Plant sterols are efficacious in lowering plasma LDL and non-HDL cholesterol in hypercholesterolemic type 2 diabetic and nondiabetic persons

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ABSTRACT
Background: Because of hyperglycemia and hyperinsulinemia, diabetic persons have higher cholesterol synthesis and lower cholesterol absorption rates than do nondiabetic persons. Differences in plant sterol efficacy between diabetic and nondiabetic persons have not been examined.

Objective: The objective was to compare the degree of response of plasma lipid concentrations and glycemic control to plant sterol consumption in a controlled diet between hypercholesterolemic type 2 diabetic and nondiabetic subjects.

Design: Fifteen nondiabetic subjects and 14 diabetic subjects participated in a double-blinded, randomized, crossover, placebo-controlled feeding trial. The diet included 1.8 g/d of either plant sterols or cornstarch placebo over 21 d, separated by a 28-d washout period.

Results: Plant sterol consumption significantly reduced ($P < 0.05$) LDL-cholesterol concentrations from baseline in both nondiabetic and diabetic subjects by 15.1% and 26.8%, respectively. The diabetic subjects had significantly ($P < 0.05$) lower absolute concentrations of total cholesterol after treatment than did the nondiabetic subjects; however, there was no significant difference in the percentage change from the beginning to the end of the trial. There was also a significant decrease ($P < 0.05$) in absolute non-HDL-cholesterol concentrations after treatment in both groups.

Conclusions: The results showed that plant sterols are efficacious in lowering LDL cholesterol and non-HDL cholesterol in both diabetic and nondiabetic persons. Plant sterol consumption may exist as a dietary management strategy for hypercholesterolemia in persons with type 2 diabetes. Am J Clin Nutr 2005;81:1351–8.

KEY WORDS Hypercholesterolemia, type 2 diabetes, plant sterols, cholesterol

INTRODUCTION

Type 2 diabetes is associated with metabolic disturbances, including hyperglycemia, insulin resistance, frequent dyslipidemia, and impaired β-cell function (1–3), and is frequently linked with several lipid aberrations, namely, hypertriglyceridemia, elevated VLDL cholesterol, and reduced HDL cholesterol (4–7). However, elevated LDL-cholesterol concentrations are not uniformly present in dyslipidemic, type 2 diabetic persons (8). Studies have shown that persons with type 2 diabetes have an increased synthesis and a decreased absorption of cholesterol (9–13). As a result, the risk of developing atherosclerotic vascular diseases, such as cardiovascular disease (CVD), in type 2 diabetic is 2- to 7-fold that in nondiabetic persons (12, 14, 15).

Diabetic patients with no history of heart disease and nondiabetic persons with a history of myocardial infarction are equally at risk of an infarction (15). Thus, the target blood lipid concentrations are more stringent for diabetic than for nondiabetic persons (16) and should be as follows: ≤2.5 mmol/L for LDL cholesterol, ≤2.0 mmol/L for triacylglycerol, and ≤4.0 for the ratio of total cholesterol (TC) to HDL cholesterol (17).

Plant sterols, which chemically resemble cholesterol, have been shown to block the absorption of dietary and endogenously derived cholesterol from the gut (18, 19). Plant sterols are not synthesized by the human body and are minimally absorbed by the human intestine (20). Daily consumption of 0.7–3.2 g plant sterols/d has been shown to reduce plasma TC by 5.0–13.0%, and LDL cholesterol by 5.6–24.4% in both normo- and hypercholesterolemic persons with (10, 21–23) and without (18, 22, 24–35) type 2 diabetes.

The efficacy of plant sterols in diabetic subjects as compared with that in nondiabetic subjects has not been well examined in previous studies. Further investigation is essential to define dietary strategies that can best normalize the risk of CVD and associated complications in type 2 diabetes. Therefore, the objective of this study was to compare the degree of response of plasma lipid concentrations and glycemic control between hypercholesterolemic type 2 diabetic and nondiabetic subjects consuming plant sterols in a precisely controlled dietary setting.

