Unesterified Plant Sterols and Stanols Do Not Affect LDL Electrophoretic Characteristics in Hypercholesterolemic Subjects

(Manuscript received 6 October 2003. Initial review completed 1 December 2003. Revision accepted 4 December 2003.)

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ABSTRACT The extent to which sterols and stanols modulate LDL particle size is unknown. We examined the effects of supplementation with unesterified plant sterols and stanols on several LDL electrophoretic characteristics. Healthy hypercholesterolemic subjects (n = 14) consumed each of four experimental diets containing plant sterols (S), plant stanols (SN), a 50:50 mixture of sterols and stanols (SSN), or cornstarch (control) in a randomized crossover design. The butter component of the diet was blended with unesterified sterols and stanols at a dose of 1.8 g/d. The LDL particles were characterized by polyacrylamide gradient gel electrophoresis of whole plasma. LDL cholesterol (LDL-C) concentrations decreased by 8.8, 13.6, and 13.1% in the S, SN, and SSN groups, respectively (P < 0.01) with a significant increase of 4.3% in the control group. None of the treatments with sterols and stanols induced significant changes in LDL peak particle diameter or in the cholesterol levels of the small LDL subfraction (<25.5 nm). The reduction in plasma LDL-C levels with SN consumption was due mainly to a decrease (P < 0.05) in the concentration of cholesterol in the large subfraction (>26.0 nm). The significant reduction in plasma LDL-C concentrations by sterol and stanol consumption in subjects was not paralleled by any beneficial changes in LDL electrophoretic characteristics.


KEY WORDS: • coronary heart disease • phytosterols • phytostanols • small, dense LDL • hypercholesterolemia

Elevated plasma LDL cholesterol (LDL-C) concentration is a well-established risk factor for coronary heart disease (CHD) (1). Accumulating evidence indicates that subclasses of LDL characterized by variations in density, size, and chemical composition may be clinically relevant (2). Indeed, a predominance of small, dense LDL particles has been suggested to affect the atherosclerotic process directly. Enhanced atherogenecity of smaller LDL vs. larger LDL is supported by evidence that smaller LDL are taken up less rapidly by LDL receptors (3), penetrate more readily into arterial tissues (4), bind more tightly to arterial proteoglycans (5), and are oxidized more rapidly than larger LDL particles (6).

The enrichment of foods with plant sterols or stanols, analogs of cholesterol, is one of the recent functional food developments to enhance the cholesterol-lowering ability of traditional food products. The serum cholesterol-lowering effect of plant sterols and stanols is believed to be caused by an inhibition of cholesterol absorption resulting from their higher affinity over cholesterol for incorporation into micelles in the intestine (7). Plant sterols and stanols appear to be equally effective in lowering plasma total and LDL-C (8). Both forms are generally esterified to increase their solubility in fat. However, recent data suggest that free sterols may be as effective as esters if they are adequately dispersed in the diet (9).

Only limited information exists concerning the effect of plant sterols and stanols on LDL particle size phenotype. In a study of healthy men fed phytosterol-supplemented ground beef, there was no effect on LDL particle size despite a reduction of total and LDL-C concentrations (10). On the other hand, Pedersen et al. (11) reported that rapeseed oil and sunflower oil had more favorable effects on blood lipids, on LDL particle size, and on the lipid content of larger and medium-size LDL subfractions compared with olive oil. Part of this difference was attributed to the variable phytosterol content of the different oils (11). Thus, the extent to which plant sterols and stanols modulate the LDL particle size phenotype remains to be firmly established. The objective of the present study was to examine and compare the effects of supplementation with unesterified plant sterols and stanols on several LDL electrophoretic characteristics in hypercholesterolemic subjects consuming strictly controlled diets.

