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Mechanisms of action of plant sterols on inhibition of cholesterol absorption

Comparison of sitosterol and sitostanol

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Summary. The effects of two different plant sterols on intestinal cholesterol absorption were compared in normal volunteers by an intestinal perfusion study during a control period followed by high dose infusion of sitosterol or sitostanol (3.6 μmol/min), to which subjects were allocated in a randomized manner. Cholesterol absorption during the control period was similar in the two groups, averaging 0.88 ± 0.48 μmol/min (32 ± 11%) for group I (sitosterol) and 0.68 ± 0.33 μmol/min (29 ± 9%) for group II (sitostanol). The infusion of a high dose of sitosterol resulted in a significant reduction of cholesterol absorption to 0.47 μmol/min (16%). Following the same dose of sitostanol, cholesterol absorption diminished significantly to 0.15 ± 0.11 μmol/min (5.1 ± 2.9%). Overall cholesterol absorption declined during sitosterol infusion by almost 50%, whereas sitostanol infusion caused a reduction of cholesterol absorption by almost 85%. These findings of a more effective inhibition of cholesterol absorption by sitostanol might confirm the observation recorded by others that an increase in hydrophobicity of a plant sterol results in a higher affinity but lower capacity to mixed micells. This may cause an effective displacement of cholesterol from micellar binding and therefore diminished cholesterol absorption.

Key words: Sitosterol, sitostanol, cholesterol absorption

Cholesterol enters the intestinal tract from two major sources, the diet and bile. In man the total amount of dietary plus biliary cholesterol ranges from 750 to 3000 mg/day [1–3]. Between 20% and 70% of this cholesterol will be absorbed [2–4] after solubilization in mixed micelles containing bile salts, mono-, and diglycerides, fatty acids and lysolecithin. Thus bile salt or pancreas lipase deficiency, disruption of micelles, or competition with cholesterol uptake in the micelles will reduce cholesterol absorption and probably serum cholesterol.

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In 1951, Peterson [5] reported for the first time that the increase of plasma cholesterol levels in chickens caused by cholesterol feeding can be prevented by including 1% soybean sterols in the diet. Since then, numerous studies have confirmed a hypocholesterolemic action of plant sterols, especially sitosterol [6–11]. Plant sterols are structurally related to cholesterol, but differ in their nuclear and/or side chain configuration or polar groups (Fig. 1). Addition of a methyl or ethyl group at the 24 carbon atom of cholesterol leads to campesterol or sitosterol, respectively. These two sterols, together with stigmasterol, are the most frequent plant sterols in nature. Chemical saturation of the delta 5 double bond leads to the 5 alpha position of the hydrogen atom (i.e., campestanol or sitostanol), whereas enzymatic transformation by bacteria in the intestine leads to the 5 beta position (i.e., methylcoprostanol, ethylcoprostanol). In contrast to cholesterol, plant sterols are absorbed to a lesser extent. Animal experiments and studies in human beings have suggested that less than 5% of ingested sitosterol is absorbed [12]. The absorption rate of campesterol is much higher, and substantial plasma levels have been measured [9]. In contrast, several studies have indicated that sitostanol is not absorbed at all [13–15], and feeding of this saturated plant sterol seems to have a more pronounced effect on reduction of serum cholesterol than does sitosterol [14–17]. In order to confirm the mode of action of sitostanol as inhibitor of intestinal cholesterol absorption, a comparison of sitostanol with sitosterol on intestinal cholesterol absorption was performed in man, using an intestinal perfusion technique described by Grundy and Mok [2].

Materials and methods

Patients

Nine male volunteers participated in the present investigation. Six subjects were studied during infusion of one plant sterol, whereas three participated during infusion of both plant sterols on two occasions. All volunteers had normal liver function tests, and none