SUBJECTS AND METHODS

Subjects

Fourteen slightly overweight hypercholesterolemic subjects with type 2 diabetes and 15 hypercholesterolemic nondiabetic
subjects were recruited from the surrounding community by advertisements in local newspapers and in private and public medical clinics. The inclusion criteria required all subjects to be ambulatory, to be between the ages of 40 and 80 y, and to have an LDL-cholesterol concentration of 3–5 mmol/L, a tricylglycerol concentration <5 mmol/L, and a body mass index (BMI; in kg/m²) between 23 and 40. The diagnostic criteria for type 2 diabetes included a fasting plasma glucose concentration ≥7 mmol/L and a glycated hemoglobin (Hb A₁c) concentration of 7–8%. Exclusion criteria included the use of β-blockers or diuretics and a personal history of CVD. Those who reported exercising at a frequency of ≥5 times/wk, being pregnant, or lactating were also excluded. Subjects were required to have refrained from using drug therapy for hypercholesterolemia for the 8-wk period before the start of the study.

Before acceptance into study, the subjects were required to undergo a complete physical examination. Fasting blood and urine samples were collected for serum biochemistry, hematology, and urinalysis. Subjects were screened for chronic illnesses—including hepatic, renal, thyroid, and cardiac dysfunction—before admission in the study. The subjects received a thorough explanation of the study protocol and were given opportunities to discuss any concerns with the principal investigator, attending physician, or research coordinators before signing a consent form. The experimental protocol was approved by the Human Ethical Review Committee of the Faculty of Agriculture and Environmental Sciences at McGill University.

**Study protocol**

The study was a randomized, double-blind, crossover, placebo-controlled clinical trial that consisted of two 21-d dietary feeding periods that were separated by a 28-d washout period. During the washout period, the subjects resumed their habitual diets without restriction.

At baseline (day 0) and at the end (day 21 and day 22) of each dietary phase, fasting blood samples were taken for the measurement of circulating lipid concentrations. In the comparison of endpoints for the 2 dietary phases, the mean of days 21 and 22 was used to account for day-to-day variation in circulating cholesterol concentrations. Day 0 and day 21 blood samples were also used for the measurement of circulating plant sterol, insulin, and Hb A₁c concentrations. At the start (day 1) and end (day 21) of each dietary phase, fasting blood samples were taken for the measurement of circulating fatty acids.

Subjects were given routine physical examinations at the beginning and end of each dietary phase by the attending physician. Throughout the trial, a physician familiar with the study protocol and diets was available in case subjects experienced discomfort with the diet.

**Experimental diets**

The baseline control diet (BCD) was planned based on the Canada Food Guide to Healthy Eating and the Good Healthy Eating Guide (36). The BCD consisted of solid foods typical of those consumed in North America and was provided as 3 meals and 1 snack per day in a 3-d rotating menu. The nutrient content of the BCD was calculated by using FOOD PROCESSOR (Esha Research, Salem, OR), a computerized dietary analysis system. The BCD was designed to meet recommended intakes for all vitamins and minerals. The Mifflin equation was used to estimate individual basal energy requirements (37), which was then multiplied by an activity factor of 1.7 to compensate for the additional energy need of mildly to moderately active healthy adults (38). If subjects gained or lost weight during the first week of each dietary phase, adjustments were made to individual energy requirements to ensure that baseline body weights were maintained (31). Body weight was monitored daily before breakfast during the feeding periods to assess changes.

The BCD contained 55% of energy as carbohydrate, of which ≥75% was complex carbohydrate; foods with a low glycemic index were selected when possible. The polyunsaturated:monounsaturated:saturated fatty acid ratio of the 30% of energy provided as fat was maintained at 1:1:1. Safflower, canola, and flaxseed oils were used to provide most of the polyunsaturated fatty acids, whereas extra virgin olive oil was selected to provide monounsaturated fatty acids. Saturated fat came from fats in the meat and palm oil. Flaxseed and canola oils were used as sources of essential fatty acids at the level of 2-3%. Protein accounted for 15% of ingested energy.

