

Comparison of the hepatic clearances of campesterol, sitosterol, and cholesterol in healthy subjects suggests that efflux transporters controlling intestinal sterol absorption also regulate biliary secretion

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Background: Recently identified ABCG5/8 transporters are responsible in part for the different absorption rates of campesterol, sitosterol, and cholesterol. These transporters are also expressed in the liver and might regulate biliary sterol secretion.

Aims: This study was therefore conducted to determine the biliary secretion rates and hepatic clearances of campesterol, sitosterol, and cholesterol.

Subjects: Six healthy, male volunteers.

Methods: Deuterium labelled sitosterol and campesterol, and unlabelled sitostanol were constantly infused together with a liquid formula using a duodenal perfusion technique. Biliary secretion and hepatic clearance rates were calculated from hourly bile and plasma samples.

Results: Plasma concentrations of cholesterol, campesterol, and sitosterol averaged 167.5 (50) mg/dl (SD), 0.50 (0.22) mg/dl, and 0.30 (0.10) mg/dl, respectively. Sitosterol showed a significantly higher biliary secretion rate (1.23 (0.87) mg/h) than campesterol (0.76 (0.54) mg/h, $p=0.0321$), but both plant sterols had significantly lower biliary secretion rates compared with cholesterol (47.7 (17.5) mg/h; $p=0.001$ for both). Hepatic clearance of cholesterol (0.31 (0.18) dl/h) was significantly lower compared with campesterol (2.11 (2.51) dl/h) and sitosterol (4.97 (4.70) dl/h; $p=0.028$ for both), and the clearance of campesterol was significant lower compared with sitosterol ($p=0.028$).

Conclusion: The observed inverse relation between hepatic clearance and known intestinal absorption of cholesterol, campesterol, and sitosterol supports the hypothesis that the ABCG5/8 transporters regulating intestinal sterol absorption might also be involved in biliary sterol excretion.

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Cholesterol absorption rates range in most subjects from 40% to 60%,^{1–6} whereas plant sterols are absorbed to a much lower extent.^{7–9} There exist also differences between the absorption rates of plant sterols.^{8–10} Sterol absorption studies indicate that the absorption rate of campesterol, differing from cholesterol by an additional methyl group at position 24 of the side chain, ranges from 9% to 18%, and that of sitosterol, with an additional ethyl group, from 4% to 8%.^{8–10} Thus, the human intestine provides an effective barrier against absorption of plant sterols. Recent studies have contributed to our understanding of the molecular mechanism of intestinal sterol absorption by identifying two ABC (ATP-Binding Cassette) transporters, ABCG5 and ABCG8.^{11–13} These transporters pump plant sterols and cholesterol selectively back from the enterocyte into the intestinal lumen thereby regulating their absorption rates. Sitosterol is pumped most effectively back into the intestinal lumen resulting in the lowest absorption rate, whereas cholesterol is least re-excreted, showing therefore the highest absorption rate of the three sterols. After absorption, plant sterols are transported in lipoproteins¹⁴ and taken up by the liver. Low concentrations are detectable in blood.^{7–15} As these ABCG transporters are also expressed in the liver^{11–13} they might regulate biliary output of both cholesterol and plant sterols. Therefore, the transporters in the liver should also selectively regulate biliary secretion resulting in different hepatic output rates. For this purpose biliary secretion rates of campesterol, sitosterol, and cholesterol were measured in healthy volunteers in this study.

METHODS

Subjects

Six male healthy volunteers (mean (SD) age 25 (2)) with normal body weight (73 (4) kg) and body mass index (22.3 (0.9) kg/m²) participated in the study. None of the subjects had impaired renal or liver function, diabetes mellitus or thyroid dysfunction, and none received any medication during the preceding six weeks. All subjects were non-vegetarians and on a typical western diet during the last six weeks before the study. In these volunteers serum concentrations and biliary secretion rates of campesterol, sitosterol, and cholesterol were measured.