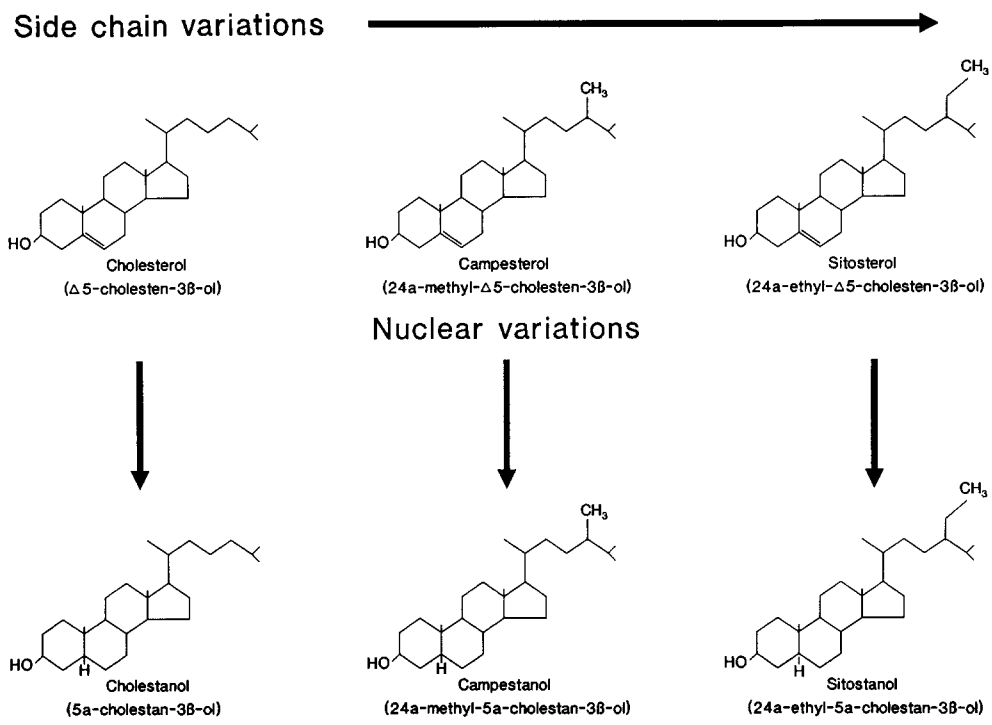

Fig. 1. Conventional chemical configurations of cholesterol, campesterol, sitosterol and the delta 5 saturated compounds. Side chain variations reflect the presence of a methyl or ethyl group at the 24α position. Nuclear variations reflect the absence of the single double bond and the hydrogen at the 5α position

showed any evidence of diabetes, thyroid dysfunction, or gastrointestinal diseases. Clinical data of all subjects and concentrations of serum lipids are given in Table 1. The study was conducted in accordance with the principles of the Helsinki Declaration, and written informed consent was obtained from each subject.

Experimental design

Measurements of biliary secretion and absorption of cholesterol were performed by a combination of the procedure developed by Grundy and Metzger [1] for biliary lipid secretion and the cholesterol absorption method of Grundy and Mok [2], as described previously [18]. The subjects were intubated with a triple-lumen polyvinyl tube on the evening before the study. After an overnight fast, the tube was placed by X-ray guidance with the most proximal outlet opposite to the ampulla of Vater and the second 10 cm distal to it. The third outlet was 50 cm beyond the second one.

A liquid formula diet containing 36% of calories as fat, 16% as protein and 48% as carbohydrates (Nutrodrip, Wander GmbH, Osthofen, FRG) was infused continuously at a constant rate of 1 ml/kg per h (1 ml = 1.07 kcal) through the most proximal lumen by a motor pump. Sitosterol dissolved in triglyceride monoleate was infused through the proximal lumen as a nonabsorbable marker at a dose of 0.8 μmol/min (Delalande, Cologne, FRG). The experimental design provided an initial equilibration period of 4 h for gallbladder contraction and stabilization of the enterohepatic circulation. Thereafter, 5-ml aliquots were collected by continuous aspiration from the second proximal and distal outlet every 30 min throughout the control period of 4 h. After the control period, sitosterol infusion was discontinued and either sitosterol (group 1) or sitostanol (group 2) was infused at a dose of 3.6 μmol/min through the proximal lumen. After another equilibration period of 1 h, samples were again collected from the two distal outlets every 30 min for the next 4 h. Using this procedure, six subjects were studied during high-dose sitosterol and six subjects during high-dose sitostanol infusion. Allocation was performed in a random manner.

After sampling, 5 ml of each sample was immediately added to 5 ml ethanol. After alkaline hydrolysis and extraction of cholesterol and the plant sterols with hexane, cholesterol, sitosterol and sitostanol were quantified by gas liquid chromatography from their trimethylsilylethers using 5-α-cholestane as an internal standard [19]. Complete separation of all sterols was achieved. After each study, the motor pumps were equilibrated for calculation of exact infusion rates.