During each feeding period, a total of 1.8 g/d of either plant sterol (plant sterol phase) powder or placebo phase cornstarch was added to margarine and served on the breakfast toast every morning under supervision. The plant sterol powder was unesterified plant sterols extracted from wood pulp byproducts (40% sitosterol, 30% campesterol, 20% dihydroß brassicasterol, and 10% others; Forbes Medi-Tech Inc, Vancouver, Canada). The placebo powder was cornstarch, because it strongly resembled the white powdery plant sterols (39). To achieve double blinding, plant sterol powder and the cornstarch were portioned in coded containers by an external party so that neither the researchers nor the subjects would know its true identity. The diets were prepared in the metabolic kitchen of the Mary Emily Clinical Nutrition Research Unit of McGill University. All subjects were required to consume breakfast at the Unit under supervision; the other 2 meals and 1 snack were available for takeout. No extra food was allowed between meals, except for decaffeinated, energy-free carbonated beverages and herbal teas, which were obtained from the kitchen’s staff. Alcoholic beverages and coffee were prohibited during the dietary phases.

**Plasma lipid concentrations**

Blood samples were drawn, after the subjects had fasted overnight for 12 h and had abstained from alcohol for ≥24 h (day 0), and collected into EDTA-containing Vacutainer (BD, Franklin Lakes, NJ) tubes. Samples were immediately centrifuged (320 × g, 15 min, room temperature), and the resulting plasma and red blood cell (RBC) subfractions were separated within 1 h of collection and stored at −80 °C until analyzed. Plasma total and HDL cholesterol and triacylglycerol were analyzed in quadruplicate with standardized reagents by using a VP Autoanalyser (Abbott Laboratories, North Chicago, IL). Calibration of the machine before each run was performed as per the standardization protocol of the Canadian Reference Laboratory (1996; Vancouver, Canada), which involved direct comparison with fresh specimen samples. Plasma HDL cholesterol was measured after precipitation of apolipoprotein B with dextran sulfate and magnesium chloride (40). LDL-cholesterol concentrations were calculated by using the Friedewald equation (41).
Fatty acid methyl ester composition

RBCs in blood collected on days 1 and 21 were analyzed in duplicate for fatty acid composition by gas-liquid chromatography (GLC) (HP 5890 Series II; Hewlett-Packard, Palo Alto, CA). A modified Folch extraction was used to extract total lipids from the samples (42), and the fatty acids were methylated as per the procedure by Al Makdessi et al (43). Packed RBCs and C17 standard (1 mg/mL) were placed into a culture tube, and MeOH was added to the sample. The culture tube was then heated to 55 °C in a water bath for 15 min. A solution of hexane:chloroform (4:1 by vol) was added and placed in a wrist action shaker for 15 min. Water (Millipore, Nepean, Canada) was added to the sample, and the sample was shaken for an additional 10 min. The sample was centrifuged at 520 × g (1500 rpm) at 4 °C for 15 min, and the organic supernatant fluid was transferred to a culture tube and dried under nitrogen at 45 °C. The aqueous layer was then reextracted by adding hexane:chloroform, and the sample was shaken for 15 min. It was centrifuged at 1500 rpm, and the supernatant fluid was added to the first extraction and dried down. Methylating reagent (7:6:7, BF₃MeOH:benzene:MeOH) was added to the sample. The tube was flushed with nitrogen, sealed with polytetrafluoroethylene tape, and mixed by vortex. The tubes were heated at 100 °C for 55 min and allowed to cool in tepid water. Hexane and Millipore water were added to the sample, after which it was vortex mixed. The top layer was transferred to a 1.5-mL crimp seal vial and dried down under nitrogen; chloroform was added and the top layer was transferred to polypropylene vials inserts.

The composition of fatty acid methyl esters in the RBCs was determined by using a Hewlett-Packard 5890 gas-liquid chromatograph equipped with a 30 m × 0.2 mm SP 2330 column (Supelco, Bellefonte, PA), flame ionization detectors, and automated injection (44). Briefly, the oven temperature was held at 100 °C for 1 min and increased to 190 °C at a rate of 3 °C/min, after which it was held at this temperature for the remainder of the run. The injector temperature was set at 210 °C and the detector temperature at 250 °C. Fatty acid methyl esters were identified based on the retention time of known standards (Supelco). All results are expressed as a percentage of total fatty acids by weight (wt/wt%).

