-measures analysis of variance with SPSS/PC+ 9.0 (SPSS Inc, Chicago). The treatment (phytosterol-supplemented and control ground beef) was the between-subject factor and the time of measurement (baseline, week 2, and week 4) was the within-subject factor. A significant treatment-by-time interaction indicated that a significant change in a variable of interest (eg, LDL cholesterol) during the study was caused by the phytosterol supplementation. In some analyses, elevated blood lipids or family history of CVD was used as another between-subject factor. The independent sample t test was used for comparison of baseline values between the 2 groups. Whenever there was a significant interaction as determined by the repeated-measures analysis of variance, we used the independent t test to examine between-group differences and Bonferroni-adjusted pairwise comparisons to evaluate changes within each group. Significance was set at an α level of 0.05, but P values of 0.1 are also reported to better balance type I and type II statistical errors. Data are presented as means ± SDs.

RESULTS

Subject baseline characteristics

The baseline characteristics of subjects in the treatment and control groups are presented in Table 2. There were no significant differences in any measured variable between the 2 groups. The apparently higher mean body weight of the treatment group was largely a result of the heaviest subject (128 kg) and the 2 lightest subjects (57 and 63 kg) being randomly assigned to the treatment or control group, respectively. The mean weight for all subjects was in the 75th percentile reported by the third National Health and Nutrition Examination Survey for weight among non-Hispanic white males aged 20–29 y (20). The subjects’
baseline mean plasma TC and HDL-cholesterol concentrations were in the 90th–95th and the 25th–50th percentiles, respectively, for non-Hispanic white males aged 20–29 y (21, 22). Eleven (32%) of the 34 subjects who completed the study knew they had high blood cholesterol before the study, 17 (50%) reported that they had a family history of premature CVD and high blood lipids, and 5 (15%) reported a family history of diabetes.

The subjects’ mean energy intake was 13249 ± 2564 kJ. Protein, carbohydrate, and fat intakes were 15.6 ± 2.5%, 49.1 ± 6.4%, and 35.6 ± 5.8% of total energy intake, respectively. These values were similar to those reported for non-Hispanic white males aged 20–29 y: 13062 kJ, 14%, 47.3%, and 34.4% for energy, protein, carbohydrate, and fat, respectively (23). Mean dietary cholesterol intake was 328 ± 95 mg for our subjects and 364 mg for non-Hispanic white males aged 20–29 y.

### Plasma lipid concentrations

At week 4, plasma TC and LDL-cholesterol concentrations and TC:HDL cholesterol had decreased 9.3%, 14.6%, and 9.1%, respectively, from baseline in subjects consuming 2.7 g soybean phytosterols/d, whereas subjects in the control group had no significant changes (Table 3). In the treatment group, 82% of the decrease in TC, 75% of the decrease in LDL cholesterol, and 80% of the decrease in TC:HDL cholesterol occurred after only 2 wk of treatment. Consumption of the treatment ground beef did not influence plasma HDL-cholesterol or triacylglycerol concentrations. The correlation between phytosterol intake expressed per kilogram of body weight and the magnitude of the decline in plasma LDL cholesterol was not significant (Figure 1).

### Lipoprotein subclass patterns

Neither the proportion of absorbance in large and small LDL nor the LDL particle diameter was significantly influenced by treatment (Table 4). Moreover, no subject changed from LDL pattern A (mean peak diameter ≥25.5 nm) to pattern B (mean peak diameter <25.5 nm) or vice versa in the treatment group. NMR analysis of the week 4 plasma samples showed that the mean LDL particle number tended to be lower ($P = 0.058$) in the

### Table 2

Baseline characteristics of subjects in the control and phytosterol-supplemented (treatment) groups and of subjects who did not meet selection criteria.