Calculation of cholesterol secretion and cholesterol absorption

The ratios of cholesterol to sitosterol or sitostanol measured at the second proximal outlet and the known amount of sitosterol (0.8 μmol/min or 3.6 μmol/min) or sitostanol infusion (3.6 μmol/min), were used to calculate the total input of cholesterol. It was then possible to calculate cholesterol absorption from the difference between cholesterol input and the disappearance of cholesterol relative to sitosterol or sitostanol, measured at the third outlet.

Statistical analysis

The results are expressed as means ± SD. Differences between the control period and high-dose sitosterol or sitostanol infusion were compared with the Wilcoxon signed rank test. Differences between the different groups were compared with the Mann-Whitney U-test.

Table 1. Clinical data of the volunteers and serum lipoprotein concentrations

Subject	Age (years)	Weight (kg)	Ideal weight[a] (%)	Cholesterol (mg/dl)[b]			
				Total	LDL	HDL	TG
1	50	74	93	183	71	90	61
2	43	83	121	272	137	48	243
3	48	77	117	141	95	38	89
4	48	100	118	308	169	61	389
5	47	95	117	219	146	53	102
6	58	65	134	242	162	61	96
7	50	78	105	237	145	44	239
8	50	74	108	285	197	64	120
9	63	76	122	343	271	43	147

[a] Ideal body weight = weight (kg)/[height (cm) − 100 − 10%] × 100
[b] Each value shown is the mean of two determinations

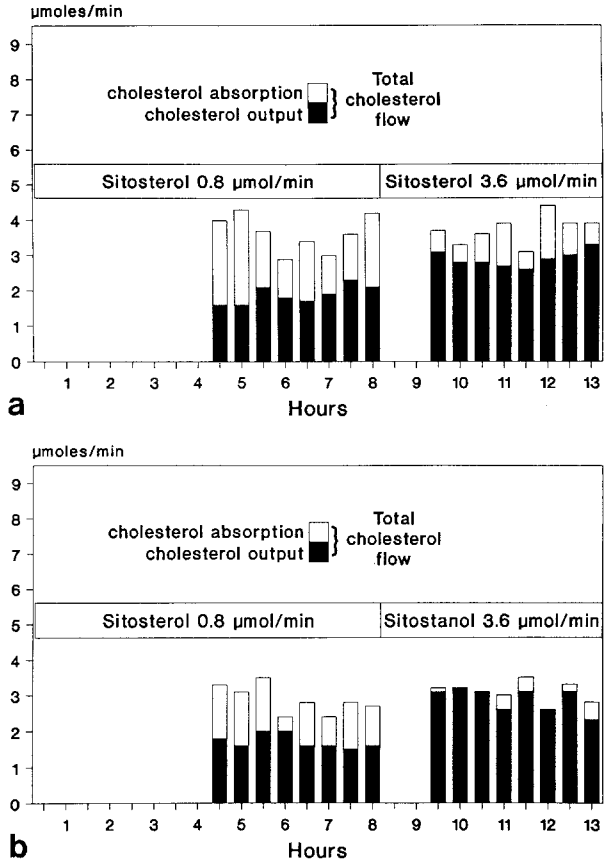

Fig. 2 a, b. Cholesterol absorption during infusion of **a** sitosterol and **b** sitostanol, each at a dose of 3.6 μmol/min

Results

Biliary secretion of cholesterol during the control period was statistically no different in group I and group II averaging 2.63 ± 0.76 μmol/min and 2.22 ± 0.45 μmol/min, respectively (Table 2). During infusion of a high dose of sitosterol (3.6 μmol/min), cholesterol input calculated from the second proximal outlet located 10 cm distal to the papilla of Vater increased in five of six subjects, on an average to 2.96 μmol/min (+ 12.5%). This increase in cholesterol input reached statistical significance ($P < 0.05$). Infusion of sitostanol (3.6 μmol/min) was followed by an even greater increase in cholesterol flow. This increase was also significant ($P < 0.01$) and averaged 23.9%. However, the difference between the increase of cholesterol input in group I and group II was not significant.

A representative study of the effect of high-dose infusion of sitosterol and sitostanol is shown in Fig. 2. Infusion of a high dose of sitosterol was followed by a consistent reduction in cholesterol absorption. In contrast, during infusion of sitostanol in a similar dose cholesterol absorption was almost completely blocked.

