Soy sterol esters and β-sitostanol ester as inhibitors of cholesterol absorption in human small bowel

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ABSTRACT

Background: Plant sterols are natural dietary components with serum cholesterol–lowering properties. The lowering of serum cholesterol by plant sterols is believed to be the result of an inhibition of cholesterol absorption in the small bowel, although increased bile acid excretion has also been suggested. The difference in effect of saturated and unsaturated plant sterols on cholesterol absorption needs to be elucidated further.

Objective: The primary aim of this study was to measure small-bowel cholesterol absorption and sterol excretion in addition to hepatic cholesterol synthesis after intake of soy sterol esters and β-sitostanol ester corresponding to 1.5 g plant sterols/d.

Design: Seven ileostomy subjects were studied during a control period and 2 intervention periods when either soy sterol esters or β-sitostanol ester was added to a basal diet. Ileostomy bags were collected every other hour and frozen immediately for analysis of nutrients and sterols.

Results: Cholesterol absorption was 56% (43–65%) in the control period and decreased to 38% (32–46%) in the soy sterol ester period (P = 0.00) and to 39% (30–48%) in the β-sitostanol ester period (P = 0.00).

Conclusion: Esterified soy sterols and β-sitostanol inhibited cholesterol absorption equally, despite the different structures of the plant sterols.

KEY WORDS Plant sterols, phytosterols, plant sterol ester, cholesterol absorption, cholesterol synthesis, lathosterol, sterol excretion, ileostomy

INTRODUCTION

Plant sterols are natural dietary components with serum cholesterol–lowering properties (1). The most common plant sterols are β-sitosterol, campesterol, and stigmasterol, which are classified as 4-desmethylsterols of the cholestane series (2). The structures of these plant sterols are similar to that of cholesterol with an extra methyl or ethyl group and a double bond in the side chain. Saturated plant sterols, referred to as stanols, have no double bond in the ring structure (3). As early as 1951 it was shown that β-sitosterol decreased serum cholesterol in chickens during a cholesterol load (4). This finding resulted in several studies of the cholesterol-lowering effects of plant sterols in humans (1, 5–12).

The serum cholesterol–lowering effect of plant sterols is believed to be caused by an inhibition of cholesterol absorption resulting from the higher affinity of plant sterols than of cholesterol for micelles (13). Additionally, specific plant sterols may increase bile acid excretion (9). Plant sterols seem more efficient as serum cholesterol–lowering agents when mixed with fat than when alone (11). Most studies have been performed with free sterols, which have a low solubility in fat and therefore a limited ability to dissolve in butter or margarine. Esterification of free plant sterols is one way to increase solubility in fat (14). A clinical study of hypercholesterolemic subjects concluded that esterified β-sitostanol was more efficient than free β-sitosterol, free β-sitostanol, or rapeseed-based margarine alone in lowering serum cholesterol (14). The superiority of β-sitostanol ester has yet to be confirmed because the intakes of the dietary plant sterols were different in the experimental groups of the trial. A comparison of free β-sitostanol with free β-sitosterol, however, showed that the saturated plant sterol increased cholesterol excretion more effectively than the unsaturated plant sterol when infused over several hours in low concentrations (3). Despite the latter finding, a recent comparison of esterified unsaturated sterols from soybeans with the ester of the saturated β-sitostanol indicated that soybean sterol esters had a similar serum cholesterol–lowering effect as the β-sitostanol ester (12). It could therefore be hypothesized that soy sterols and β-sitostanol inhibit cholesterol absorption equally when both fractions are esterified.

The conventional sterol balance technique can be criticized for being imprecise when used to measure cholesterol and bile acids because of the considerable variation in colon transit time and bacterial degradation (15). To improve the precision of measurement of small-bowel excretion, sterol excretion can be studied in ileostomy subjects. Analysis of ileostomy bags after immediate freezing reveals an almost complete absence of sec-

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ordinary bile acids, which might correspond with a low rate of fermentation (16). The primary aim of this study was to measure small-bowel cholesterol absorption and sterol excretion and hepatic cholesterol synthesis after the intake of soy sterol esters and β-sitostanol ester corresponding to 1.5 g plant sterols/d.