<table>
<thead>
<tr>
<th>Variable and group</th>
<th>Baseline</th>
<th>Week 2</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>Control group</td>
<td>5.70 ± 0.05</td>
<td>5.80 ± 0.70</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>Control group</td>
<td>4.00 ± 0.45</td>
<td>3.95 ± 0.60</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>Control group</td>
<td>1.04 ± 0.15</td>
<td>1.05 ± 0.20</td>
</tr>
<tr>
<td>Total:HDL cholesterol</td>
<td>Control group</td>
<td>5.6 ± 1.0</td>
<td>5.6 ± 1.2</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>Control group</td>
<td>1.58 ± 0.64</td>
<td>1.70 ± 0.75</td>
</tr>
</tbody>
</table>

1. $\bar{x} \pm SD$; $n = 17$ in the control group and 17 in the treatment group.
2. Initial screening was done 6 wk before the study; the data were not included in any analysis but are provided as additional information.
3. Significant treatment-by-time interaction, $P \leq 0.001$ (repeated-measures ANOVA).
4. Significantly different from baseline, $P < 0.001$ (Bonferroni-adjusted pairwise comparisons).
treatment than in the control group (1467 ± 212 compared with 1696 ± 424 nmol/L). The LDL particle diameter measured by NMR (n = 34) was strongly correlated (r = 0.784, P ≤ 0.0001) with the LDL particle diameter determined by gradient gel electrophoresis. Correlations between changes in small and large LDL particle distribution and changes in plasma concentrations of phytosterols were not significant.

The proportion of large HDL2a+2b increased after 4 wk in the treatment and control groups combined (Table 4). However, there was no significant treatment-by-time interaction on large-HDL subclasses. The modest increase in the proportion of large HDL was primarily due to an increase in HDL2a. The increase in large HDL corresponded with a trend for a decrease in the proportion of small HDL3a+3b+3c particles.

**Plasma neutral sterol concentrations**

In the treatment group, the plasma campesterol concentration increased from 8.0 ± 4.0 μmol/L at baseline to 13.1 ± 4.1 μmol/L after 4 wk (P ≤ 0.001). Plasma β-sitosterol concentrations tended to increase (3.9 ± 2.4 to 5.9 ± 1.8 μmol/L; P = 0.1). Plasma stigmasterol concentrations did not change significantly (0.50 ± 0.70 to 0.78 ± 0.62 μmol/L).

**Family history of CVD and plasma LDL cholesterol**

Approximately one-half of the subjects in each group reported a family history of premature CVD and elevated blood lipids. In the treatment group, 8 subjects reported a family history and 9 subjects reported no family history of CVD. At baseline, subjects in the treatment group who had a family history had higher TC (6.3 ± 0.9 mmol/L) and LDL-cholesterol (5.55 ± 0.5 mmol/L) concentrations (P < 0.01) than did subjects without a family history (4.35 ± 0.8 and 3.85 ± 0.5 mmol/L for TC and LDL cholesterol, respectively). However, at week 4, the magnitude of decreases in TC and LDL cholesterol was not significantly different between these subgroups of the treatment group. The mean reductions in TC and LDL cholesterol, respectively, were 8.7% and 12.6% for the subjects with a family history and 8.9% and 15.6% for the subjects without a family history of CVD.

**Body weight and other plasma metabolic variables**

Subjects in the treatment and control groups tended (P = 0.09, time effect) to gain a small amount of weight during the 4-wk experimental period (0.34 ± 1.1 kg; range: −2.2 to +2.6 kg). Treatment had an effect on blood urea nitrogen:creatinine, which increased from baseline 11.9 ± 3.0 to 13.8 ± 2.8 in the treatment group and decreased from 14 ± 3.0 to 13.8 ± 2.6 in the control group (P = 0.045, treatment-by-time interaction). Phytosterol intake tended to influence total blood urea nitrogen, which increased from 5.0 ± 1.0 at baseline to 6.0 ± 1.5 mmol/L in the treatment group but did not change significantly in the control group (6.0 ± 1.0 to 6.0 ± 1.0) (P = 0.061, treatment-by-time interaction). There was no effect of treatment on plasma total protein, albumin, or creatinine. However, in both groups, there were small but significant (P < 0.001, time effect) changes in plasma total protein from 75 ± 4.0 to 76 ± 4.0 g/L, albumin from 47 ± 2 to 48 ± 2 g/L, and creatinine from 109 ± 10 to 110 ± 10 μmol/L. Plasma concentrations of protein, creatinine, and nitrogen; nitrogen:creatinine; and all other metabolic variables were nonetheless within the reported normal range (24). The serum liver enzymes alkaline phosphatase and aspartate aminotransferase were not influenced by treatment.

