Lack of cholesterol-lowering efficacy of Cuban sugar cane policosanols in hypercholesterolemic persons

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ABSTRACT

Background: More than 50 studies have reported substantial reductions in plasma lipid concentrations in response to 2–40 mg Cuban sugar cane policosanol (SCP) mixtures/d. However, several animal and human trials conducted outside of Cuba that used non-Cuban mixtures have failed to reproduce the efficacy of policosanols observed in earlier studies.

Objective: The objective was to evaluate lipid-modulating actions of the authentic Cuban SCPs on plasma lipids in healthy hypercholesterolemic volunteers.

Design: Twenty-one volunteers consumed, under supervision, 10 mg SCPs/d or a placebo incorporated in margarine as an afternoon snack, for a period of 28 d with the use of a randomized, double-blind crossover study design. Subjects maintained their habitual diet and physical activity and were weighed daily throughout the study period. Blood was collected at days 1, 2, 28, and 29 of the feeding trial, and lipid concentrations were measured.

Results: Body weights did not vary significantly throughout the trial and did not affect plasma lipid values. No significant difference was observed between treatment and control groups in plasma total, LDL-, HDL-cholesterol, and triacylglycerol concentrations.

Conclusion: Present results show no beneficial effects of Cuban SCPs on plasma lipids in healthy hypercholesterolemic volunteers.

Subjects and treatments

Otherwise healthy hypercholesterolemic men and postmenopausal women (n = 21), aged 40–80 y and with body mass index (in kg/m²) of 23–30, were recruited for the clinical trial. The volunteers were asked to visit the research unit for 2 screenings, including a blood draw to measure their lipid profile and other health indicators and a medical examination. The subjects accepted in the study had plasma LDL-cholesterol concentrations that ranged from 3.0 to 5.0 mmol/L and triacylglycerol concentrations <4.0 mmol/L at screening. Exclusion criteria included a history of recent or chronic use of oral hypolipidemic therapy or chronic use of insulin, systemic antibodies, corticosteroids, androgens, or phenytoin. Subjects were also excluded if they had experienced a myocardial infarction, coronary artery bypass, or other major surgical procedures within the past 6 mo or reported the policosanol treatment groups compared with 10% in the plant sterol groups, compared with placebo (30).

All clinical trials that studied the lipid-lowering effect of policosanols were almost without exception conducted by one group of researchers based in Cuba. However, recently other investigators have contradicted the positive outcomes of the original research laboratory (31–34). Although similar to the authentic Cuban product, the policosanol mixtures used by those trials differed in source or composition of minor alcohols, possibly explaining the discrepancies between findings. Indeed, researchers initially providing data for the remarkable efficacy of SCPs suggested that failure by other groups to observe cholesterol-lowering effects could be explained by differences in the policosanol ratios in the mixtures tested (8). The purpose of the present study was to evaluate the authentic Cuban mixture of policosanols, as used in previous efficacy studies, as an ingredient for cholesterol lowering in moderately hypercholesterolemic persons.

SUBJECTS AND METHODS

Subjects and treatments

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recent onset of angina, congestive heart failure, inflammatory bowel disease, pancreatitis, or hypothyroidism. Significant pre-existing diseases, including cancer, chronic use of laxatives, smoking, or consumption of >2 drinks per day were also considered to be part of the exclusion criteria. Before they were enrolled in the study, the subjects were asked to sign a consent form that outlined the details of the trial. Policosanols derived from sugar cane wax (Lipex; Dalmer Laboratories, La Havana, Cuba) were purchased in the form of 5-mg pills which were crushed and incorporated into margarine. The composition of the SCP treatment was 65.6% octacosanol, 13.4% triacontanol, and 4.5% hexacosanol (unpublished data, 2006). The daily dose of treatment provided 10 mg policosanols mixed with 10 g margarine and was served on a slice of bread.

Protocol
The feeding trial was designed as a double-blind crossover study, whereby subjects were randomly assigned to either policosanol or control margarines during 2 phases of 28 d each, separated by a washout phase of the same duration. The subjects were asked to maintain their habitual diet and physical activity patterns throughout the study. They were required to visit the clinic daily to consume the treatment as a late afternoon snack under staff supervision at the Mary Emily Clinical Research Centre, to ensure absolute compliance. The subjects were asked to abstain from alcohol throughout the 2 study phases, and caffeinated drinks were restricted to 1 cup/d. Body weights were recorded daily to monitor weight fluctuations throughout the study period. At the beginning and end of each feeding phase, blood draws were scheduled to check for health abnormalities at the onset of the trial and as a result of treatment. All procedures included in the protocol were approved by the ethics committee of the medical faculty of McGill University.