SUBJECTS AND METHODS

Subjects

Seven ileostomy subjects, 5 men and 2 women, were studied. All subjects had undergone proctocolectomies for ulcerative colitis and had well-functioning ileostomies. The median (range) time since surgery was 13 y (2–26 y). The subjects had a median body mass index (BMI; in kg/m²) of 26 (18–30) and a median age of 54 y (29–73 y). Three subjects took medications for hypertension, 1 subject took medication for hypothyroidism, and 1 subject took medication for sacroiliitis. The subjects were otherwise healthy and showed no signs of anemia, inflammation, or hepatic or thyroid disease as confirmed by history and standard laboratory tests. An inclusion criterion for subjects was a total bile acid excretion of <1 g/d. Higher bile acid excretion reflects a large resection of the small bowel, which would break the normal enterohepatic circulation of bile acids. Informed consent was obtained from all subjects and approval for the study was granted by the ethics committee of Göteborg University in February 1996.

Design

The study consisted of 1 control period and 2 intervention periods of 3 d each. The order of periods was assigned randomly and the subjects completed the study over 3 consecutive weeks. There was a minimum washout period of 4 d between every study period. A basal diet was served during all 3 periods. During the intervention periods, 2.5 g soy sterol ester or β-sitostanol, corresponding to 1.5 g free plant sterols, was added to the basal diet daily. The first day of every period was an adaptation day to minimize carryover effects from the subjects’ habitual diets. On study days 2 and 3, the subjects changed ileostomy bags every second hour. The night bag was collected separately. Bags were frozen on dry ice immediately to minimize bacterial degradation. Collection started at 0730 with a change of the first bag at 1000 and continued until 2200. Each subject’s bags were freeze-dried, pooled (24 h), and stored at −20°C until analyzed for energy, nitrogen, starch, resistant starch, dietary fiber, and sterol contents.

A blood sample was drawn before the start of the study to determine each subject’s apolipoprotein (apo) E phenotype. On the third day of each period, a blood sample was drawn while subjects were in a fasting state to measure serum concentrations of plant sterols and the hepatic cholesterol synthesis marker lathosterol.

Basal diet and plant sterols

The basal diet was designed to have a low content of plant sterols and a high content of cholesterol (Table 1). The high cholesterol intake was chosen so that there would be enough cholesterol for the plant sterols to inhibit. Food items with a known high cholesterol concentration were chosen, eg, eggs, milk products, and meat. The composition of the diet was calculated from Swedish national food composition tables (17) and a computer program was used for nutrient calculations (DIETIST; Näringssdata AB, Bromma, Sweden). Analyses of starch, resistant starch, fiber, and sterols were performed on duplicate portions of the diet. Breakfast was served in the study center every morning of the 3 experimental days. The remaining meals were provided by the study center for subjects to consume at home or at work.

The basal diet consisted of breakfast (0730), fruit (1000), lunch (1200), coffee (1500), dinner (1700), and an evening snack (2000). All foods were prepared from the same batches and were stored in special containers at −20°C. Subjects were allowed to eat the prepared food only. Leftovers were weighed and recorded each day. Subjects drank tap water but were asked to drink the same amount at the same time every day.

The daily amount of plant sterol esters was divided into 3 portions, which were weighed directly onto small buns spread with butter. The buns were eaten at breakfast, lunch, and dinner. The 2.5 g soy sterol esters contained 722 mg β-sitosterol, 408 mg campesterol, 225 mg stigmasterol, 50 mg Δ-5-avenasterenol, 38 mg brassicasterol, 20 mg campesterol, and 8 mg Δ-7-stigmasterol, which gave a total sum of 1.47 g plant sterols. The 2.5 g β-sitostanol ester contained 1363 mg β-sitostanol, 119 mg campesterol, 25 mg β-sitosterol, 8 mg campesterol, and 2 mg brassicasterol, resulting in a total dose of 1.52 g plant sterols. The soy sterols were purchased from Archer Daniels Midland (Decatur, IL). The source of the β-sitostanol was tall oil (The Raisio Group, Raisio, Finland). Transesterification of both plant sterol fractions with oleic acid from rapeseed oil was performed by The Raisio Group. The esterification rates were 94% for soy sterols and 98% for β-sitostanol and the purity of both products was >95%.

