# Dietary Sitostanol and Campestanol: Accumulation in the Blood of Humans with Sitosterolemia and Xanthomatosis and in Rat Tissues

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**ABSTRACT:** Dietary sitostanol has a hypocholesterolemic effect because it decreases the absorption of cholesterol. However, its effects on the sitostanol concentrations in the blood and tissues are relatively unknown, especially in patients with sitosterolemia and xanthomatosis. These patients hyperabsorb all sterols and fail to excrete ingested sitosterol and other plant sterols as normal people do. The goal of the present study was to examine the absorbability of dietary sitostanol in humans and animals and its potential long-term effect. Two patients with sitosterolemia were fed the margarine Benecol (McNeill Nutritionals, Ft. Washington, PA), which is enriched in sitostanol and campestanol, for 7–18 wk. Their plasma cholesterol levels decreased from 180 to 167 mg/dL and 153 to 113 mg/dL, respectively. Campesterol and sitosterol also decreased. However, their plasma sitostanol levels increased from 1.6 to 10.1 mg/dL and from 2.8 to 7.9 mg/dL, respectively. Plasma campestanol also increased. After Benecol withdrawal, the decline in plasma of both sitostanol and campestanol was very sluggish. In an animal study, two groups of rats were fed high-cholesterol diets with and without sitostanol for 4 wk. As expected, plasma and liver cholesterol levels decreased 18 and 53%, respectively. The sitostanol in plasma increased fourfold, and sitostanol increased threefold in skeletal muscle and twofold in heart muscle. Campestanol also increased significantly in both plasma and tissues. Our data indicate that dietary sitostanol and campestanol are absorbed by patients with sitosterolemia and xanthomatosis and also by rats. The absorbed plant stanols were deposited in rat tissues. Once absorbed by sitosterolemic patients, the prolonged retention of sitostanol and campestanol in plasma might increase their atherogenic potential.

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In the 1950s, Peterson demonstrated that plant sterols had a plasma cholesterol-lowering effect in chickens fed cholesterol (1). Further studies in other animals, and later in humans, documented that the decreased plasma cholesterol levels occurred because of decreased cholesterol absorption (2,3). Sitosterol, the most common plant sterol, was later marketed as a cholesterol-lowering drug, but its effect was mild and it fell into disuse. Excellent reviews of this subject are found in Kritchevsky and others (2,3).

Recently, there has been interest in both sitosterol when esterified and a saturated sterol derived from sitosterol, sitostanol. Like sitosterol, sitostanol interferes with cholesterol absorption (4–6). When consumed in margarine, sitostanol produced, on average, a 10% reduction of plasma cholesterol and a 12% LDL cholesterol-lowering effect (7). Sitostanol may be helpful in the therapy of hypercholesterolemic patients and in sitosterolemia in which hyperabsorption of all sterols occurs (8,9).

Sitosterolemia with xanthomatosis was first described in 1974 by Bhattacharyya and Connor (10). High levels of plant sterols were found in the patients' blood and tissues. The cause was twofold: (i) hyperabsorption of all sterols, including the usually poorly absorbed plant sterols, and (ii) poor excretion of sterols by the liver (10–13). The major clinical manifestations included tendon xanthomas of the extensor tendons, and tuberous xanthomas of the skin of the elbows and knees, premature atherosclerosis and coronary heart disease (11–14). Recent studies indicated that this Niemann-Pick C1 like 1 (NPC1L1) gene is critical for cholesterol absorption (15) and also suggested that this disorder is caused by mutations in either of two genes that encode the ATP binding cassette (ABC) half transporters, ABCG5 and ABCG8 (16,17).