Plasma lipid analyses
Fasting blood samples were drawn from the subjects on days 1, 2, 28, and 29 into EDTA-coated tubes and centrifuged at 805 × g for 20 min at 4 °C. Then, plasma was separated from red blood cells and stored at −80 °C for further analysis. Initial lipid values were measured from blood samples taken on days 1 and 2, whereas endpoint values were taken from days 28 and 29. Plasma total, HDL-cholesterol, and triacylglycerol concentrations were measured with enzymatic kits, standardized reagents, and standards with the use of a VP Autoanalyzer (Abbott Laboratories, North Chicago, IL). The Friedewald equation was used to calculate LDL-cholesterol concentrations (35).

Statistical analyses
The sample size (n = 21) for the clinical trial was calculated to provide an 80% probability of detecting an anticipated difference in indicators tested of 20%, by using a CV of 15–20%. Effects of SCP treatment on indicators of interest were compared with control with the use of a one-factor analysis of covariance for crossover models and subsequent Scheffe’s post hoc test. The effect of sequence, treatment period, and weight change was included in the model as covariates to test for potential confounders. Statistical significance was set at a level of P < 0.05. All data was analyzed by using SAS statistical software for WINDOWS version 8.02 (SAS Institute Inc, Cary, NC).

TABLE 1
Baseline characteristics of the subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n = 12)</th>
<th>Women (n = 9)</th>
<th>All (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>54.0 ± 2.9a</td>
<td>60.1 ± 2.6a</td>
<td>57.8 ± 2.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84.8 ± 4.9a</td>
<td>71.1 ± 3.8b</td>
<td>76.3 ± 3.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.9 ± 1.0a</td>
<td>26.3 ± 0.8a</td>
<td>26.5 ± 0.6</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.4 ± 0.4a</td>
<td>6.09 ± 0.3a</td>
<td>5.8 ± 0.2</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.5 ± 0.3a</td>
<td>3.8 ± 0.3a</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.2 ± 0.1a</td>
<td>1.6 ± 0.1b</td>
<td>1.4 ± 0.09</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/L)</td>
<td>1.5 ± 0.4a</td>
<td>1.7 ± 0.3a</td>
<td>1.6 ± 0.2</td>
</tr>
</tbody>
</table>

1 All values are x ± SEM. The men-to-women ratio was 1.3. Values in a row with different superscript letters are significantly different, P < 0.05.

RESULTS
Subjects and dropout rate
Twenty-two subjects were enrolled in the clinical trial. Baseline characteristics, recorded the first 2 d of the trial, are shown in Table 1. One subject dropped out because of conflicting schedules. Therefore, 21 subjects successfully completed the feeding trial. Most subjects tolerated the afternoon snack well. However, 4 subjects reported abdominal pain, soft stools, and increased frequency of bowel movements. Two of these subjects were consuming the treatment and the other 2 were receiving the placebo.

Blood biochemistry and weight changes across the study period
Blood biochemistry and hematology values remained within normal ranges throughout the study period. Initial and final body weights were calculated as average weights of the first and last 5 d of each phase, respectively. These averages are presented in Figure 1 and show that subjects maintained a stable weight throughout the study phases. In fact, average final body weights varied by <2 kg from the initial weight during the 2 treatment periods. Only one participant, subject 4, lost 3 kg during the control phase.

FIGURE 1. Average initial and final body weights across treatment phases. C(in), control phase initial value; C(fn), control phase final value; P(in), policosanol phase initial value; P(fn), policosanol phase final value.
Plasma lipid concentrations across the study period

**Total cholesterol responses to treatment**

Individual total cholesterol values across treatment phases are shown in Figure 2. Authentic SCPs did not significantly affect plasma total cholesterol concentration endpoints or percent changes across each phase compared with control. Mean (±SEM) plasma total cholesterol was 5.77 ± 0.21 mmol/L at the end of the SCP treatment phase compared with 5.60 ± 0.21 mmol/L in response to placebo (P = 0.25). Percent changes from baseline were (P = 0.18) 1.8 ± 3.0% and −4.0 ± 3.0% in the SCP and control groups, respectively.