Measurement of biliary secretion

The evening before the study, the subjects were admitted to the metabolic ward of the Department of Clinical Pharmacology, University of Bonn, where they swallowed a triple lumen tube. Next morning the tube was positioned under radiological guidance with the two proximal outlets adjacent to the ampulla of Vater and the third outlet 10 cm distally just beyond the ligament of Treitz. Biliary secretion of cholesterol was measured by the intestinal perfusion method of Grundy and Metzger,¹⁶ as described previously,^{17,18} using sitostanol as non-absorbable marker. Briefly, through the most proximal outlet of the tube a liquid formula (Fresubin, Fresenius Kabi, Bad Homburg, Germany) containing 15% of calories as protein, 55% as carbohydrates, 30% as fat, and also containing small amounts of natural sitostanol, was infused constantly with an infusion rate of 1.42 kcal/kg/h. Fivefold deuterated

Table 1 Plasma concentrations of cholesterol and plant sterols and their ratio to cholesterol in six healthy volunteers

Subject	Cholesterol (mg/dl)*	Campesterol (mg/dl)*	Sitosterol (mg/dl)*	Campesterol/cholesterol (µg/mg)*	Sitosterol/cholesterol (µg/mg)*
1	137 (3)	0.28 (0.01)	0.20 (0.014)	2.05 (0.03)	1.45 (0.03)
2	117 (12)	0.22 (0.02)	0.16 (0.01)	1.87 (0.04)	1.35 (0.13)
3	173 (4)	0.55 (0.01)	0.35 (0.01)	3.18 (0.02)	2.05 (0.05)
4	261 (5)	0.77 (0.02)	0.43 (0.01)	2.96 (0.04)	1.65 (0.04)
5	168 (8)	0.69 (0.03)	0.34 (0.02)	4.10 (0.03)	2.05 (0.05)
6	150 (7)	0.50 (0.02)	0.29 (0.02)	3.30 (0.04)	1.90 (0.08)
Mean (SD)	167.5 (50)	0.50 (0.22)	0.30† (0.10)	2.91 (0.83)	1.74‡ (0.30)

*Mean (SD) of five hourly samples. †Significantly lower compared with campesterol ($p=0.0089$). ‡Significantly lower compared with campesterol/cholesterol ratio ($p=0.0038$).

2,2,4,4,6-²H₅-sitosterol and 2,2,4,4,6-²H₅-campesterol (Medical Isotopes, Concord, NH) were constantly infused with the liquid formula after solubilisation in lecithin. During the perfusion study the liquid formula was stirred constantly. The exact infusion rates of sitostanol, deuterated sitosterol, and campesterol were measured for every subject. After allowing four hours for gallbladder contraction and for stabilisation of hepatic bile secretion, hourly samples were obtained for the next five hours from the distal and the second proximal outlets by continuous slow aspiration. Hourly blood samples for sterol measurements were obtained during the perfusion time.

Sterol measurement

Total concentrations of sitosterol, campesterol, and cholesterol in serum, and cholesterol in the liquid formula and duodenal samples were analysed as trimethylsilyl ethers by gas-liquid chromatography (GLC; Hewlett Packard 5890) using an automatic injection system (Automatic Sampler Hewlett Packard 7673 A) with 5 α -cholestane as internal standard.⁸ Quantification of natural and deuterated sitosterol and campesterol as well as natural sitostanol was done in duodenal samples and liquid formula by means of gas-liquid chromatography-mass spectrometry (GLC-MS) on a Hewlett-Packard (HP 5970) quadrupole type mass spectrometer with an HP 5890 gas chromatograph and an HP 7698 A automatic sample injector as described previously.⁹ The following ions were traced: natural and deuterated campesterol: M⁺ 472 and M⁺ 477; natural and deuterated sitosterol: M⁺ 486 and M⁺ 491; natural sitostanol: M⁺ 488.

Biliary sterol secretion

Biliary cholesterol secretion was calculated from the ratio of cholesterol to sitostanol from the distal samples multiplied by the constant hourly infusion rate of sitostanol minus the infusion rate of cholesterol with the liquid formula. The biliary secretion rates of campesterol and sitosterol were calculated from the constant ratio of natural to deuterated sterols infused with the formula and the enrichment with biliary campesterol and sitosterol from the distal samples, multiplied by the known infusion rate of sitostanol. Hourly samples were analysed from the liquid formula to measure the ratio of natural to deuterated campesterol and sitosterol as well as natural sitostanol. The coefficients of variation of the ratio of campesterol and sitosterol of the five hourly samples ranged from 0.7% to 1.2% in the six subjects, indicating homogeneity of the mixture. The "hepatic sterol clearance" was calculated by dividing hourly biliary sterol secretion by the respective serum sterol concentration. The results were expressed as dl/h.