Cholesterol absorption

Cholesterol absorption in the small bowel was measured with 3H-labeled cholesterol and 14C-labeled β-sitosterol according to a modification of the method of Grundy (18). On the second day of each study period a total dose of 3.9 kBq (106 nCi) [β-4-14C]sitosterol and 8.6 kBq (233 nCi) [1α, 2α, 7α,3H]cholesterol dissolved in 750 mg rapeseed oil was weighed onto 3 sugar cubes. The sugar cubes were served with the same meals as the

| TABLE 1 |
| Composition of the basal diet |
| Energy (MJ) | 10.0 |
| Total fat (% of energy) | 37 |
| Protein (% of energy) | 17 |
| Carbohydrates (% of energy) | 45 |
| Starch (g) | 102 |
| Resistant starch (g) | 5 |
| Dietary fiber (g) | 14 |
| Sterols (mg) |  |
| Cholesterol | 775 |
| Total plant sterols | 172 |
| β-Sitosterol | 94 |
| β-Sitostanol | 13 |
| Campesterol | 43 |
| Campestanol | 7 |
| Brassicasterol | 30 |
| Stigmasterol | 10 |
| 5-Avenasterol | 5 |

1 Calculated from the Swedish Food Composition Tables (17).
2 Analyzed value.
plant sterol–fortified buns. β-Sitosterol was purchased from Amersham International (Buckinghamshire, United Kingdom) and the tritium was from Duomedical (Stockholm).

Analytic procedures

The energy content of the basal diet was analyzed by bomb calorimetry (automatic adiabatic bomb calorimetry; Gallenkamp, Loughborough, United Kingdom). Nitrogen content was determined by a modified micro-Kjeldahl method (19). Total starch and resistant starch were analyzed by the modified enzymatic procedure of Englyst et al (20) and dietary fiber was measured by the method of Asp et al (21). Radioactive isotopes of β-sitosterol and cholesterol in the ileostomy effluents were extracted according to the method of Miettinen et al (22). The samples were analyzed in a Beckman Tri-Carb liquid scintillation counter (model 1900 TR; Packard Instruments, Meriden, CT) with automatic external standardization. Recoveries of isotopes added to the diet in ileostomy excreta were 92% for β-sitosterol and 89% for cholesterol, which was taken into account during the calculations of cholesterol absorption. Plant sterols were analyzed according to the method of Jonker et al (23), with some modifications (24, 25). In short, the method is based on acid hydrolysis, alkaline hydrolysis, silylation with trimethylsilyl ether, and finally gas-liquid chromatography (24). Sterols in the ileostomy excreta were analyzed as described by Bosaeus and Anderson (16). A cholesterol precursor, lathosterol, and plant sterols were analyzed in the nonsaponifiable part of serum by gas-liquid chromatography (26). Phenotyping was performed according to the method of Johansson et al (27). Serum cholesterol, HDL, and triacylglycerols were analyzed by an enzymatic procedure developed by Bayer Technicon AB (Stockholm).

Calculations and statistics

LDL-cholesterol concentrations were calculated with the Friedewald formula (28). The Quintao formula was used to calculate fractional cholesterol absorption as follows (29):

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\text{Fractional cholesterol absorption (\%)} = 100 \times (1 - \frac{[\text{administered } ^{14}\text{C}]\beta\text{-sitosterol}}{[\text{administered } ^{14}\text{C}]\beta\text{-sitostanol}}) \quad (1)
\]

Results are presented as medians and ranges in the text and tables. Statistical analyses were performed with the statistical package SYSTAT for WINDOWS (version 7.0; SPSS Inc, Chicago). Cholesterol absorption, ratios of lathosterol to cholesterol, and cholesterol and bile acid excretion were analyzed by analysis of variance. Significantly different pairwise comparisons were identified by a one-sided Dunnett test. Adjustment was made for multiple comparisons according to Bonferroni. \( P < 0.05 \) was chosen for statistical significance.

RESULTS

Before the beginning of the study, the subjects’ median serum cholesterol concentration was 5.2 mmol/L (4.9–6.5 mmol/L) and their median LDL-cholesterol concentration was 3.2 mmol/L (2.6–4.0 mmol/L). Serum triacylglycerol concentrations were 0.99 mmol/L (0.81–2.0 mmol/L), LDL concentrations were 3.9 mmol/L (3.1–5.0 mmol/L), and HDL concentrations were 1.5 mmol/L (1.2–2.1 mmol/L). The individual subject’s apo E phenotypes were as follows: E4,3 (subject 3); E3,3 (subjects 1, 2, 4, and 6); E3,2 (subject 5); and E2,3 (subject 7).

On day 3, the subjects had median serum β-sitosterol concentrations of 8.0 μmol/L (4.7–13.1 μmol/L) during the control period, 8.6 μmol/L (4.7–15.2 μmol/L) during the soy sterol ester period, and 9.6 μmol/L (6.4–17.1 μmol/L) during the β-sitostanol ester period. Median serum campesterol concentrations were 12.5 μmol/L (8.2–19.3 μmol/L) during the control period, 14.3 μmol/L (10.2–29.7 μmol/L) during the soy sterol ester period, and 14.2 μmol/L (10.6–20.9 μmol/L) during the β-sitostanol ester period. On day 3, the subjects had median serum concentrations of total cholesterol of 5.5 mmol/L (4.4–7 mmol/L) during the control period, 5.3 mmol/L (4.4–7.2 mmol/L) during the soy sterol ester period, and 6 mmol/L (4.5–7.2 mmol/L) during the β-sitostanol ester period.