Plasma plant sterol concentrations

Plant sterols were measured by GLC (HP 5890 Series II; Hewlett-Packard) facilitated with flame ionization detection and auto-injector system as described (26, 31, 45). A 30-m SAC-5 column (Sigma-Aldrich Canada Ltd, Oakville, Canada) was used. Briefly, an internal standard, 5 α-cholestanol, was added to each plasma sample. Samples were saponified and sterols were extracted, resuspended in chloroform, and injected into the gas-liquid chromatograph. The column temperature was 285 °C. Isothermal running conditions were maintained for 42 min. The injector and detector were set at 300 and 310 °C, respectively. The carrier gas (helium) flow rate was 1.2 mL/min and inlet splitter set at 100:1. Plant sterols were identified compared with authentic standards (Sigma-Aldrich Canada Ltd). Internal standards were used to calculate detector response factors.

Glycemic control

Insulin concentrations were measured, in duplicate, in the day 0 and day 21 plasma samples with the use of a commercially available radioimmunoassay kits (ICN Pharmaceuticals, Inc, Costa Mesa, CA) with 125I as a tracer. Radioactivity was determined by gamma counting (1282 compugamma CS, LKB Wallac, Fisher Scientific, Montreal, Canada) and collected as counts per min. Plasma values were quantified by using a standard curve and automated data reduction procedures. Insulin values were expressed as μU/mL. Hb A₁c, was measured in day 0 and day 21 blood samples at a clinical diagnostic laboratory (LDS Laboratories, Montreal, Canada).

Statistics

The results are presented as means ± SEMs. Differences between groups at baseline were analyzed by using an analysis of variance (ANOVA) model. When a significant difference was found, a Tukey’s post hoc test was performed to determine the differences between group means. When baseline differences were noted for a specific variable, an analysis of covariance was performed with the baseline value as a covariate. Differences between group posttreatment values, and the percentage change from the beginning to the end of the trial, were analyzed by using a two-factor ANOVA model that identified diabetic state and plant sterol effects and their interactions. Statistical significance was set at a P value <0.05 in all analyses. Tests for normality were included in the model. Data were analyzed by using SAS software (version 8.0; SAS Institute Inc, Cary, NC).

RESULTS

Subject compliance and dropout rate

Sixteen hypercholesterolemic nondiabetic and 16 diabetic persons were enrolled in the study. One nondiabetic subject dropped out at the first week of the second feeding cycle because of a myocardial infarction. Two diabetic subjects dropped out at the first week of the first feeding cycle because of personal reasons. Therefore, complete data from 15 nondiabetic and 14 diabetic subjects were collected and analyzed as per the study protocol. The BCD was well tolerated overall. However, some subjects reported minor gastrointestinal discomfort, which did not require medical intervention or lead to the withdrawal of any subject from the study. During the first week of the first feeding trial, some subjects reported that they were given too much food, ie, the meal sizes were larger than those of their habitual diets. However, the subjects consumed all of the food provided.

Subject characteristics

Baseline characteristics of the study subjects are presented in Table 1. Lipid concentrations denoted in the table are based on the values obtained from the initial blood screen. There were no significant differences in age, weight, or TC, LDL, and HDL concentrations. However, BMI, triacylglycerol, fasting blood glucose, and Hb A₁c were significantly higher in the diabetic than in the nondiabetic group. There were no significant differences in weight at the endpoint and no changes across either phase for any group (data not shown).

Plasma lipid profile in response to treatment

The mean plasma lipid concentrations at baseline (day 0) and at the end (day 21) of each dietary phase are shown in Table 2. There were no significant differences in mean TC concentrations.
Mean LDL-cholesterol concentrations were shown to be significantly ($P < 0.05$) different between groups at baseline. After further analysis, it was shown that the mean baseline LDL-cholesterol concentrations of the diabetic subjects who received placebo were significantly lower ($P < 0.05$) than those of the nondiabetic subjects. Results of the two-factor ANOVA showed no significant diabetic state $\times$ sterol interaction. When LDL-cholesterol concentrations were expressed as the percentage change between pre- and posttreatment values, with baseline as covariate, a significant ($P < 0.05$) main effect of sterols was noted for nondiabetic and diabetic subjects combined.

Triacylglycerol concentrations were not significantly different between groups at baseline. Results of the two-factor ANOVA showed no significant diabetic state $\times$ sterol interaction. Similarly, there were no significant changes in triacylglycerol across groups or treatments.