**LDL-cholesterol responses to treatment**

Calculated LDL-cholesterol endpoints and percent changes were not significantly affected by SCP supplementation. Individual responses to treatment and control are presented in Figure 2. Mean (±SEM) LDL-cholesterol values for SCP treatment and control groups were (P = 0.52) 3.69 ± 0.18 mmol/L and 3.61 ± 0.18 mmol/L, respectively. Average LDL-cholesterol values were higher than baseline by 4.5 ± 4.0% in response to the SCP treatment and 1.6 ± 4.0% lower than baseline in response to control. However, differences between the groups did not reach significance (P = 0.28).

**HDL-cholesterol responses to treatment**

SCP treatment did not show any significant effect on HDL-cholesterol endpoints or percent changes compared with control. Each subject’s HDL-cholesterol responses to treatments are shown in Figure 2. Mean (±SEM) endpoint values for SCP and control groups were (P = 0.36) 1.37 ± 0.81 mmol/L and 1.33 ± 0.81 mmol/L, respectively. Percent changes in HDL cholesterol were (P = 0.19) −2.7 ± 3.0% in the SCP group compared with −7.4 ± 3.0% in the control group.

**Triacylglycerol responses to treatment**

As for the other lipid indicators, no significant effect of SCPs was observed on plasma triacylglycerol endpoints (P = 0.72) and percent changes (P = 0.36). Individual triacylglycerol responses to treatment and control are shown in Figure 2. Mean (±SEM) endpoint values for treatment and control groups were 1.63 ± 0.24 mmol/L and 1.59 ± 0.24 mmol/L, respectively; percent change in the SCP group was −3.2 ± 6.0% from baseline compared with −4.3 ± 6.0% for the control group.

**Effect of sequence, period, and body weight change on lipid indicators**

When study sequence, treatment period, and weight changes across periods were included in the statistical model as covariates, no significance was recorded (P > 0.05) in their effect on plasma mean endpoint values and percent changes of total, LDL-cholesterol, HDL-cholesterol, and triacylglycerol concentrations.

**DISCUSSION**

Contradicting the previous literature on Cuban SCPs, the results of the present study do not show that authentic SCPs alter...
total cholesterol, LDL-cholesterol, HDL-cholesterol, or triacylglycerol concentrations in healthy, hypercholesterolemic persons. Original research on the effect of SCPs on plasma lipids in humans showed highly promising results in terms of risk reduction for coronary heart disease (1–28). Tested on numerous study populations and for various durations, SCP treatments proved to be effective regardless of diet control and compliance monitoring. In fact, SCPs were often tested in a free-living context in which subjects were instructed to follow a National Cholesterol Education Program Step 1 diet and were given the treatment for consumption under no supervision (9, 16, 17, 24). In addition, follow-up visits, including tablet counting, were scheduled once a month (2, 9, 10, 15, 24), in some cases once every 3 mo (20). Still, doses of SCPs as low as 2 mg/d were seen to reduce total and LDL cholesterol by 14.8% and 15.6%, respectively (2). The design of the present study was such that 10 mg SCPs/d were administered under staff supervision, to ensure that subjects were following the study protocol in terms of treatment dose and time of consumption. On the one hand, we were not able to replicate the results obtained by previous research on authentic Cuban SCPs. On the other hand, the lack of efficacy seen in this trial agrees with the data reported by the few human studies conducted outside the original research laboratory. Lin et al (33) used 20 mg wheat-germ policosanols/d with similar octacosanol content to the authentic mixture for a period of 4 wk. No benefit was attributed to the effect of policosanols on blood lipids. In 2 recent randomized double-blind placebo-controlled crossover trials (31, 32), 10 mg rice policosanols/d was administered to hypercholesterolemic subjects for a period of 8 wk. Although the treatment significantly reduced total cholesterol, it did not affect LDL cholesterol, HDL cholesterol, or triacylglycerols. Because the cholesterol-lowering effect of SCPs is mainly mediated by a decrease in LDL cholesterol (36), significant reductions would be expected as can be observed in original policosanol studies. A longer duration of treatment was used in a recent crossover study by Greyling et al (34), in which participants received 20 mg Lesstanol Octa-60 policosanols/d for a period of 12 wk. Agreeing with results of a previous animal trial on the same product (37), the investigators reported no significant effect on total and LDL cholesterol in hypercholesterolemic humans.