SUBJECTS AND METHODS

Subjects. The analyses presented in this report were conducted as part of a study from which data on plasma lipid levels were already reported (12). Briefly, 16 (10 men and 6 women) otherwise healthy, free-living volunteers with primary familial hyperlipidemia who had...
no history of chronic illness, including hepatic, renal, thyroid, and cardiac dysfunction, before admission to the study and aged between 35 and 58 y (Table 1) were recruited as described previously (12). Women were either postmenopausal or had undergone a hysterectomy. Subjects were selected on the basis of screening values for total circulating cholesterol and triacylglycerol concentrations. Criteria for acceptance were plasma total cholesterol concentration between 5.2 and 9.0 mmol/L and circulating triacylglycerol concentration < 3.5 mmol/L. Subjects were required to refrain from using drug therapy for hypercholesterolemia during and for ≥8 wk before the start of the study. Blood samples from fasting subjects and urine samples were collected for serum biochemistry, hematology, and urinalysis. The experimental protocol was approved by the Human Ethical Review Committee of the Faculty of Agriculture and Environmental Sciences for the School of Dietetics and Human Nutrition at McGill University. Before study commencement, all subjects were informed of the protocol before signing the consent form.

Experimental design and diets. The study was performed as a randomized, crossover, double-blind clinical trial with 4 dietary treatments of 21 feeding days, each separated by a 4-wk washout period during which the subjects consumed their habitual diets (12). To reduce the error term associated with diet sequencing, subjects were randomly assigned to 1 of 4 predetermined Latin squares, each of which possessed 4 sequenced phases and 4 subjects.

The diets contained plant sterols (S), plant stanols (SN), a 50:50 mixture of sterols and stanols (SSN), or cornstarch (control). The S supplement consisted of purified phytosterols derived from soybeans and contained 43% sitosterol, 26% campesterol, 17% stigmasterol, and 14% other identified phytosterols. The same soybean phytosterols were hydrogenated to produce the SN supplement, a composition of sitostanol (66%) and campestanol (33%). For the SSN treatment, an emulsion of phytosterols derived from soybeans was added to the diet. The control product was cornstarch, which strongly resembled the white, powdery phytosterol-containing mixtures. Sterols, stanols, the mixture of sterols and stanols, and the cornstarch control containers were coded to achieve double blinding, randomly assigned to 1 of 4 predetermined Latin squares, each of which required to consume a minimum of 2 meals/d, including breakfast, under supervision at the Nutrition Research Unit. The third meal was available for take-out.

Plasma lipid analysis. Blood samples were taken from fasting subjects at the start (d 1) and end (d 22) of each dietary phase for measurement of circulating lipid concentrations as described previously (12). The LDL-C concentration was calculated using the Friedewald equation (14).

LDL particle size characterization. Nondenaturing 2–16% polyacrylamide gel electrophoresis was performed on whole plasma taken at d 21 and 22 using procedures described previously (15). Briefly, LDL particle size phenotype was determined on 8 × 8-cm polyacrylamide gradient gels. A 15-min prerun at 75 V preceded electrophoresis of the plasma samples at 150 V for 3 h. Gels were stained for 1 h with Sudan black (0.07%) and stored in a 0.81% acetic acid:4% methanol solution. The Imager 1-D Prime computer software (Amersham Pharmacia Biotech) was used to analyze the gels. LDL size was extrapolated from the relative migration of 4 standards. The LDL diameter was adjusted accordingly where necessary.

Meals were prepared in the Mary Emily Clinical Nutrition Research Unit metabolic kitchen of McGill University. Subjects were required to consume a minimum of 2 meals/d, including breakfast, under supervision at the Nutrition Research Unit. The third meal was available for take-out.

### Statistical analyses
All data are expressed as means ± SD. Postdiet values as well as absolute and relative changes in lipoprotein cholesterol, triacylglycerol levels, and LDL electrophoretic characteristics were analyzed for within and between differences using a repeated-measures ANOVA. When treatment effects were identified as significant, a Tukey test was used for identifying significant effects between diets. Inclusion of a “diet × sex” interaction term in the models indicated that sex did not modulate the LDL size response to experimental diets. Because the comparison of postdiet values and of absolute and relative changes yielded similar results, only the comparisons of postdiet values across the 4 dietary regimens are presented. A level of significance at P < 0.05 was used in all analyses. The data were analyzed using the PROC general linear model procedure in SAS (version 8.1; SAS Institute).