Absolute values and percentages of cholesterol absorption in all subjects during the control periods and high-dose sitosterol or sitostanol infusion are summarized in Table 3. Cholesterol absorption during the control period was not different in groups I and II, averaging 0.88 ± 0.48 μmol/min and 0.68 ± 0.33 μmol/min (32 ± 11% and 29 ± 9%), respectively. Sitosterol infusion was associated with a reduction in absorption of cholesterol, on an average to 0.47 μmol/min. The percentage of cholesterol absorption was also reduced significantly to 16% ($P < 0.05$). Thus, infusion of sitosterol at 3.6 μmol/min resulted in an overall reduction of 50% in intestinal cholesterol absorption. When sitostanol was infused at 3.6 μmol/min, cholesterol absorption declined significantly, from 0.68 ± 0.33 μmol/min during the control period to 0.15 ± 0.11 μmol/min during sitostanol infusion ($P < 0.01$). The percentage of cholesterol absorption was also markedly decreased with sitostanol infusion, from 29 ± 9% to 5.1 ± 2.9% ($P < 0.01$). Overall cholesterol absorption during sitostanol infusion diminished by almost 85%. This effective inhibition of cholesterol absorption occurred even though the input of cholesterol was increased significantly (Table 2). Compared with the results obtained with high-dose sitosterol infusion, the mass and percentage of cholesterol absorption was inhibited more effectively by sitostanol infusion ($P < 0.05$).

Discussion

The purpose of the present study was to compare two different plant sterols – sitosterol and sitostanol – for their potency of inhibition of intestinal cholesterol absorption in man. The technique of intestinal intubation for measurement of cholesterol absorption has been described elsewhere [2, 18] and has been validated by another method [3]. The perfusion technique has an advantage in that it allows for direct measurement of the mass and the percentage of biliary cholesterol absorption. In addition, absorption of both endogenous (biliary) and exogenous (dietary) cholesterol entering the intestinal tract can be determined. The possibility of differentiating between the mass and the percentage of biliary cholesterol absorption might be important, because the hepatic secretion of cholesterol can vary and a change in biliary cholesterol secretion must be followed by a change in either percentage or mass of cholesterol absorption or both. Infusion of cholesterol with a liquid formula can also be changed to vary cholesterol input. In the present study cholesterol infusion

Table 2. Mean input of cholesterol during control period and infusion of sitosterol (group I) and sitostanol (group II) in a dose of 3.6 μmol/min

Cholesterol input μmol/min (mean ± SD)[a]					
Group I			Group II		
Subject	Control	Sitosterol	Subject	Control	Sitostanol
1	1.92 ± 0.80	2.21 ± 0.53	1	1.83 ± 0.45	2.28 ± 0.52
2	3.63 ± 0.50	3.72 ± 0.39	2	2.01 ± 0.25	2.41 ± 0.28
3	2.44 ± 0.39	2.68 ± 0.44	3	2.57 ± 0.51	2.72 ± 0.53
4	3.21 ± 1.03	3.67 ± 0.14	7	1.73 ± 0.18	1.80 ± 0.67
5	2.96 ± 0.59	2.97 ± 0.54	8	2.32 ± 0.29	4.17 ± 1.19
6	1.68 ± 0.26	2.53 ± 0.63	9	2.87 ± 0.41	3.14 ± 0.53
Mean	2.64	2.96[b]		2.22	2.75[b]
± SD	0.76	0.62		0.45	0.83

[a] Values for each subject represent the average for eight determinations during the steady state period of formula infusion

[b] Significantly different from corresponding control values ($P < 0.05$)

Table 3. Mean cholesterol absorption during control period and infusion of sitosterol (group I) and sitostanol (group II) in a dose of 3.6 μmol/min

Cholesterol absorption [μmol/min (%)]									
Group I					Group II				
Subject	Control		Sitosterol		Subject	Control		Sitostanol	
1	0.58	30	0.42	19	1	0.49	28	0.08	3.5
2	1.73	48	0.84	23	2	0.39	19	0.02	1.7
3	1.06	43	0.42	16	3	1.02	40	0.26	9.5
4	0.90	28	0.62	17	7	0.49	26	0.12	4.9
5	0.64	22	0.27	9.1	8	0.50	22	0.31	7.6
6	0.39	23	0.24	10	9	1.17	41	0.12	3.5
Mean	0.88	32	0.47[a]	16[a]		0.68	29	0.15[a, b]	5.1[a, b]
± SD	0.48	11	0.23	5		0.33	9	0.11	2.9

[a] Significantly different from corresponding control values ($P < 0.05$).
[b] Significantly different from absorption during sitosterol infusion ($P < 0.01$).

with the liquid formula remained constant and was less than 0.3 μmol/min. Thus, the change in cholesterol absorption observed in the present study was due to changes in biliary cholesterol absorption.