In 1995, Lutjohann *et al.* (4) treated two sitosterolemic patients with sitostanol for 4 wk. They observed decreased plasma cholesterol and sitosterol levels in these patients. These authors reported that sitostanol was not absorbed to a significant degree in patients with sitosterolemia. They therefore concluded that oral administration of sitostanol was a new approach for the treatment of these patients. However, after feeding 2063 mg a day of a plant sterol mixture as a spread for 10–12 weeks to heterozygotes for sitosterolemia, Kwiterovich *et al.* (18) recently found significant increases in both campesterol and sitosterol levels in the plasma of these relatives. No sitostanol was fed. The authors discussed their concern about implications for development of coronary heart disease when plasma plant sterol levels were elevated.

In the present study, we examined the changes of plasma sitostanol and campestanol of two patients with sitosterolemia

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Abbreviations: ABC, ATP binding cassette; GCRC, General Clinical Research Centers.

and xanthomatosis after consuming a diet rich in sitostanol and campestanol. In a parallel study, we determined the effects of dietary sitostanol and campestanol on the levels of these stanols in both blood and tissues of a rat model.

## METHODS

*Human study. (i) Description of patients*. The first patient was a 70-yr-old Japanese–Canadian woman with sitosterolemia and xanthomatosis, diagnosed in 1994. She weighed 44 kg and was 1.5 m in height. Her plasma cholesterol was 180 mg/dL, and her plasma plant sterol/stanol levels totaled 25.2 mg/dL (12% of the total sterol/stanol levels). The second patient was a 14-yr-old Hispanic girl diagnosed with sitosterolemia in 1994 at the age of five. She weighed 34 kg and was 1.46 m in height. Her plasma cholesterol level was 153 mg/dL, and her plasma total plant sterol/stanol levels were 36.5 mg/dL (19% of the total sterol/stanol levels).

*(ii) Diet and Benecol margarine supplement.* Throughout the study period, patients were instructed to maintain a sterol-free diet. Counseling with General Clinical Research Centers (GCRC) dietitians and obtaining special sterol-free diet recipes designed by the GCRC dietitians achieved this. Each patient was given three packages of Benecol margarine (McNeill Nutritionals, Ft. Washington, PA) a day. Benecol margarine contained mainly stanols (campestanol and sitostanol). Each package of Benecol contained 744 mg sitostanol, 281 mg campestanol, and 14 mg sitosterol. The dietitians recorded the actual consumption.

On average, patient #1 consumed 2.7 packets of Benecol margarine per day, containing a total of 2009 mg/d of sitostanol, for 7 wk. Blood samples were collected at baseline and at 7 wk during the Benecol supplement, and 12 wk after Benecol was withdrawn from the diet.

Patient #2 consumed 2.3 packages of Benecol margarine per day (1713 mg/d sitostanol) for 18 wk. Blood samples were taken at baseline and at 3 and 10 wk during Benecol feeding. Because of logistic difficulty, the 18-wk sample was not obtained. Blood was analyzed 43 wk after Benecol was withdrawn from the diet.

*The design of the rat study of dietary sitostanol and campestanol*. Eighteen young male Wistar rats (weight 130–150 g) were divided into two groups. The experimental group was fed standard rat chow containing 5% lard, 0.5% cholesterol, and 0.5% stanols (sitostanol/campestanol comparable to Benecol provided by B.C. Chemicals, Vancouver, Canada). The control group was fed the same diet without stanols. The sterol composition of the control and experimental diet is presented in Table 1. After consuming the respective diets for 4 wk, these rats were killed; and the plasma, liver, heart, and skeletal muscle were collected for analysis.

