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The metabolism of plant sterols is disturbed in postmenopausal women with coronary artery disease

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Abstract

In postmenopausal coronary artery disease (CAD) women, serum plant sterols are elevated. Thus, we investigated further whether serum plant sterols reflect absolute cholesterol metabolism in CAD as in other populations and whether the *ABCG5* and *ABCG8* genes, associated with plant sterol metabolism, were related to the risk of CAD. In free-living postmenopausal women with (n = 47) and without (n = 62) CAD, serum noncholesterol sterols including plant sterols were analyzed with gas-liquid chromatography, cholesterol absorption with peroral isotopes, absolute cholesterol synthesis with sterol balance technique, and bile acid synthesis with quantitating fecal bile acids. In CAD women, serum plant sterol ratios to cholesterol were 21% to 26% ($P < .05$) higher than in controls despite similar cholesterol absorption efficiency. Absolute cholesterol and bile acid synthesis were reduced. Only in controls were serum plant sterols related to cholesterol absorption (eg, sitosterol; in controls: $r = 0.533$, $P < .001$; in CAD: $r = 0.296$, P = not significant). However, even in CAD women, serum lathosterol (relative synthesis marker) and lathosterol-cholestanol (relative synthesis-absorption marker) were related to absolute synthesis and absorption percentage (P range from .05 to <.001) similarly to controls. Frequencies of the common polymorphisms of *ABCG5* and *ABCG8* genes did not differ between coronary and control women. In conclusion, plant sterol metabolism is disturbed in CAD women; so serum plant sterols only tended to reflect absolute cholesterol absorption. Other relative markers of cholesterol metabolism were related to the absolute ones in both groups. *ABCG5* and *ABCG8* genes were not associated with the risk of CAD.

1. Introduction

Coronary artery disease (CAD) is a frequent cause of morbidity and mortality also in women. Elevated serum cholesterol level is an important risk factor for the atheromatous artery disease. It has been shown earlier that, in postmenopausal coronary women, absolute whole-body cholesterol metabolism is perturbed so that cholesterol synthesis is downregulated and cholesterol turnover is lower compared with healthy controls [1]. In this previous study, cholesterol absorption percentage was similar between CAD and control women; but the absolute absorption of cholesterol was lower in CAD than in controls owing to lower biliary secretion of cholesterol [1].

In addition to cholesterol, serum contains also small amounts of noncholesterol sterols. Their serum levels have been compared with the direct whole-body measurements of cholesterol metabolism, that is, sterol balance technique, the criterion standard of cholesterol synthesis, and peroral cholesterol absorption percentage. It has turned out that the cholesterol precursors desmosterol and lathosterol and especially their ratios to cholesterol reflect cholesterol synthesis in general [2-5] and that those of cholestanol (a metabolite of cholesterol) and plant sterols campesterol, sitosterol, and avenasterol reflect cholesterol absorption [4,6,7]. Squalene, a nonsterol precursor of cholesterol, is a cholesterol synthesis marker in some [2,3,5] but not all populations. In CAD women, squalene, desmosterol, campesterol, and sitosterol in serum were elevated compared

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with controls [8,9]. These results, however, revealed some peculiarities.

Despite reduced absolute cholesterol synthesis and lower serum lathosterol level in CAD women compared with control women, serum desmosterol and squalene levels were elevated in CAD [8,9]. In addition, despite similar cholesterol absorption percentage and serum cholestanol value in CAD and control women, serum campesterol and sitosterol levels were paradoxically elevated in CAD [8,9]. There are also other reports of elevated serum plant sterol levels in CAD including also men [10-13]. These observations could suggest that the metabolism of plant sterols is abnormal in CAD, and their serum levels may not any longer reflect cholesterol absorption.

Two mechanisms have been revealed to regulate the absorption of cholesterol and plant sterols, that is, the Niemann-Pick C1 Like 1 (NPC1L1) and the ABCG5 and ABCG8 intestinal proteins [14,15]. Mutations in the *ABCG5* and *ABCG8* genes cause phytosterolemia, an inherited disease with high absorption and low biliary secretion of cholesterol and plant sterols, high serum plant sterol levels, and aggressive atherosclerosis [15-17]. Sequence variations in D19H [18,19] and T400K [18] of *ABCG8* were associated with low serum plant sterol [18,19] and high serum synthesis marker [19] ratios to cholesterol in earlier studies. The question now arises whether the sequence variation in *ABCG5* and *ABCG8* is associated with the presence of CAD. To this end, we evaluated further the relationships between relative and absolute variables of sterol metabolism to find out how plant sterol metabolism is perturbed and whether serum noncholesterol sterols and squalene can be used as surrogate markers of absolute cholesterol metabolism (daily synthesis and absorption) in postmenopausal women with CAD. We also compared the frequencies of the common sequence variants of *ABCG5* and *ABCG8* genes between women with and without CAD.