Previously, some investigators have reported the effect of sitosterol on cholesterol absorption in man [6, 19]. In general, a reduction of between 25% and 65% in cholesterol absorption could be observed. This magnitude of inhibition of cholesterol absorption by sitosterol was confirmed in the present investigation. Studies with sitostanol in man are lacking, but sitostanol has been compared previously with sitosterol for inhibition in cholesterol absorption and serum cholesterol in experimental animals [13, 16, 17]. Sugano and coworkers [14] added 0.5% of cholesterol to rat diet and observed an increase in serum cholesterol. Addition of 0.5% of sitosterol diminished the increase in serum cholesterol, but the addition of 0.5% of sitostanol produced a significantly greater effect. These authors and others [13] were able to prove by fecal excretion or lymphatic drainage that sitostanol is almost unabsorbable, in contrast to small amounts of sitosterol, which can always be detected in human blood.

In the present study a direct comparison of sitosterol with sitostanol on intestinal cholesterol absorption confirmed a marked effect of sitostanol on inhibition of cholesterol absorption in man, although these two plant sterols differ only in the double bond in the B ring. It has been noted before that modification of the sterol nucleus has a profound influence on its absorbability. For example, cholestanol, generated by hydrogenation of the double bond in the B ring of cholesterol, is absorbed to a lesser extent than cholesterol [20], although whether it inhibits cholesterol absorption is not known. In addition, in animal experiments it has been demonstrated that an inverse relationship exists between the intestinal absorbability of plant sterols and their effects on inhibition of cholesterol absorption [13]. This relationship could be one explanation for the present results obtained with sitostanol. Another explanation could be derived from recent in vitro studies by Armstrong and Carey [21]. These investigators observed a close relationship between hydrophobicity of neutral sterols and number of side chain carbon atoms on the one hand and decrease of micellar solubility on the other. Moreover, the sitosterol binding on trihydroxy bile salt micelles seemed to be energetically favored compared with cholesterol. These findings of a high affinity but low capacity of hydrophobic plant sterols for micelles may cause an effective displacement of cholesterol from micellar binding. If these authors are correct in supposing that hydrogenation of the delta-5-nucleus double bond leads to a moderate enhancement of the hydrophobicity of sterols, their findings could explain why sitostanol is more effective in inhibition of cholesterol absorption than sitosterol.

Another interesting finding was observed in the present study, which is worthy of discussion. Cholesterol input measured at the second proximal opening increased significantly during high-dose infusion of both plant sterols. How can we account for this increased flow of cholesterol with high-dose infusion of sitosterol and sitostanol? Three possibilities might be considered.

First, biliary cholesterol output increased during the second part of the infusion because of the long duration of the study (13 h). However, previous studies have clearly indicated that biliary cholesterol secretion is quite constant during continuous liquid formula infusion, even over a longer period [1, 3].

A second possibility must also be considered. Both plant sterols decrease the absorption of cholesterol. Therefore less cholesterol is incorporated into chylomicrons and returns to the liver. As a consequence, cholesterol synthesis in the liver might increase, resulting in a higher biliary cholesterol secretion. Although long-term inhibition of cholesterol absorption stimulates cholesterol synthesis, it seems unlikely that the short period of reduced cholesterol absorption (5 h) will be enough to stimulate cholesterol synthesis and thereby cholesterol output.

The third possible explanation for why both plant sterols increase cholesterol input at the second proximal outlet seems more plausible. From the results of the present study there is no doubt that infusion of sitosterol or sitostanol in a dose of 3.6 μmol/min results in inhibition of cholesterol absorption between the second proximal and the distal outlets. Why should the plant sterols not inhibit cholesterol absorption even between the site of the infusion, the first proximal opening adjacent to the ampulla of Vater, and the aspiration site 10 cm distal to it? This explanation for an increase in cholesterol flow is a logical consequence from the results of the current study. Under these circumstances, cholesterol absorption during the control period is underestimated. Taking into account that on an average 23% of cholesterol is absorbed in the 10-cm mixing segment (difference in cholesterol input in group II between sitostanol infusion and the control period), cholesterol flow has to be corrected for this factor, resulting in higher rates of cholesterol absorption. Thus cholesterol absorption in subjects of group I (sitosterol) would decline from 45% to 16% (an overall reduction of 64%) and in subjects of group II (sitostanol) from 42% to 5.1% (a reduction of 88%). Moreover, all previous studies for measurement of biliary secretion of cholesterol might have underestimated the amount of hepatic cholesterol output because of choles-

terol absorption in the mixing segment. Further studies using high-dose sitostanol as a marker will clarify this possibility.