Ethics

The study protocol was approved by the local ethics committee and all participants gave written informed consent. The study was conducted in accordance with the revised Declaration of Helsinki.

Statistical procedures

All parameters were expressed as mean (SD). Correlation analysis were calculated as Pearson's product moment using Fisher's test for significance testing. Normal distribution was tested with the Shapiro-Wilks test. Non-normally distributed data such as the hepatic clearances were compared with the two sided Wilcoxon signed rank test. All other parameters were normally distributed and compared with the two sided Student's *t* test for dependent samples. A *p* value <0.05 was considered as statistically significant. *p* Values <0.10 were considered as indicators for a statistical trend. Because of the exploratory character of the study no adjustments for multiple comparisons were made.

RESULTS

Mean plasma concentrations of cholesterol, campesterol, and sitosterol from five hourly samples showed a wide variation in the six subjects (table 1). On average the concentration of cholesterol was 370 and 590 times higher than of campesterol and sitosterol. The concentrations of campesterol were 1.7 times higher than of sitosterol. A positive correlation between the concentration of cholesterol and campesterol ($r=0.855$; $p=0.030$), and sitosterol ($r=0.897$; $p=0.015$) was observed. The slope of the regression curve was approximately two times higher for campesterol compared with sitosterol (data not displayed). The correlation between campesterol and sitosterol concentrations was also highly significant ($r=0.972$; $p=0.001$). Data of biliary secretion rates of cholesterol, campesterol, and sitosterol are given in table 2. Secretion rates of cholesterol in the six volunteers averaged 48 mg/h. Secretions rates of cholesterol compared with campesterol and sitosterol were 79% and 56% higher, and those of sitosterol were 1.6 times higher than of campesterol. A highly positive correlation was found between biliary secretion of cholesterol and both plant sterols (fig 1). The correlation between the secretion rates of campesterol and sitosterol was also highly significant ($r=0.954$; $p=0.003$).

Table 2 Biliary secretion of cholesterol, campesterol, and sitosterol in six healthy volunteers

Subject	Cholesterol (mg/h)*	Campesterol (mg/h)*	Sitosterol (mg/h)*
1	38 (6)	0.36 (0.04)	0.85 (0.10)
2	79 (10)	1.53 (0.18)	2.12 (0.40)
3	53 (9)	1.22 (0.50)	2.34 (0.77)
4	48 (7)	0.92 (0.24)	1.39 (0.61)
5	39 (10)	0.33 (0.20)	0.50 (0.37)
6	29 (6)	0.21 (0.05)	0.19 (0.09)
Mean (SD)	47.7 (17.5)†‡	0.76 (0.54)	1.23 (0.87)§

*Mean (SD) of five hourly samples. †Significantly higher compared with campesterol ($p=0.001$). ‡Significantly higher compared with sitosterol ($p=0.001$). §Significantly higher compared with campesterol ($p=0.0321$).

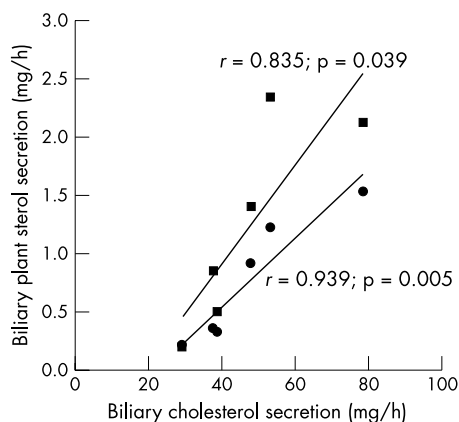


Figure 1 Correlation of the biliary secretion of cholesterol compared with the biliary secretion of campesterol (filled circles) and sitosterol (filled squares).

Table 3 Hepatic clearance of cholesterol, campesterol, and sitosterol in six healthy volunteers

Subject	Cholesterol clearance (dl/h)*	Campesterol clearance (dl/h)*	Sitosterol clearance (dl/h)*
1	0.28 (0.05)	1.29 (0.12)	4.29 (0.50)
2	0.68 (0.08)	7.06 (1.20)	13.56 (2.33)
3	0.31 (0.06)	2.23 (0.90)	6.60 (2.11)
4	0.18 (0.02)	1.19 (0.32)	3.22 (1.36)
5	0.23 (0.06)	0.48 (0.30)	1.47 (1.09)
6	0.20 (0.05)	0.44 (0.12)	0.69 (0.38)
Mean (SD)	0.31 (0.18)†	2.11 (2.51)	4.97 (4.70)‡

*Mean (SD) of five hourly samples. †Significantly lower compared with campesterol and sitosterol ($p=0.028$, for both). ‡Significantly higher compared with campesterol ($p=0.028$).