Mean HDL-cholesterol concentrations at baseline were not significantly different between groups. In addition, diabetic state $\times$ sterol interactions were not significant. Moreover, no significant main effects of diabetic state or sterol were noted for absolute HDL-cholesterol concentrations or percent changes post-treatment.

Mean non-HDL-cholesterol concentrations at baseline were not significantly different between groups. In addition, diabetic

### TABLE 1

Baseline characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Nondiabetic ($n = 9$ F, 6 M)</th>
<th>Diabetic ($n = 9$ F, 5 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td>55.1 $\pm$ 2.2</td>
<td>54.5 $\pm$ 1.8</td>
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<tr>
<td><strong>Weight (kg)</strong></td>
<td>76.6 $\pm$ 2.6</td>
<td>82.8 $\pm$ 2.4</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>26.9 $\pm$ 0.8</td>
<td>30.2 $\pm$ 1.1 $^2$</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/L)</strong></td>
<td>6.12 $\pm$ 0.21</td>
<td>6.11 $\pm$ 0.17</td>
</tr>
<tr>
<td><strong>LDL cholesterol (mmol/L)</strong></td>
<td>3.95 $\pm$ 0.17</td>
<td>3.62 $\pm$ 0.14</td>
</tr>
<tr>
<td><strong>HDL cholesterol (mmol/L)</strong></td>
<td>1.39 $\pm$ 0.09</td>
<td>1.22 $\pm$ 0.07</td>
</tr>
<tr>
<td><strong>Triacylglycerol (mmol/L)</strong></td>
<td>1.71 $\pm$ 0.18</td>
<td>3.00 $\pm$ 0.36 $^2$</td>
</tr>
<tr>
<td><strong>Fasting glucose (mmol/L)</strong></td>
<td>4.83 $\pm$ 0.09</td>
<td>9.66 $\pm$ 0.79 $^2$</td>
</tr>
<tr>
<td><strong>Glycated hemoglobin (% of total hemoglobin)</strong></td>
<td>0.055 $\pm$ 0.002</td>
<td>0.073 $\pm$ 0.004 $^2$</td>
</tr>
</tbody>
</table>

$^1$ All values are $\bar{x} \pm$ SEM.

$^2$ Significantly different from nondiabetic subjects, $P < 0.05$ (ANOVA).

between groups at baseline. When the data were analyzed by using a two-factor ANOVA, diabetic state $\times$ sterol interactions were not significant. Additionally, there was no significant main effect of sterol on posttreatment absolute TC values. However, a significant ($P < 0.05$) main effect of diabetic state was noted on posttreatment absolute TC values.