2. Materials and methods

2.1. Subjects

The study population has been described earlier in detail [9]. In short, postmenopausal women, aged 50 to 55 years, who had been successfully treated for CAD at the University Central Hospital of Helsinki from 1988 to 1996 were recruited to the study (n = 47). Coronary artery disease was verified by coronary angiography as at least 50% occlusion in 2 coronary vessels. Women of similar age were randomly chosen from the population registry of Helsinki as controls (n = 62). The controls were free of chest pain and dyspnea, and results of their electrocardiograms were normal. *Postmenopause* was defined by amenorrhoea and elevated serum follicle-stimulating hormone level (>30 U/L). Subjects on hypolipidemic medication or consuming plant sterol–enriched food, or with severe heart, liver, thyroid, or digestive tract diseases or malignancies or diabetes mellitus were excluded.

All subjects volunteered for the study and gave their written informed consent. The study was carried out in accordance with the principles of the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the Department of Medicine, University of Helsinki.

2.2. Study design

All subjects were free-living and on their habitual diet. After a 12-hour fast, a blood sample was drawn, of which serum lipids, squalene and sterols, and routine laboratory measurements were taken to ensure normal health. Twenty-seven CAD and 43 control women volunteered to the metabolic studies revealing the absolute whole-body cholesterol synthesis, bile acid synthesis, and peroral cholesterol absorption percentage. Cholesterol synthesis was assayed with the sterol balance technique [20,21]. Bile acid synthesis was evaluated with the quantitation of fecal bile acids. All these methods are considered as the criterion standards for the direct whole-body metabolic studies of cholesterol.

2.3. Laboratory measurements

Serum total cholesterol and triglycerides and high-density lipoprotein (HDL) cholesterol after precipitation were measured with enzymatic photometric methods with commercial kits (Boehringer Diagnostica, Mannheim, Germany). Squalene and noncholesterol sterols were quantified from nonsaponifiable serum material with capillary gas-liquid chromatography (GLC) (HP 5890 Series II plus; Hewlett Packard, Wilmington, DE) equipped with a 50-m–long Ultra 2 capillary column (5% phenylmethyl siloxane) (Agilent Technologies, Wilmington, DE) with a method developed by Miettinen [22]. The procedure uses 5α-cholestane as internal standard; and it measures the concentrations of squalene, cholesterol, cholestanol, desmosterol, lathosterol, campesterol, sitosterol, and avenasterol in the increasing order of refractory time. The variability percentage was 3.9% for cholesterol, 4.4% for cholestanol, 7.1% for desmosterol, 5.0% for lathosterol, 4.9% for campesterol, and 3.0% for sitosterol.

The values of squalene and noncholesterol sterols were expressed also in terms of 10^2 × micromoles per millimole of cholesterol (called *ratio* in the text) dividing their concentrations by the cholesterol value of the same GLC run to eliminate different low-density lipoprotein (LDL) cholesterol concentrations. To avoid extra decimals because of low concentrations of the noncholesterol sterols, the values were multiplied with 100.

2.4. Metabolic studies

The participants took a capsule containing 200 mg of Cr_2O_3, ^{3}H-sitostanol, and ^{14}C-cholesterol 3 times a day with each major meal for a week. During the last 3 days, stools were collected and pooled for analysis of labels, neutral sterols, and bile acids. The fecal sterols and bile acids were measured with GLC [23] using recovery of Cr_2O_3 or labeled sitostanol for measurement of fecal flow. During the week,

the participants kept a food record, of which dietary intake of energy, fat, and cholesterol was quantified with a computerized program [24]. The program is not accurate enough to measure the dietary intake of plant sterols, but their fecal values are usually considered to reflect their dietary intakes. The following calculations were performed:

Absorption percentage of dietary cholesterol
= $^{14}C/^{3}H$ in stools − $^{14}C/^{3}H$ in capsules consumed.
Cholesterol synthesis = dietary cholesterol
− (fecal cholesterol + bile acids).

2.5. Polymorphisms of ABCG5 and ABCG8

Previously identified 5 common polymorphisms of the *ABCG5* and *ABCG8* genes [18,25] were assayed by polymerase chain reaction amplification and restriction fragment length polymorphism analysis as described earlier [19].

2.6. Statistical analyses

All statistical analyses were performed with SPSS for Windows 11.5 statistics program (SPSS, Chicago, IL). The results are given as means ± SEM. Normal distribution was checked before further analyses. Student *t* test was used to compare the values between the groups. For variables of interest, Pearson or Spearman correlation coefficients were calculated. To control the overall α level, Bonferroni adjustment was used. Variables, which were neither normally distributed even after different transformations nor noncontinuous, were tested with Mann-Whitney test or Fisher exact test. A value of *P* less than .05 was considered statistically significant.