### RESULTS

**Subject baseline characteristics.** Sixteen subjects commenced the study protocol and 15 completed all 4 treatments. As indicated in a previous report (12), 1 man dropped out at the end of the first feeding cycle because of difficulties with daily transportation to the unit. Also, we were not able, despite several attempts, to analyze the polyacrylamide gel electrophoresis of 1 man. Therefore, complete data for 8 men and 6 women were analyzed. All 4 regimens were well tolerated, and participants were unable to distinguish among dietary treatments. There were no significant mean group weight changes across any of the 4 dietary phases.

**Plasma lipid concentrations.** As reported previously (12), plasma total cholesterol concentrations decreased by 6.1, 10.9, and 12.2% at the end of the dietary period in the S, SN, and SSN groups, respectively (P < 0.002) (Table 2). LDL-C concentrations decreased by 8.8, 13.6, and 13.1 in the S, SN,
and SSN groups, respectively, \( P < 0.01 \) with a significant increase of 4.3% in the control group. Plasma triacylglycerol and HDL cholesterol concentrations did not differ among the treatments.

**LDL size phenotype.** The baseline LDL characteristics in this sample of hypercholesterolemic subjects reflected a relatively favorable profile with large LDL-PDD (~26.1 nm) and with <25% of LDL-\( C_{<25.5 \text{ nm}} \) and >50% of LDL-\( C_{>26.0 \text{ nm}} \) (Table 2). There was no change after any of the experimental diets in the LDL-PDD and the LDL integrated size. S and SSN did not affect plasma LDL-\( C_{<25.5 \text{ nm}} \) and LDL-\( C_{>26.0 \text{ nm}} \) levels. Men and women did not differ in their LDL electrophoretic characteristic response to dietary sterols and stanols (data not shown). There was a trend \( (P = 0.07) \) toward a decrease in plasma LDL-\( C_{>26.0 \text{ nm}} \) and LDL-\( C_{25.5-26.0 \text{ nm}} \) levels with SSN supplementation, whereas the reduction in plasma LDL-C levels with SN was due almost exclusively to a decrease in the concentration of LDL-\( C_{26.0 \text{ nm}} \) \( (P < 0.05) \). It must be stressed that overall, the effect of each supplement on the electrophoretic characteristics of LDL did not differ from those in response to the control diet.

**DISCUSSION**

The objective of this strictly controlled dietary experiment in hypercholesterolemic men and women was to investigate the effects of phytosterol consumption on several LDL electrophoretic characteristics and to compare, for the first time, the effects of unesterified plant sterols and stanols on size and composition of LDL subclasses. The major finding of this study was that the treatment with sterols and stanols in hypercholesterolemic diets did not affect LDL particle size phenotype, although supplementation with stanols tended to lower LDL-C \( (P = 0.07) \) in the large LDL subfraction (LDL-\( C_{>26.0 \text{ nm}} \)).

Cholesterol levels in various LDL subclasses were shown to be influenced by dietary and pharmacologic interventions, particularly those that affect plasma triacylglycerol concentrations (19). Thus, diets rich in SFA or low in fat have been associated with increased and decreased LDL particle size, respectively (20). Information about the effect of phytosterols on LDL subclasses is limited. Matvienko et al. (10) showed that phytosterols had virtually no effect on LDL particle size phenotype, whereas Pederson et al. (11) suggested that the effect of sunflower and rapeseed oils on LDL size phenotype compared with olive oil might be attributed to the variable phytosterol content of the experimental oils. In the present study, the reduction in plasma total and LDL-C levels by the phytosterol or phytostanol supplements was not paralleled by significant shifts from smaller to larger LDL or by an increase in LDL particle size.