### TABLE 2

Plasma lipid concentrations at baseline and after treatment

<table>
<thead>
<tr>
<th>Lipid variable and intervention group</th>
<th>Baseline $^1$</th>
<th>After treatment $^1$</th>
<th>Change $^3$</th>
<th>Main effect of diabetic state $^4$</th>
<th>Main effect of PS $^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol/L</td>
<td>mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic, PS ($n = 14$)</td>
<td>5.84 $\pm$ 0.39</td>
<td>4.89 $\pm$ 0.29</td>
<td>0.04</td>
<td>0.74</td>
<td>0.51</td>
</tr>
<tr>
<td>Diabetic, placebo ($n = 14$)</td>
<td>5.55 $\pm$ 0.32</td>
<td>4.94 $\pm$ 0.35</td>
<td>0.39</td>
<td>0.25</td>
<td>0.56</td>
</tr>
<tr>
<td>Nondiabetic, PS ($n = 15$)</td>
<td>6.06 $\pm$ 0.24</td>
<td>5.39 $\pm$ 0.18</td>
<td>0.67</td>
<td>0.25</td>
<td>0.56</td>
</tr>
<tr>
<td>Nondiabetic, placebo ($n = 15$)</td>
<td>6.21 $\pm$ 0.25</td>
<td>5.49 $\pm$ 0.14</td>
<td>0.72</td>
<td>0.25</td>
<td>0.56</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic, PS ($n = 12$)</td>
<td>3.24 $^{14}$ $\pm$ 0.35</td>
<td>2.48 $\pm$ 0.33</td>
<td>0.19</td>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>Diabetic, placebo ($n = 14$)</td>
<td>2.59 $^{14}$ $\pm$ 0.31</td>
<td>2.67 $\pm$ 0.35</td>
<td>0.18</td>
<td>0.25</td>
<td>0.05</td>
</tr>
<tr>
<td>Nondiabetic, PS ($n = 14$)</td>
<td>3.92 $^{14}$ $\pm$ 0.27</td>
<td>3.29 $\pm$ 0.23</td>
<td>0.63</td>
<td>0.35</td>
<td>0.05</td>
</tr>
<tr>
<td>Nondiabetic, placebo ($n = 13$)</td>
<td>3.84 $^{14}$ $\pm$ 0.29</td>
<td>3.59 $\pm$ 0.20</td>
<td>0.25</td>
<td>0.35</td>
<td>0.05</td>
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<tr>
<td>HDL cholesterol</td>
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</tr>
<tr>
<td>Diabetic, PS ($n = 14$)</td>
<td>1.37 $\pm$ 0.13</td>
<td>1.37 $\pm$ 0.18</td>
<td>0.09</td>
<td>0.20</td>
<td>0.38</td>
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<tr>
<td>Diabetic, placebo ($n = 14$)</td>
<td>1.18 $\pm$ 0.07</td>
<td>1.08 $\pm$ 0.08</td>
<td>0.10</td>
<td>0.20</td>
<td>0.38</td>
</tr>
<tr>
<td>Nondiabetic, PS ($n = 15$)</td>
<td>1.32 $\pm$ 0.10</td>
<td>1.23 $\pm$ 0.11</td>
<td>0.11</td>
<td>0.20</td>
<td>0.38</td>
</tr>
<tr>
<td>Nondiabetic, placebo ($n = 15$)</td>
<td>1.34 $\pm$ 0.10</td>
<td>1.22 $\pm$ 0.09</td>
<td>0.12</td>
<td>0.20</td>
<td>0.38</td>
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<tr>
<td>Triacylglycerol</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Diabetic, PS ($n = 14$)</td>
<td>2.71 $\pm$ 0.53</td>
<td>2.75 $\pm$ 0.49</td>
<td>0.11</td>
<td>0.79</td>
<td>0.84</td>
</tr>
<tr>
<td>Diabetic, placebo ($n = 14$)</td>
<td>3.12 $\pm$ 0.47</td>
<td>2.63 $\pm$ 0.42</td>
<td>0.49</td>
<td>0.79</td>
<td>0.84</td>
</tr>
<tr>
<td>Nondiabetic, PS ($n = 15$)</td>
<td>1.98 $\pm$ 0.31</td>
<td>2.07 $\pm$ 0.34</td>
<td>0.09</td>
<td>0.79</td>
<td>0.84</td>
</tr>
<tr>
<td>Nondiabetic, placebo ($n = 15$)</td>
<td>2.26 $\pm$ 0.27</td>
<td>1.97 $\pm$ 0.40</td>
<td>0.29</td>
<td>0.79</td>
<td>0.84</td>
</tr>
<tr>
<td>Non-HDL cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic, PS ($n = 14$)</td>
<td>4.47 $\pm$ 0.41</td>
<td>3.51 $\pm$ 0.34</td>
<td>0.05</td>
<td>0.37</td>
<td>0.34</td>
</tr>
<tr>
<td>Diabetic, placebo ($n = 14$)</td>
<td>4.37 $\pm$ 0.34</td>
<td>3.87 $\pm$ 0.35</td>
<td>0.50</td>
<td>0.37</td>
<td>0.34</td>
</tr>
<tr>
<td>Nondiabetic, PS ($n = 15$)</td>
<td>4.74 $\pm$ 0.22</td>
<td>4.16 $\pm$ 0.16</td>
<td>0.58</td>
<td>0.37</td>
<td>0.34</td>
</tr>
<tr>
<td>Nondiabetic, placebo ($n = 15$)</td>
<td>4.87 $\pm$ 0.21</td>
<td>4.28 $\pm$ 0.15</td>
<td>0.59</td>
<td>0.37</td>
<td>0.34</td>
</tr>
</tbody>
</table>

$^1$ PS, plant sterols. Means with different superscript letters are significantly different, $P < 0.05$ (Tukey’s post hoc test).