**TABLE 1**
**Sterol and Stanol Content of the Control and Experimental Diets in Rat Studies (mg/kg chow)**

| Sterols | Control | Experimental |
|---|---|---|
| Cholesterol | 5,280 | 5,278 |
| Campesterol | 38 | 38 |
| Stigmasterol | 16 | 16 |
| Sitosterol | 178 | 177 |
| Campestanol | 6 | 1,006 |
| Sitostanol | 27 | 4,027 |
| Total | 5,545 | 10,542 |

*Biochemical analyses.* The sterols and stanols of plasma were analyzed by a method reported previously (19). Plasma samples were saponified with alcoholic KOH, and the sterols were extracted with hexane. Trimethylsilyl ether derivatives of these sterols were subjected to analysis by a gas–liquid chromatograph equipped with a hydrogen FID (Model 8500; PerkinElmer, Norwalk, CT) and containing a nonpolar 30-m SE-30 capillary column (Alltech, Deerfield, IL) with 0.25-mm i.d. and 0.25-μm film thicknesses. Cholestane was used as the internal standard. The temperatures of column, detector, and injection port were 240, 280, and 280°C, respectively. Helium was used as the carrier gas. The relative retention time (related to cholestane) was 2.07 for cholesterol, 2.65 for campesterol, 2.86 for stigmasterol, 3.28 for sitosterol, and 3.39 for sitostanol.

The lipids of the Benecol margarine supplement and rat tissues were extracted by the method of Folch, Lees, and Sloane Stanley (20). The lipid extracts were saponified with alcoholic KOH. The recovered sterols and stanols were analyzed by the GLC system just described. To avoid blood contamination, tissues were washed three times with saline and blotted dry before lipid extractions..

*Statistical analysis.* Statistical analysis was performed using the SPSS statistical software package 10 (SPSS, Chicago, IL) (21). All results were expressed as a mean ± SD.

## RESULTS

*Human studies in the sitosterolemic patients.* After consuming Benecol in the diet, there were uniform decreases in plasma sterols and increases in the plasma stanols of these patients (Table 2, Fig. 1). After 7 wk of Benecol feeding, the plasma cholesterol level in patient #1 decreased from 180 to 167 mg/dL and rebounded to 179 mg/dL 12 wk after Benecol was withdrawn from the diet. The plasma campesterol level decreased from 8.4 mg/dL at baseline to 6.0 mg/dL after 7 wk of Benecol feeding, and it did not change at 12 wk after the subsequent Benecol-free diet. Sitosterol decreased from 14.7 mg/dL at baseline to 10.1 mg/dL at 7 wk and increased slightly after withdrawing Benecol from the diet. In contrast, the plasma campestanol level of this patient increased from 0.5 to 5.2 mg/dL at 7 wk and had only decreased to 3.6 mg/dL after 12 wk of the Benecol-free diet (Fig. 1). Sitostanol increased from 1.6 mg/dL at baseline to 10.1 mg/dL at 7 wk and only dropped to 6.2 mg/dL after 12 wk on the Benecol-free diet.

Similar results occurred in patient #2 (Table 2, Fig. 1). Her plasma cholesterol decreased from 153 mg/dL at baseline to 146 mg/dL at 3 wk and to 113 mg/dL at 10 wk after Benecol feeding. The cholesterol level returned to 137 mg/dL after

**TABLE 2**
**Changes of Plasma Sterols and Stanols of Sitosterolemic Patients Before, During, and After the Administration of Benecol Margarine**

| | Plasma sterols and stanols (mg/dL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Patient 1 | | | Patient 2 | | | |
| | Baseline | Benecol (+) | Benecol (–) | Baseline | Benecol (+) | | Benecol (–) |
| Sterols and stanols | | 7[a] | 12[b] | | 3[a] | 10[a] | 43[b] |
| Cholesterol | 180 | 167 | 179 | 153 | 146 | 113 | 137 |
| Campesterol | 8.4 | 6.0 | 6.0 | 12.4 | 6.2 | 7.9 | 9.2 |
| Stigmasterol | — | — | — | 0.6 | — | — | 0.9 |
| Sitosterol | 14.7 | 10.1 | 11.6 | 20.1 | 17.2 | 16.3 | 17.0 |
| Campestanol | 0.5 | 5.2 | 3.6 | 0.6 | 0.7 | 2.0 | 2.0 |
| Sitostanol | 1.6 | 10.1 | 6.2 | 2.8 | 3.7 | 7.9 | 5.6 |
| Total sterols plus stanols | 205 | 198 | 206 | 190 | 174 | 147 | 172 |

[a]Weeks after Benecol feeding.
[b]Weeks after withdrawing Benecol.