One of the main points of controversy in policosanol research is the appropriateness of the product used in studies yielding negative results. Octacosanol is the main alcohol in policosanol mixtures and was suggested to be the main active component of the treatments (38). The octacosanol content of wheat germ, rice, and Lesstanol Octa-60 policosanols is similar (33) or close (31, 34) to the authentic product. However, mixtures with diverging minor alcohol compositions appear to have different lipid-lowering activities despite their similar octacosanol contents (8). To date, the original policosanol mixture was used in only one clinical trial (39), in which the raw material was provided by Dalmer Laboratories and the tablets were manufactured in Germany. Results from that recent trial agree with conclusions of external studies that found no effect of policosanols on the lipid profile in hypercholesterolemic patients. Consequently, in the present study, the use of authentic SCP tablets manufactured in Cuba is of great importance to compare our results with those of the original research.

Dissimilarities between original and external research on policosanol include the length of the study period. Unlike external clinical trials with durations ranging from 4 to 12 wk (31–34, 39), original researchers tested SCPs in the context of clinical trials covering 6 wk to 3 y (1–5, 9, 10, 12, 15, 17, 19, 23). However, note that, when interim lipid measurements were performed, SCP efficacy was already manifested by the fourth week of the trial (15, 19). The present study did not show any changes in plasma lipids at the end of the 4-wk trial, therefore agreeing with external researchers on the ineffectiveness of policosanols.

The form of administration of a treatment often plays a role in determining its efficacy. Most studies conducted in Cuba used policosanol treatments in the form of a pill ingested with a meal. The objective of this study was to evaluate SCPs incorporated in a food; therefore, policosanols were mixed in 10 g margarine. Fatty matrices, especially margarine, have been used as a vehicle for fat-soluble substances such as plant sterols (40, 41). Previous literature shows that these matrices resulted in better cholesterol lowering than did others, such as low-fat beverages and capsules (42, 43). Knowing that policosanols are not soluble in water (44), the fatty matrix used was considered appropriate for better mixing and distribution of SCPs. Therefore, the lack of efficacy seen in the present study is not likely to be related to the form of administration of policosanols.

Very-long-chain fatty alcohols are rather abundant in nature, more precisely in plants and oils that constitute a significant part of the human diet (45). Specific human intakes of very-long-chain fatty alcohols are yet to be determined; however, estimates of the typical US intake of the wax ester hexacosanoate are reported to be 12–40 mg/d (45). Octacosanol is found in many common market foods, such as rice, wheat, apples, plums, spinach, and various nuts and seeds (45); therefore, a diet already providing a high concentration of octacosanol could potentially mask the effect of a supplementation of this alcohol. Investigating variations in dietary intakes of policosanols in general and octacosanol in particular across populations may determine the involvement of an important factor (ie, the typical octacosanol content of traditional foods consumed). Furthermore, the use of Cuban subjects in original positive research compared with most European or North American participants in external negative research draws the attention to the possible effect of genetics or dietary habits on policosanol action, potentially making this treatment specific to certain populations.

Despite the extensive research published on the efficacy of SCPs, their mechanism of action is still unclear. SCPs are believed to be absorbed by the gut (46) and transported to the hepatocytes where they reduce cholesterol biosynthesis by suppressing 3-hydroxy-3-methylglutaryl coenzyme A reductase (47, 48) and increasing LDL uptake by the cells (49). Radioisotope labeling was used in most studies that looked at policosanol absorption and their effect on cholesterol synthesis. In both cases, labeling policosanols could lead to erroneous results because of their degradation in the body into their metabolites (50). This would suggest that this detection method is nonspecific. Also, research has reported poor absorption rates for policosanols in the gut, possibly because of their extreme hydrophobicity (45, 46, 51). To understand the mechanism by which a significant amount of SCPs can reach the hepatocytes and directly or indirectly induce changes in cholesterol synthesis, more research on the alcohols’ bioavailability and their effect on cholesterol kinetics is necessary.

In conclusion, the present study did not find SCPs to be efficacious for the reduction of plasma cholesterol in hypercholesterolemic persons. Additional studies are needed to fully understand...
their mechanism of action, their metabolism in the body, and the genetic and dietary factors affecting their action to determine the reasons for controversy in policosanol research.

We thank the study participants for their good will and compliance throughout the trial period. ANK designed the study, collected and analyzed the data, and wrote the manuscript. PHJ designed the study, supervised and provided significant advice and consultation, and wrote the manuscript. None of the authors had any conflicts of interest.

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