Cholesterol absorption was 56% (43–65%) in the control period and decreased to 38% (32–46%) in the soy sterol ester period and to 39% (30–48%) in the β-sitostanol ester period (\( P = 0.00 \); Figure 1). Ratios of lathosterol to cholesterol (mmol/mol cholesterol) were 1.41 (1.06–2.33) during the control period, 1.80 (0.87–3.09) in the soy sterol ester period, and 1.76 (1.17–2.69) in the β-sitostanol ester period. The ratio of lathosterol to cholesterol was not significantly different between the intervention periods and the control period.

Data on sterol excretion are shown in Table 2. Cholesterol and bile acid excretion were not significantly different between periods. Total plant sterol excretion, ie, the sum of brassicasterol, β-sitosterol, β-sitostanol, campesterol, campestanol, stigmasterol, and avenasterol, was 205 mg/d (186–256 mg/d) in the control period, 1447 mg/d (1250–1522 mg/d) in the soy sterol ester period, and 1592 mg/d (1484–1783 mg/d) in the β-sitostanol ester period. The median total recoveries corresponded to 88% with the soy sterol ester and 94% with the β-sitostanol ester.

DISCUSSION

To our knowledge, this is one of the first comparisons of the effects of plant sterols with unsaturated or saturated esterified structures on cholesterol absorption. Our results contribute to the ongoing plant sterol debate, which has centered mostly on the hypothesized stronger effects on cholesterol absorption of saturated plant sterols than of unsaturated plant sterols. Our findings contradict the suggestion that saturated plant sterols, especially β-sitostanol, are more potent inhibitors of cholesterol absorption than unsaturated plant sterols (14). In fact, we found that unsaturated soy sterol esters inhibited cholesterol absorption as efficiently as β-sitostanol. Free plant sterols, however, have been shown to differ in their ability to reduce cholesterol absorption (3) and to lower serum cholesterol concentrations (9). A comparison of free β-sitosterol with free β-sitostanol showed that the saturated plant sterol decreased cholesterol absorption more efficiently than the unsaturated one (3). A comparison of the result from that single intubation study with the results of the present ileostomy study suggests that esterification makes the unsaturated soy sterols comparable to β-sitostanol in this respect.

An earlier comparison of free β-sitosterol with β-sitosteryl oleate in humans showed that the free plant sterol decreased cholesterol absorption more efficiently than the ester during a breakfast meal with a high cholesterol content (30). In this study,
however, the plant sterols were added to the meal differently: the free form was mixed into the breakfast omelet and the esterified form was blended with the frying fat. To what extent this affected the inhibition of cholesterol absorption is unknown. The difference found between the free and esterified forms may have been due to an incomplete hydrolyzation of the plant sterol esters in the gut, because it is the sterol monohydrate that affects micellar binding (31). Still, it is important to emphasize that in the present ileostomy study, the different plant sterol esters were consumed in exactly the same way.

An important finding supporting the results of the present study was described recently by Weststrate and Meijer (12), who showed that soy sterol esters and \( \beta \)-sitostanol ester result in a similar hypocholesterolemic response. The source of the esters used by Weststrate and Meijer was fortified margarines. Note, however, that the incorporation of plant sterol esters into margarines changes the physicochemical environment of the plant sterol ester. In the present study, the plant sterol esters were not incorporated into the butter, which may explain the different effect on cholesterol absorption from that of the fortified products. However, as in the study by Weststrate and Meijer, the effects of soy sterol esters and \( \beta \)-sitostanol ester seemed to also be comparable when not blended with fat.

In vegetable oils, plant sterols exist in both free and esterified forms. This aspect of plant sterols was addressed in a study comparing 3 dietary regimens: 1) corn oil with a naturally high plant sterol concentration, 2) olive oil with a naturally low concentration of plant sterols, and 3) olive oil enriched in free plant sterols (32). Addition of the mixture of free plant sterols to the olive oil in the third regimen did not result in the same serum cholesterol–lowering effect as produced by the corn oil regimen, even though the third regimen contained 2-fold more plant sterols than the first. Serum plant sterol concentrations in the 3 periods were not significantly different, despite the differing dietary intakes of plant sterols. Plant sterols are absorbed after micellar incorporation in the human small bowel (33) and the lack of increase in serum concentrations indicates that the plant sterols had not been dissolved in the micelles. To make the addition of plant sterols comparable, they should have been added as a mix of free and esterified plant sterols, as in the natural oils. This may explain the unexpected response in serum cholesterol elicited by the third regimen.