The lack of effect of phytosterol and phytostanol supplementation on LDL peak particle size may be largely attributable to a lack of change in plasma triacylglycerol levels. This agrees with results in a previous study examining the effects of plant sterols on plasma lipid concentrations (8,12). Elevated triacylglycerol levels are associated with an increased risk of CHD, possibly through physiologically important alterations in the lipid compositions of LDL and HDL particles (21). Patients with hypertriglyceridemia are more likely to possess small, dense LDL particles (2). Indeed, triacylglycerol-enriched LDL, common in hypertriglyceridemic states, are more susceptible to hydrolysis by intravascular lipolytic enzymes such as hepatic lipase (22). Through the same pathway, interventions aimed at modifying plasma triacylglycerol levels are most likely to influence LDL particle size phenotype. As described previously, the treatment period lasted 3 wk, which was long enough to induce significant reductions in plasma cholesterol levels. It remains possible that this period was not long enough to modify LDL electrophoretic characteristics.

We recently reported, based on the large population-based Québec Cardiovascular Study (15), that individuals with small, dense LDL were at greater risk for CHD. We also suggested that information on the distribution of cholesterol among LDL of various sizes may contribute to a more adequate characterization of CHD risk than the more traditional mea-

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**TABLE 2**

**Plasma lipid concentration and LDL electrophoretic characteristics in hypercholesterolemic men and women at baseline and d 22 of control, S, SN, and SSN dietary treatments**

<table>
<thead>
<tr>
<th>LDL subclasses, ( \text{mmol/L} )</th>
<th>Control Baseline</th>
<th>Control d 22</th>
<th>S Baseline</th>
<th>S d 22</th>
<th>SN Baseline</th>
<th>SN d 22</th>
<th>SSN Baseline</th>
<th>SSN d 22</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total cholesterol</strong></td>
<td>6.1 ± 1.3</td>
<td>6.2 ± 1.0*</td>
<td>6.0 ± 1.1</td>
<td>5.6 ± 0.9†</td>
<td>6.2 ± 0.9</td>
<td>5.5 ± 0.9†</td>
<td>6.4 ± 1.3</td>
<td>5.6 ± 1.0†</td>
</tr>
<tr>
<td><strong>LDL-C</strong></td>
<td>4.0 ± 1.1</td>
<td>4.1 ± 0.8</td>
<td>4.0 ± 0.8</td>
<td>3.8 ± 0.7</td>
<td>4.1 ± 0.7</td>
<td>3.5 ± 0.7</td>
<td>4.2 ± 1.0</td>
<td>3.6 ± 0.7</td>
</tr>
<tr>
<td><strong>Triacylglycerols</strong></td>
<td>1.93 ± 0.77</td>
<td>2.16 ± 1.29</td>
<td>1.85 ± 0.81</td>
<td>1.88 ± 0.95</td>
<td>2.15 ± 0.98</td>
<td>1.89 ± 0.80</td>
<td>2.28 ± 1.44</td>
<td>1.99 ± 1.28</td>
</tr>
<tr>
<td><strong>LDL peak particle size, nm</strong></td>
<td>26.07 ± 0.84</td>
<td>26.13 ± 0.75</td>
<td>26.17 ± 0.69</td>
<td>26.09 ± 0.76</td>
<td>26.07 ± 0.87</td>
<td>25.97 ± 0.93</td>
<td>26.19 ± 0.52</td>
<td>26.00 ± 0.72</td>
</tr>
<tr>
<td><strong>LDL integrated size, nm</strong></td>
<td>26.14 ± 0.39</td>
<td>26.11 ± 0.47</td>
<td>26.13 ± 0.43</td>
<td>26.14 ± 0.42</td>
<td>26.09 ± 0.47</td>
<td>26.02 ± 0.57</td>
<td>26.12 ± 0.34</td>
<td>26.04 ± 0.44</td>
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<tr>
<td><strong>Relative distribution among LDL subclasses, %</strong></td>
<td></td>
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<tr>
<td>LDL-( C_{&lt;25.5 \text{ nm}} )</td>
<td>15.6 ± 17.3</td>
<td>14.7 ± 14.6</td>
<td>15.0 ± 16.0</td>
<td>15.9 ± 16.3</td>
<td>17.6 ± 14.8</td>
<td>22.0 ± 21.7</td>
<td>15.9 ± 15.7</td>
<td>20.0 ± 20.1</td>