**DISCUSSION**

The present study differs from previous studies (1, 25, 26) on the effects of phytosterol consumption on plasma cholesterol concentrations in several respects. First, the phytosterols were mixed into a ground beef product that was low in fat and consumed only once daily. Previous experiments examined the effects of phytosterols in high-fat products such as margarine or spreads that were usually consumed 3 times/d (12, 25, 26). Second, to the best of our knowledge, the present study is the first to report the effects of phytosterol consumption on LDL and HDL subclass patterns. Lipoprotein subclasses are influenced by dietary and pharmacologic interventions that affect LDL-cholesterol concentrations (27–33). Finally, the present study tested the effects of phytosterols in young adult men with at least 3 risk

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**FIGURE 1.** The correlation between the percentage change in LDL cholesterol (LDL-C) from baseline and soybean phytosterol consumption was not significant (r = 0.32; n = 17). The percentage change in LDL-C was calculated by subtracting LDL-C plasma concentrations at baseline from LDL-C at week 4. Phytosterol consumption was calculated by dividing the phytosterol intake by the mean weight at baseline, week 2, and week 4.

**TABLE 4**

<table>
<thead>
<tr>
<th>Lipoprotein subclass and group</th>
<th>Baseline</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL peak particle diameter (nm)</td>
<td>26.1 ± 0.7</td>
<td>26.1 ± 0.8</td>
</tr>
<tr>
<td>Control group</td>
<td>26.4 ± 0.5</td>
<td>26.3 ± 0.6</td>
</tr>
<tr>
<td>Treatment group</td>
<td>79.0 ± 14.6</td>
<td>79.4 ± 19.1</td>
</tr>
<tr>
<td>Control group</td>
<td>88.0 ± 10.1</td>
<td>84.4 ± 12.8</td>
</tr>
<tr>
<td>Treatment group</td>
<td>21.0 ± 14.6</td>
<td>20.6 ± 19.1</td>
</tr>
<tr>
<td>Control group</td>
<td>12.0 ± 10.1</td>
<td>15.6 ± 12.8</td>
</tr>
<tr>
<td>Treatment group</td>
<td>45.6 ± 6.8</td>
<td>47.6 ± 6.5</td>
</tr>
<tr>
<td>Control group</td>
<td>40.5 ± 9.0</td>
<td>43.6 ± 9.0</td>
</tr>
<tr>
<td>Small HDL3a+3b+3c (%)</td>
<td>50.4 ± 8.1</td>
<td>49.0 ± 8.3</td>
</tr>
<tr>
<td>Treatment group</td>
<td>55.3 ± 9.5</td>
<td>51.6 ± 13.3</td>
</tr>
</tbody>
</table>

1 SD; n = 17 in the control group and 17 in the treatment group.
2 Significant time effect, P = 0.007 (repeated-measures ANOVA).
3 Time effect, P = 0.068 (repeated-measures ANOVA).
factors for developing CVD. Most other studies tested the effect of phytosterol-supplemented food products primarily in older normo- and hypercholesterolemic adults (12, 25, 26).

Our results show that lean ground beef is a viable food matrix to deliver an effective dose of phytosterols to lower plasma TC and LDL cholesterol in young men. The 9% and 15% declines in TC and LDL cholesterol, respectively, after the consumption of a single daily dose of 2.7 g of soybean phytosterols in ground beef are similar to the 8–13% decline in plasma TC and LDL cholesterol after consumption of multiple daily doses of a similar amount and type of soybean phytosterols in margarine (25). Furthermore, the magnitude of the decline in TC and LDL cholesterol in our study is comparable with the 8–12% decline observed in studies with stanol ester–fortified margarine (12). Thus, the phytosterol mixture in beef, like phytosterol esters in margarine (26), can lower plasma TC and LDL-cholesterol concentrations as effectively as can margarine containing stanol esters.

Phytosterols are generally thought to lower plasma cholesterol by interfering with absorption of dietary and biliary cholesterol (34–38). It has been suggested that phytosterols act most effectively when consumed along with cholesterol-containing foods (35, 39, 40). Thus, many published studies report feeding phytosterol-fortified food products at most if not all meals. A recent study by Plat et al (41) showed that consumption of 2.5 g of phytosterol esters at one meal was as effective in lowering LDL cholesterol as consumption of the same phytosterol dose divided over 3 meals. Our findings show that soybean phytosterols consumed in a single daily dose also have a significant hypocholesterolemic effect. The efficacy of a single relatively large dose may result from saturating the enteroocyte and prolonging the presence of the phytosterol within the cell, thereby inhibiting cholesterol absorption during subsequent meals. There is experimental evidence suggesting that such a hypothesis is tenable. Approximately 5% of β-sitosterol is absorbed, and substantial amounts are incorporated into intestinal mucosal cell membranes (42–44). In enterocytes, phytosterols may inhibit cholesterol esterification and cause nonesterified cholesterol to diffuse or to be transported back into the intestinal lumen (45, 46).