Benecol was removed from her diet for 43 wk. Campesterol dropped from 12.4 to 6.2 mg/dL at 3 wk and was 7.9 mg/dL at 10 wk. It increased to 9.2 mg/dL after the Benecol-free diet. Plasma sitosterol decreased from 20.1 mg/dL at baseline to 17.2 mg/dL at 3 wk and 16.3 mg/dL at 10 wk. It remained at 17 mg/dL without Benecol in the diet. Opposite changes were seen in the plasma stanols (Fig. 1). The plasma campestanol increased from 0.6 mg/dL at baseline to 0.7 mg/dL at 3 wk and 2.0 mg/dL at 10 wk. After withdrawing Benecol from the diet for 43 wk, its level remained at 2.0 mg/dL. The plasma sitostanol rose from 2.8 to 3.7 mg/dL at 3 wk and 7.9 mg/dL at 10 wk. Plasma sitostanol remained high at 5.6 mg/dL after the patient had consumed a Benecol-free diet for 43 wk.

*The effects of dietary sitostanol and campestanol in the rat.* Dietary sitostanol and campestanol significantly increased the plasma stanols in the rats consuming the diet containing stanols (Table 3, Fig. 2). The plasma sitostanol increased from 0.05 to 0.2 mg/dL. Campestanol increased from zero to 0.3 mg/dL. These stanols were subsequently deposited into various tissues (Table 3, Fig. 2). In the liver, campestanol increased from zero to 19.0 μg/g while the sitostanol concentration remained the same. In skeletal muscle, campestanol increased from 1.1 to 3.7 μg/g and sitostanol increased from 2.6 to 8.4 μg/g. In heart muscle, campestanol increased from 8.7 to 18.1 μg/g and sitostanol increased from 6.0 to 14.6 μg/g. Thus, these two stanols not only were absorbed but also were deposited in the tissues.

## DISCUSSION

It was not too surprising that dietary sitostanol decreased plasma cholesterol levels in both the sitosterolemic patients and the animals. Several studies had already demonstrated that dietary sitostanol decreases plasma cholesterol by reducing cholesterol absorption in normal subjects as well as in sitosterolemic patients (4–6). It was, however, unexpected to find the sharp increases of plasma stanols after Benecol feeding because we had assumed that these dietary stanols would not be absorbed. For our two patients, who consumed 2009 and 1713 mg of sitostanol per day for 7 and 10 wk, their plasma sitostanol level increased 531 and 182%, respectively. These results are quite different from the results of Lutjohann *et al.* (4). They fed 1500 mg sitostanol per day to two sitosterolemic patients for 4 wk. They observed decreased plasma cholesterol levels and reduced cholesterol absorption, with little to no change in plasma stanol concentrations. Because the recovery of deuterated sitostanol was similar to that of $Cr_2O_3$, a nonabsorbable marker, these authors concluded that sitostanol was not absorbed to a significant degree in patients with sitosterolemia. The reason for these divergent results may be that the sitostanol in gelatin capsules was poorly absorbed in contrast to our study in which the sitostanol was incorporated in a margarine as a sitostanol ester and given with other foods. Micellar formation of sterols is enhanced by the concurrent presence of dietary fat. Further, in contrast to only 4 wk of sitostanol feeding, we fed Benecol to our patients for 7 and 10 wk, respectively.