### TABLE 2

Sterol composition of ileostomy excreta during the 3 study periods.\(^1\)

<table>
<thead>
<tr>
<th>Sterol</th>
<th>Basal diet</th>
<th>Basal diet + 2.5 g soy sterol ester</th>
<th>Basal diet + 2.5 g ( \beta )-sitostanol ester</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/24 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1066 (776–1277)</td>
<td>1216 (1074–1288)</td>
<td>1204 (1018–1323)</td>
</tr>
<tr>
<td>Cholic acid</td>
<td>320 (80–646)</td>
<td>230 (56–502)</td>
<td>297 (58–560)</td>
</tr>
<tr>
<td>Chenodeoxycholic acid</td>
<td>232 (42–310)</td>
<td>136 (36–263)</td>
<td>182 (44–325)</td>
</tr>
</tbody>
</table>

\(^1\)Median; range in parentheses. Changes in cholesterol absorption were tested by using ANOVA and the Dunnett test. There were no significant differences between any of the study periods.
The present study showed that soy sterol ester and β-sitostanol ester inhibited cholesterol absorption, although there were no major changes in sterol excretion. The 4 possible mechanisms for serum cholesterol lowering, as suggested by Spritz et al (34), are 1) decreased cholesterol synthesis; 2) increased excretion of cholesterol, bile acids, or both; 3) shift of plasma cholesterol to other tissues; or 4) a combination of the 3 prior mechanisms. The main explanation for the serum cholesterol-lowering effect of plant sterols seems to be an inhibition of cholesterol absorption, which thereby increases cholesterol excretion. The lack of a significant increase in cholesterol excretion in the present study may be explained by the low power of the study as a result of the low number of subjects.

An increase in bile acid excretion, as suggested by Becker et al (9), is not supported by the results of the present study. Bile acid excretion was slightly decreased with soy sterol esters, although not significantly, and not changed at all with β-sitostanol ester. Earlier studies of plant sterols in humans and animals also showed conflicting results for bile acid excretion, probably because of the small group sizes and variations in fecal output (7, 9, 14, 35–39). Moreover, sterol excretion in response to a plant sterol intervention may depend on the plant sterol and cholesterol dose (37). Studies have been performed with ratios of plant sterol to cholesterol of 2–250; furthermore, some authors have given no description of cholesterol intake. Different plant sterols may also induce various effects on bile acid excretion. A study of pure stigmasterol showed increased bile acid excretion (38). In a clinical study of children with familial hypercholesterolemia, the addition to the diet of saturated β-sitostanol increased bile acid excretion significantly, whereas β-sitostanol had no significant effect on bile acid excretion (9). The wide range of ratios of plant sterol to cholesterol used in supplementation studies and the lack of systematic testing of different isolated structures makes it difficult to evaluate the effects of plant sterols on bile acid excretion.

Because lathosterol-to-cholesterol ratios reflect hepatic cholesterol synthesis (40), increased ratios found after the addition of plant sterol esters seem to reflect increased hepatic synthesis as a result of the reduced uptake of cholesterol by the gut. Six-week regimens with intakes of β-sitostanol ester corresponding to 3.4 g and < 1 g β-sitostanol/d were shown to result in increased lathosterol-to-cholesterol ratios (14, 40). Increased hepatic cholesterol synthesis does not seem to be sufficient to balance the inhibition of cholesterol absorption. The lack of statistical increase in the present study was probably the result of the small number of subjects.

In conclusion, we showed that esterified soy sterols and β-sitostanol ester corresponding to 3.4 g β-sitostanol/d were shown to result in increased lathosterol-to-cholesterol ratios (14, 40). Increased hepatic cholesterol synthesis does not seem to be sufficient to balance the inhibition of cholesterol absorption. The lack of statistical increase in the present study was probably the result of the small number of subjects.

In conclusion, we showed that esterified soy sterols and β-sitostanol ester corresponding to 3.4 g β-sitostanol/d were shown to result in increased lathosterol-to-cholesterol ratios (14, 40). Increased hepatic cholesterol synthesis does not seem to be sufficient to balance the inhibition of cholesterol absorption. The lack of statistical increase in the present study was probably the result of the small number of subjects.

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REFERENCES