Neither the most effective dose nor the optimal cholesterol-lowering ratio of esterified to free soybean phytosterols was determined in this study. There was no significant association between the percentage of change in the plasma LDL-cholesterol concentration and total phytosterol intake in a range of doses from 0.025 to 0.0425 g/kg body wt (1.75–3.0 g/d for a 70-kg individual). Decreases in LDL cholesterol, similar to those in the present study, were reported in subjects consuming sterol esters in margarine at either 0.02 or 0.045 g/kg body wt (1.6–3.4 g/d, 26).

To our knowledge no studies have examined the effects of phytosterol consumption on changes in lipoprotein subclass patterns. Dietary interventions can reduce LDL cholesterol by decreasing LDL particle number, particle size, or both in some individuals (28). A reduction in particle size is undesirable because smaller LDL is associated with an atherogenic lipid profile (47), although some researchers do not think this evidence is compelling (48). In our study, subjects fed phytosterol-supplemented beef did not have a significant shift from larger to smaller LDL. This suggests that the decrease in LDL cholesterol resulted primarily from a decrease in the number of LDL particles. Indeed, the NMR analysis performed at week 4 showed that the subjects in the treatment group tended to have a lower mean LDL particle number than did the control subjects. There is evidence that LDL particle size is influenced by metabolic and dietary factors affecting plasma triacylglycerol (49–51) and is strongly and inversely correlated with plasma triacylglycerol concentrations (52–54). Perhaps phytosterol supplementation had no effect on LDL particle size in our study because plasma triacylglycerol was not affected.

Phytosterol treatment did not significantly affect HDL-cholesterol concentrations or HDL subclasses. However, the percentage of HDL as large particles (HDLaglass) increased and as small particles (HDLaglass) tended to decrease in both groups during the experiment. Larger HDL particles are thought to be more protective against atherosclerosis than are smaller particles (55–57). A shift from larger to smaller HDL particles is observed in some subjects receiving dietary and drug treatments to lower LDL cholesterol (27, 29–33). Studies with a greater number of subjects are necessary to determine whether phytosterols significantly affect HDL subclasses.

Ground beef is a suitable food for phytosterol supplementation for several reasons. First, ground beef is the major single source of protein for young adult white men (58, 59). Second, consumption of moderate amounts of meat as part of a healthy diet is important in meeting recommended dietary allowances for vitamin B-12, niacin, and zinc (60). Third, lean ground beef (≤15% fat by weight) as part of 170 g (6 oz) cooked meat, poultry, or fish/d can be included in a healthy diet (61, 62). Phytosterol-supplemented foods available to consumers today are spreads such as Benecol (McNeil Consumer Products, Fort Washington, PA) and Take Control (Lipton, Englewood Cliffs, NJ). When used as directed (3 times/d) these foods add 12–27 g extra fat but provide no other nutrients (63, 64). The high prevalence of overweight and obesity in the United States makes limiting fat consumption a priority. The phytosterol-supplemented lean ground beef used in this study has less total fat and is considerably more nutritious than the currently marketed phytosterol spreads. One disadvantage of fortifying foods with phytosterols is that phytosterols reduce the absorption of β-carotene (65, 66). Although we did not measure plasma concentrations of β-carotene, Plat et al (41) reported that a decrease in plasma β-carotene concentrations was similar when stanol ester–supplemented food was consumed either 1 or 3 times/d.

The results of our study show that lean ground beef is an excellent vehicle for delivering a dose of plant phytosterols to effectively lower plasma TC and LDL-cholesterol concentrations and improve TC:HDL cholesterol while providing ≈29 g protein and only ≈13 g fat. Clearly, ground beef supplemented with phytosterols could become a hypocholesterolemic functional food consumed as part of a healthy diet to lower the risk of heart disease in mildly hypercholesterolemic young adults.

REFERENCES