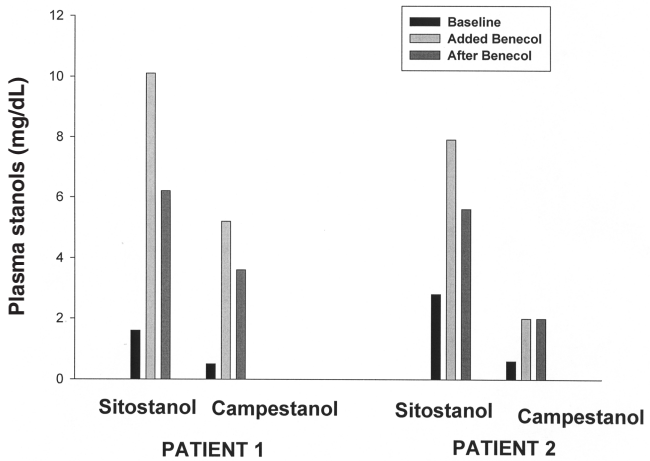


**FIG. 1.** The changes of plasma campestanol and sitostanol of two sitosterolemic patients consuming diets with and without Benecol (patient #1 after 7 wk Benecol and 12 wk after its withdrawal; patient #2 after 10 wk Benecol and 43 wk after its withdrawal).

**TABLE 3**
**Effects of Dietary Sitostanol/Campestanol on the Sterol and Stanol Composition of Plasma (mg/dL plasma) and Tissues (mg/g dried wt) in Control ($n$ = 9) and Experimental Rats ($n$ = 9)**

| Sterols and | Plasma | | Liver | | Skeletal muscle | | Heart muscle | |
|---|---|---|---|---|---|---|---|---|
| Stanols | Control | Expl.[a] | Control | Expl.[a] | Control | Expl.[a] | Control | Expl.[a] |
| Cholesterol | 98.6‡ ± 7.1 | 80.7± 5.2 | 16,109 ± 3,154 | 7,579$^{+}$ ± 1,944 | 948 ± 359 | 891 ± 513 | 2,407 ± 1,043 | 2,781 ± 1,035 |
| Campesterol | 0.8 ± 0.2 | 0.5*** ± 0.1 | 91 ± 25 | 37$^{+}$ ± 16 | 6.1 ± 2.4 | 5.9 ± 2.8 | 22.0 ± 9.4 | 19.4 ± 8.0 |
| Stigmasterol | 0.1 ± 0.05 | 0.03*** ± 0.02 | 35 ± 17 | 13*** ± 9 | 2.4 ± 1.3 | 5.5 ± 5.4 | 15.1 ± 10.4 | 10.9 ± 7.2 |
| Sitosterol | 0.6 ± 0.01 | 0.4*** ± 0.08 | 37 ± 17 | 24 ± 14 | 8.0 ± 4.0 | 21.3* ± 18.4 | 24.7 ± 12.1 | 30.8 ± 12.1 |
| Campestanol | —[b] | 0.3$^{+}$ ± 0.07 | —[b] | 19 ± 6.6 | 1.1 ± 0.05 | 3.7* ± 3.3 | 8.7 ± 2.2 | 18.1*** ± 7.7 |
| Sitostanol | 0.05 ± 0.02 | 0.2$^{+}$ ± 0.06 | 7.3 ± 4.1 | 8.7 ± 5.6 | 2.6 ± 1.7 | 8.4* ± 7.7 | 6.0 ± 3.5 | 14.6* ± 9.2 |

[a]Experimental vs. control group: *$P$ < 0.05; **$P$ < 0.01; ***$P$ < 0.005; $^{+}P$ < 0.001; ‡ mean ± SD.
[b]Not detectable.

In the rat study, the feeding of the stanol mixture (comparable to Benecol) for 4 wk resulted in significant increases in the concentrations of plasma campestanol and sitostanol, a result similar to the increased plasma stanols observed in our two sitosterolemic patients. Concurrently, there was a significant increase of these two stanols in the liver, skeletal muscle, and heart muscle of these rats. Based on these observations, we hypothesize that the increase of stanols in the plasma after sitostanol feeding probably would result in higher tissue stanol content in these sitosterolemic patients. Incidentally, previous studies showed that plasma sterols were deposited in all the tissues in these patients (except brain) in the same proportion as theywere present in plasma (10,11).