$^2$ There were no significant differences between intervention groups for total cholesterol, triacylglycerol, HDL-cholesterol, and non-HDL-cholesterol concentrations (Tukey’s test).

$^3$ Diabetic state–by-sterol interactions were not significant.

$^4$ Two-factor ANOVA. Analysis of covariance was performed with baseline as a covariate for LDL cholesterol.
state × sterol interactions were not significant. However, a significant \( P < 0.05 \) main effect of diabetic state was noted for posttreatment absolute non-HDL-cholesterol values.

### Plasma plant sterol concentrations in response to treatment

Plasma plant sterol concentrations and ratios relative to TC are presented in Table 3. When the data were analyzed by using a two-factor ANOVA, diabetic state × sterol interactions were not significant. Absolute plasma campesterol and \( \beta \)-sitosterol concentrations were not significantly different between groups at baseline or at the end of each dietary phase. Moreover, there were no differences between groups at baseline and there were no changes at the end of each dietary phase for the campesterol:TC, campesterol:sterol, and \( \beta \)-sitosterol:TC ratios. When campesterol concentrations were expressed as the percentage change between pre- and posttreatment values, a significant main effect of both diabetic state \( P < 0.04 \) and sterol \( P < 0.02 \) was observed. When sitosterol concentrations were expressed as the difference between pre- and posttreatment values, a significant \( P < 0.02 \) main effect of sterol was also observed. A significant main effect of sterol \( P < 0.02 \) was also noted for the percentage change in both the campesterol:cholesterol and sitosterol:cholesterol ratios.

### Changes in red blood cell fatty acid composition within each phase

There were no significant differences in the percentage of fatty acids in RBCs between groups at baseline. When the data were analyzed by using a two-factor ANOVA, diabetic state × sterol interactions were not significant. Additionally, there was no significant main effect of either diabetic state or sterol treatment on absolute posttreatment concentrations. A significant \( P < 0.03 \) main effect of sterol was noted for the change in oleic acid and the sum of monounsaturated fatty acids, relative to baseline.

### Glycemic control in response to treatment

Plasma insulin concentrations at baseline were significantly different \( P < 0.01 \) between diabetic and nondiabetic subjects. When the data were analyzed by using a two-factor ANOVA, diabetic state × sterol interactions were not significant. Absolute concentrations of insulin remained significantly different \( P < 0.01 \) between diabetic and nondiabetic subjects; however, changes in plasma insulin concentrations between the 2 groups were not significantly different with and without plant sterol consumption.

Plasma Hb A1c concentrations at baseline were significantly different \( P < 0.001 \) between diabetic and nondiabetic subjects. When the data were analyzed by using a two-factor ANOVA,
diabetic state × sterol interactions were not significant. Absolute concentrations of Hb A1c remained significantly different (P < 0.001) between diabetic and non-diabetic subjects; however, there was no significant change in Hb A1c concentrations for either group after consumption of each diet for 21 d.

**Associations between plasma lipid and plasma plant sterol concentrations**

Across all subjects, both plasma TC (r = 0.30, P = 0.0017) and LDL-cholesterol (r = 0.38, P = 0.0001) concentrations varied directly with plasma β-sitosterol concentrations. However, neither plasma TC nor LDL-cholesterol was associated with plasma campesterol or the campesterol:β-sitosterol ratio.

**DISCUSSION**

In the current study, overall lipid changes were more favorable in diabetic subjects than in non-diabetic control subjects. There was a significant effect of diabetic state on mean endpoint non-HDL-cholesterol concentrations; the diabetic subjects had lower mean endpoint non-HDL-cholesterol values than did the non-diabetic subjects. Plant sterol consumption also led to significant decreases in LDL cholesterol in diabetics and non-diabetic subjects. Although the 26.8% change in LDL in the diabetic subjects who consumed plant sterols is numerically attractive, it was not statistically different from the value in the control subjects. These effects may have been due to a relatively low absorption efficiency (9), as part of the insulin resistance syndrome (13) in type 2 diabetic subjects. In general, the results of this study agree well with those of earlier studies of various designs using plant sterols, which showed reductions in TC and LDL cholesterol in the range of 5.0–13.0% and 5.6–24.4%, respectively (10, 18, 21–26, 29–32, 34, 35). In the current study, TC decreased from baseline by 14.6% and LDL cholesterol by 26.8% in the diabetic subjects after 21 d. The extent of reduction is generally above that observed in previous studies. On the other hand, the non-diabetic subjects had a 10.4% reduction in TC and a 15.1% reduction in LDL cholesterol; these values are within the previously reported reduction range.