The individual “hepatic clearance rates” of cholesterol, campesterol, and sitosterol are summarised in table 3. The lowest clearance was observed for cholesterol. Clearance rates of campesterol and sitosterol were 6 and 14 times higher than of cholesterol, and the one of sitosterol was 2.6 times higher than the hepatic clearance of campesterol. The clearance of campesterol and sitosterol were significantly higher compared with cholesterol ($p=0.028$, for both). The positive correlation between the hepatic clearance rates of the plant sterols and cholesterol was highly significant for both campesterol ($r=0.982$; $p=0.0005$) and sitosterol ($r=0.957$; $p=0.0028$) and was also highly significant for the correlation of the hepatic clearance rates of campesterol and sitosterol ($r=0.979$; $p=0.001$).

DISCUSSION

Using deuterated campesterol and sitosterol in a liquid formula as marker makes it possible to quantify hepatic output of the two plant sterols. From the simultaneous measurements of cholesterol, campesterol, and sitosterol in serum, the “hepatic clearance” of the three sterols could also be calculated. Thus, this study describes for the first time biliary secretion and hepatic clearance of campesterol and sitosterol together with cholesterol. However, it has to be realised that the calculation of total cholesterol clearance by this approach gives not the true clearance rate because cholesterol is also synthesised and metabolised in the liver. Otherwise, the hepatic clearances for sitosterol and campesterol actually reflect the total clearance of these sterols, as they do not undergo extensive metabolism in humans.^{19,20}

From the chemical structure of the plant sterols it could have been anticipated that biliary secretion rates of plant sterols correlate positively with cholesterol output. Indeed, a

highly significant correlation between hepatic cholesterol output and the secretion rates of campesterol and sitosterol was detected in this study. Furthermore, a marked difference between the “hepatic clearance” of the three sterols was observed. The highest clearance was detected for sitosterol and the lowest for cholesterol. The clearance of campesterol was intermediate. These results are in line with previous findings from Salen *et al* and Bhattacharyya *et al*^{21,22} who demonstrated a faster turnover of sitosterol compared with cholesterol after intravenous injection of radio labelled tracers. The observed lower campesterol clearance explains also why the serum concentrations of campesterol in adults are always higher than those of sitosterol.^{7,9,23} Whereas the dietary intake of campesterol is on average only one third compared with sitosterol²⁴ the absorption rate is three times higher,^{8,10} and the “hepatic clearance” of campesterol is more than two times lower compared with sitosterol, resulting in higher plasma concentrations compared with sitosterol. The positive correlation of biliary cholesterol and plant sterol secretion rates might be unexpected as it could be supposed that there would be a competitive inhibition of different sterols, which might result in a negative correlation. Assuming identical transporters at the biliary membrane one possible explanation could be that the biliary sterol secretion rate is a function of the number of available sterol transporters. Thus, the higher the biliary secretion of one sterol the higher is the number of transporters resulting also in higher biliary secretion rates of other substrates of these transporters. This hypothesis is supported by the work of Repa and colleagues who found the liver X receptors (LXRs) mediated increase in ABCG5/8 mRNA expression in cholesterol fed mice in both intestine and liver.²⁵ Also the “hepatic clearance” rates—which have been shown to be inversely related to their absorption rates^{8,10} in this study—indicate that the same transporters which regulate intestinal cholesterol absorption of sterols are also involved in their biliary secretion. These results are also in line with the findings in the rare inherited disease of phytosterolaemia, where at least one of the ABCG5/8 transporters is defective.^{11–13} Hyperabsorption, but also diminished biliary excretion of cholesterol and plant sterols are the main metabolic defects in this disease.^{26–28}

The results of this study—that sterols with lower intestinal absorption efficiency express higher hepatic clearance rates—support the hypothesis that identical transporters regulate intestinal sterol absorption by re-excretion into the gut and hepatic output into bile in humans. However, more studies with different expression of the ABC transporters have to be performed to confirm the results.

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