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References

1. Grundy SM, Metzger AL (1972) A physiological method for estimation of hepatic secretion of biliary lipids. Gastroenterology 62: 1200–1217
2. Grundy SM, Mok HYI (1977) Determination of cholesterol absorption in man by intestinal perfusion. J Lipid Res 18: 263–271
3. Mok HYI, von Bergmann K, Grundy SM (1979) Effects of continuous and intermittent feeding on biliary lipid outputs in man: application for measurements of intestinal absorption of cholesterol and bile acids. J Lipid Res 20: 389–398
4. Samuel P, McNamara DJ, Ahrens EH, Crouse JR, Parker T (1982) Further validation of the plasma isotope ratio method for measurement of cholesterol absorption. J Lipid Res 23: 480–489
5. Peterson DW, Nichols CW, Peek NF, Chaikoff IL (1956) Depression of cholesterol in human subjects consuming butter containing soy sterols. Fed Proc 15: 569
6. Grundy SM, Mok HYI (1976) Effects of low dose phytosterols on cholesterol absorption in man. In: Greten H (ed) Lipoprotein metabolism. Springer, New York Berlin Heidelberg, pp 112–118
7. Lees AM, Mok HYI, Lees RS, McCluskey MA, Grundy SM (1977) Plant sterols as cholesterol – lowering agents: clinical trials in patients with hypercholesterolemia and studies of sterol balance. Atherosclerosis 28: 325–338
8. Lederle RM (1983) Langzeitbehandlung der Hypercholesterinämie bei terminaler Niereninsuffizienz. Med Klin 78: 136–137
9. Lees RS, Lees AM (1976) Effects of sitosterol therapy on plasma lipid and lipoprotein concentrations. In: Greten H (ed) Lipoprotein metabolism. Springer, New York Berlin Heidelberg, pp 119–124
10. Farquhar JW, Smith RE, Dempsey ME (1956) The effect of beta sitosterol on the serum lipids of young men with arteriosclerotic heart disease. Circulation 14: 77–82
11. Oster P, Schlierf G, Heuck C, Greten H, Grundert-Remy U, Haase W, Klose G, et al. (1976) Sitosterol in tpye II hyperlipoproteinemia. In: Greten H (ed) Lipoprotein metabolism. Springer, New York Berlin Heidelberg, pp 125–130
12. Salen G, Ahrens EH Jr, Grundy SM (1970) Metabolism of beta-sitosterol in man. J Clin Invest 49: 952–967
13. Hassan AS, Rampone AJ (1979) Intestinal absorption and lymphatic transport of cholesterol and beta-sitostanol in the rat. J Lipid Res 20: 646–653
14. Sugano M, Morioka H, Ikeda I (1977) A comparison of hypocholesterolemic activity of beta-sitosterol and beta-sitostanol in rats. J Nutr 107: 2011–2019
15. Heinemann T, Leiss O, von Bergmann K (1986) Effects of low-dose sitostanol on serum cholesterol in patients with hypercholesterolemia. Atherosclerosis 61: 219–223
16. Ikeda I, Sugano M (1978) Comparison of absorption and metabolism of beta-sitosterol and beta-sitostanol in rats. Atherosclerosis 30: 227–237
17. Chandler RF, Hooper SN, Ismail HA (1979) Antihypercholesterolemic studies with sterols: beta-sitosterol and stigmasterol. J Pharm Sci 68: 245–247
18. Leiss O, von Bergmann K, Streicher U, Strotkoetter H (1984) Effect of three different dihydroxy bile acids on intestinal cholesterol absorption in normal volunteers. Gastroenterology 87: 144–149
19. Mattson FH, Grundy SM, Crouse JR (1982) Optimizing the effect of plant sterols on cholesterol absorption in man. Am J Clin Nutr 35: 697–700
20. Ivy AC, Lin TM, Karvinen F (1955) Absorption of dihydrocholesterol and soya sterols by the rat's intestine. Am J Physol 183: 79–85
21. Armstrong MJ, Carey MC (1987) Thermodynamic and molecular determinants of sterol solubilities in bile salt micelles. J Lipid Res 28: 1144–1155