Sitosterolemic patients may have normal plasma cholesterol levels and elevated plasma plant sterols, although their plasma total sterols levels are usually much lower than in hypercholesterolemic patients. Yet, premature atherosclerosis with death has been observed in these patients (11–13). Thus, dietary sitosterol is pathologic in the patients with sitosterolemia (12). Dietary sitosterol might even be more atherogenic than dietary cholesterol. In a cross-sectional study, Glueck and coworkers (22) reported such modestly elevated levels of plant sterols in probands and relatives from families with premature coronary heart disease. Sitostanol may be similar to sitosterol in this aspect. Recently, we found that sitosterol and sitostanol were much less esterified by the ACAT enzyme than was cholesterol (Connor, W.E., and Lin, D.S., unpublished data). This may result in more of the free form of these sterols in the tissues. Free sterols are more toxic than esterified sterols (23). Furthermore, in the current study, the turnover of plasma sitostanol was very sluggish (Fig. 1). It has already been documented that the turnover of sitosterol in these patients was very slow (12,24). Therefore, the increased plasma stanols from sitostanol and campestanol feeding to these patients could result in undesirable consequences. It is noteworthy that in a recent report, ezetimibe, a compound known to reduce cholesterol absorption, reduced both plasma cholesterol and plant sterols (25).

Our data indicate that the rat is capable of absorbing stanols from the diet and develops increased sitostanol and campestanol levels in both plasma and tissues after sitostanol feeding. As rats and humans absorb dietary cholesterol similarly (26,27), the rat seems a good model for sterol metabolism in the human intestinal tract.

Sitostanol and campestanol have been incorporated into a margarine, Benecol, which is recommended to the public for daily use to decrease plasma cholesterol levels. From our study, sitostanol and campestanol are absorbed by rats and by humans as well. The consequences of long-term daily consumption of Benecol and the possible accumulation of these stanols in the tissues should be considered. Similar considerations would apply to the use of another margarine, Take Control (Lipton, Englewood Cliffs, NJ), which contains a sitosterol ester. Clearly, neither of these margarines would be recommended for sitosterolemic patients. However, even their use in other patients would present the human intestinal tract with enormous quantities of plant sterols and stanols, up to 10 times or more the usual intake of plant sterols in the United States (28).

Thus, margarines containing sitostanol (Benecol) or sitosterol itself (Take Control) could be hazardous to patients with sitosterolemia and xanthomatosis, and we recommend that they be strictly avoided just as other margarines and liquid vegetable oils containing other plant sterols should be. Medium-chain TG oil would be an exception since it is virtually sterol-free (29). Furthermore, the balance of benefit and possible drawbacks of the long-term feeding of pharmacological amounts of sitostanol and sitosterol in the human diet needs to be evaluated.

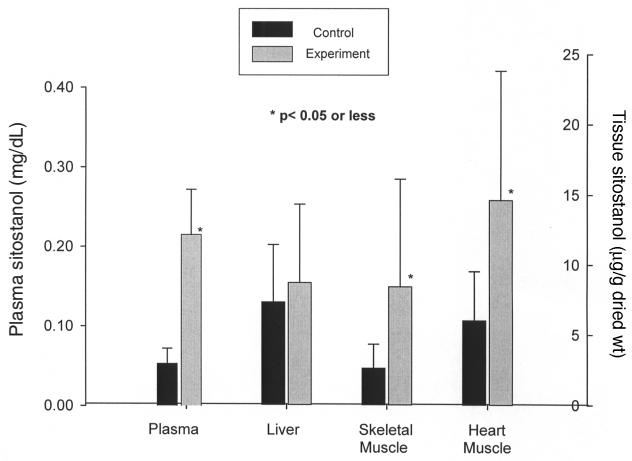


**FIG. 2.** Sitostanol content in plasma and tissues of control and experimental rats ($n$ = 9 each).

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