The decrease in TC did not differ significantly between the plant sterol and placebo phases across groups, except for a significant main effect of diabetic state on posttreatment absolute TC values. This result, which was not consistent with the results of most other studies that examined the efficacy of plant sterols, was perhaps due to several features of the study design, including the plasma cholesterol–modifying characteristics of the control diet, subject-specific type of lipid disorder, or plant sterol dose and composition (18). The results of the third National Health and Nutrition Examination Survey showed that moderately high carbohydrate (50–55% of energy) diets, such as the BCD in this study, are associated with low CVD risks and favorable lipid metabolites. However, various plant sterols are absorbed and metabolized differently. Sitosterol made up 40% of the plant sterol composition from day 1 to day 21 within the 2 dietary phases. These data provide indirect evidence that there was good compliance with the dietary modification because the absence of changes in RBC fatty acid composition reflects that there were no changes in the diet within any of the dietary phases. Moreover, given that all diets in this study were isocaloric, there were no statistically significant changes in body weight in either subject group after 2 dietary phases (data not shown). These data provide additional indirect evidence that there was good compliance by the subjects with the diets.

Previous studies have shown that plasma campesterol and sitosterol concentrations increase (31) or remain unchanged (51) with sitosterol feeding. In the current study there was no significant difference between groups in plasma plant sterol concentrations after sterol supplementation. Plasma plant sterol concentrations have been used as an indirect measure of cholesterol absorption. Specifically, serum campesterol concentrations and the campesterol/cholesterol ratio have been shown to correlate positively with intestinal cholesterol absorption. With controlled diets, this association would be expected to reflect cholesterol absorption. However, various plant sterols are absorbed and metabolized differently. Sitosterol made up 40% of the plant sterol regimen used in this study, which has been shown to increase plasma sterol concentrations (31). Thus, it was not appropriate in this study to use plasma campesterol as an indicator of cholesterol absorption.

Another objective of this study was to examine possible effects of plant sterol consumption on glycemic control. There was no improvement in either Hb A1c or insulin with plant sterol feeding. This result disagrees with the recent work of Lee et al (23), in which a significant reduction in Hb A1c occurred in type 2 diabetic subjects, under free-living conditions, after using a plant sterol–enriched spread for 4 wk. However, the duration of the...
PLANT STEROLS LOWER CHOLESTEROL IN TYPE 2 DIABETES

Plant sterol diet and the degree of glycemic control of type 2 diabetic subjects were different compared with our study. In summary, the current study showed that the consumption of plant sterols is efficacious in lowering LDL-cholesterol and non-HDL-cholesterol concentrations in both type 2 diabetic and non-diabetic persons. Plant sterols, therefore, serve as a potential adjunct to dietary management of hypercholesterolemia in patients with type 2 diabetes. The risk of developing CVD is 2- to 7-fold higher in type 2 diabetic than in nondiabetic persons, and this study showed that plant sterol consumption decreases the risk of CVD in this population. In conclusion, incorporation of plant sterols into a low-saturated-fat and low-cholesterol diet for persons at increased risk of CVD mortality could have a positive effect on reducing the mortality rate associated with type 2 diabetes.

VWYL and MJ recruited the subjects; performed the screening, selection, and randomization; planned the 3-d cycle menu; prepared the daily meals in the kitchen; supervised the subjects at the research unit; and coded the blood samples. VWYL performed the blood lipid and insulin measurements, completed the gas chromatographic analyses of plasma plant sterols and RBCs, and performed the statistical analysis of the data. PJHJ designed the study and acted as the principal investigator.

PJHJ is a consultant to Forbes Medi-Tech Inc, which provided the plant sterol mixture. None of the other authors had a conflict of interest.

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