</tr>
<tr>
<td>LDL-( C_{25.5-26.0 \text{ nm}} )</td>
<td>27.0 ± 7.8</td>
<td>29.3 ± 8.0</td>
<td>28.8 ± 4.4</td>
<td>28.2 ± 9.4</td>
<td>29.7 ± 7.8</td>
<td>30.8 ± 10.4</td>
<td>32.7 ± 10.6</td>
<td>29.0 ± 7.5</td>
</tr>
<tr>
<td>LDL-( C_{&gt;26.0 \text{ nm}} )</td>
<td>57.4 ± 21.6</td>
<td>52.2 ± 21.2</td>
<td>55.9 ± 21.6</td>
<td>53.9 ± 21.9</td>
<td>52.7 ± 17.9</td>
<td>47.2 ± 22.8</td>
<td>52.7 ± 19.3</td>
<td>51.0 ± 21.9</td>
</tr>
<tr>
<td><strong>Cholesterol levels among LDL subclasses, mmol/L</strong></td>
<td></td>
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<tr>
<td>LDL-( C_{&lt;25.5 \text{ nm}} )</td>
<td>0.60 ± 0.59</td>
<td>0.73 ± 0.74</td>
<td>0.50 ± 0.61</td>
<td>0.72 ± 0.74</td>
<td>0.68 ± 0.55</td>
<td>0.82 ± 0.74</td>
<td>0.66 ± 0.68</td>
<td>0.76 ± 0.88</td>
</tr>
<tr>
<td>LDL-( C_{25.5-26.0 \text{ nm}} )</td>
<td>1.26 ± 0.64</td>
<td>1.21 ± 0.41</td>
<td>1.08 ± 0.33</td>
<td>1.01 ± 0.34</td>
<td>1.20 ± 0.36</td>
<td>1.08 ± 0.44</td>
<td>1.36 ± 0.55</td>
<td>1.06 ± 0.44</td>
</tr>
<tr>
<td>LDL-( C_{&gt;26.0 \text{ nm}} )</td>
<td>2.14 ± 0.60</td>
<td>2.11 ± 1.04</td>
<td>2.31 ± 0.96</td>
<td>2.07 ± 1.01</td>
<td>2.20 ± 0.97</td>
<td>1.65 ± 0.86</td>
<td>2.16 ± 0.88</td>
<td>1.75 ± 0.72</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, \( n = 14 \).
2 Means in a row with different superscripts differ, \( P < 0.05 \). * Different from baseline, \( P < 0.05 \); † different from baseline, \( P < 0.01 \); ‡ different from baseline, \( P < 0.001 \).
ures corresponding to the LDL peak particle diameter. We chose therefore, to investigate the effect of sterol and stanol supplementation on the relative and absolute cholesterol levels of large and small particles. Our data suggest that sterols in their esterified or unesterified form have very little effect, if any, on the distribution of cholesterol among various LDL subclasses. Further studies will be required to conclude definitively that phytosterols and phytostanols have no effect on LDL subclasses. It is also possible that the relatively favorable baseline LDL size profile in subjects of the present study may have impeded any further change in the various electrophoretic characteristics of LDL.

Another novel aspect of this study is the strictly controlled design, and compliance was considered to be optimal. In the stanol-supplemented groups with or without sterols, the reduction in LDL-C resulted primarily from a decrease in the cholesterol found in the large-size LDL subclass (LDL-C_{>26.0 nm}) with no change in the relative or absolute levels of small LDL particles. The S supplement was not associated with any significant change in LDL subclasses. On the basis of several clinical trials, it is anticipated that the reduction in plasma LDL-C levels with consumption of phytosterols and phytostanols will lead to significantly reduced CHD risk (8,10,12). Although the independent association between a predominance of small, dense LDL and an increased risk of CHD is not well recognized, the extent to which a decrease in LDL-C_{>26.0 nm} will contribute to modulate CHD risk is also not clear.

In conclusion, the results of the present study indicated that the cholesterol-lowering properties of unesterified plant sterols and stanols were accompanied by only minor modifications in LDL electrophoretic characteristics, including a reduction in the LDL-C_{>26.0 nm} levels. The clinical relevance of these findings in terms of CHD risk remains to be determined.

**LITERATURE CITED**