3. Results

The CAD and control women were of similar age; and their body mass index, blood pressures, and total cholesterol levels were identical. However, LDL cholesterol and serum triglycerides were higher and HDL cholesterol concentration was lower in CAD women than in control women (Table 1). According to the dietary records, cholesterol (265 ± 19 and 268 ± 19 mg/d) and total plant sterol (4.2 ± 0.2 and 4.6 ± 0.2 mg/[kg d]) intakes were similar in CAD and control women, respectively.

Serum squalene and desmosterol (*P* = .056) concentrations were higher and that of lathosterol was lower in CAD women compared with control women (Table 2). Serum sitosterol, avenasterol, and campesterol (*P* = .051) concentrations were higher in CAD women compared with control women. Practically similar results were observed after adjusting squalene and noncholesterol sterol concentrations with that of serum cholesterol. Neither the concentration nor the ratio of serum cholestanol differed between the groups. The ratio of squalene to cholestanol was 61% (*P* < .001) and that of desmosterol to cholestanol was 41% (*P* = .119) higher in CAD women than in control women.

Cholesterol absorption percentage was identical between the groups; but fecal bile acids, neutral sterols, and

Table 1
Clinical characteristics, plasma glucose, serum insulin, and lipids in postmenopausal women with and without CAD

Variables	CAD n = 47	Control n = 62	*P*
Age, y	53.5 ± 0.6	53.7 ± 0.4	.132
Weight, kg	69.0 ± 1.6	70.8 ± 1.7	.547
Height, cm	161.6 ± 0.8	163.2 ± 0.6	.581
Body mass index, kg/m^2	26.2 ± 0.6	26.8 ± 0.7	.566
Systolic blood pressure, mm Hg	134 ± 4	133 ± 3	.824
Diastolic blood pressure, mm Hg	83 ± 2	85 ± 2	.481
Serum cholesterol, mmol/L	6.00 ± 0.14	5.76 ± 0.14	.182
LDL cholesterol, mmol/L	4.08 ± 0.12	3.67 ± 0.13	.018
HDL cholesterol, mmol/L	1.30 ± 0.04	1.50 ± 0.05	.001
Serum triglycerides, mmol/L	1.38 ± 0.09	1.25 ± 0.10	.025

Mean ± SEM.

Table 2
Serum squalene and noncholesterol sterols in postmenopausal women with and without CAD

Variables	CAD n = 47	Control n = 62	*P*
Concentrations, μg/dL			
Squalene	93 ± 5	62 ± 2	<.001
Desmosterol	210 ± 18	167 ± 6	.056
Lathosterol	370 ± 20	431 ± 17	.011
Campesterol	642 ± 54	494 ± 24	.051
Sitosterol	348 ± 25	271 ± 13	.017
Avenasterol	100 ± 6	83 ± 3	.047
Cholestanol	286 ± 14	283 ± 11	.920
Ratios to cholesterol, 10^2 × μmol/mmol of cholesterol			
Squalene	42 ± 2	28 ± 1	<.001
Desmosterol	96 ± 8	76 ± 2	.005
Lathosterol	168 ± 8	196 ± 7	.018
Campesterol	287 ± 21	227 ± 11	.047
Sitosterol	156 ± 10	124 ± 6	.014
Avenasterol	45 ± 2	38 ± 1	.037
Cholestanol	130 ± 6	129 ± 4	.893
Squalene-cholestanol, μg/μg	37 ± 4	23 ± 1	<.001
Desmosterol-cholestanol, μg/μg	90 ± 12	64 ± 3	.119

Mean ± SEM.

Table 3
Variables of absolute cholesterol metabolism in postmenopausal women with and without CAD

Variables	CAD n = 27	Control n = 43	*P*
Cholesterol absorption efficiency, %	41.7 ± 2.1	43.7 ± 1.4	.336
Bile acid synthesis, mg/(kg d)	4.8 ± 0.3	6.3 ± 0.5	.029
Fecal neutral sterols, mg/(kg d)	10.4 ± 0.6	12.4 ± 0.6	.018
Fecal campesterol + sitosterol, mg/(kg d)	3.50 ± 0.24	3.54 ± 0.19	.529
Cholesterol synthesis, mg/(kg d)	10.8 ± 0.8	14.5 ± 0.9	.002
Cholesterol synthesis/absorption, mg/kg/d/%	0.30 ± 0.04	0.35 ± 0.03	.088

Mean ± SEM.

cholesterol synthesis were significantly lower in CAD women than in control women (Table 3). The ratio of absolute cholesterol synthesis to absorption was 17% lower in CAD women than in control women (P = .088). Fecal campesterol + sitosterol concentrations did not differ between the groups.

In CAD and control women, cholesterol absorption percentage and cholesterol synthesis were interrelated (r =

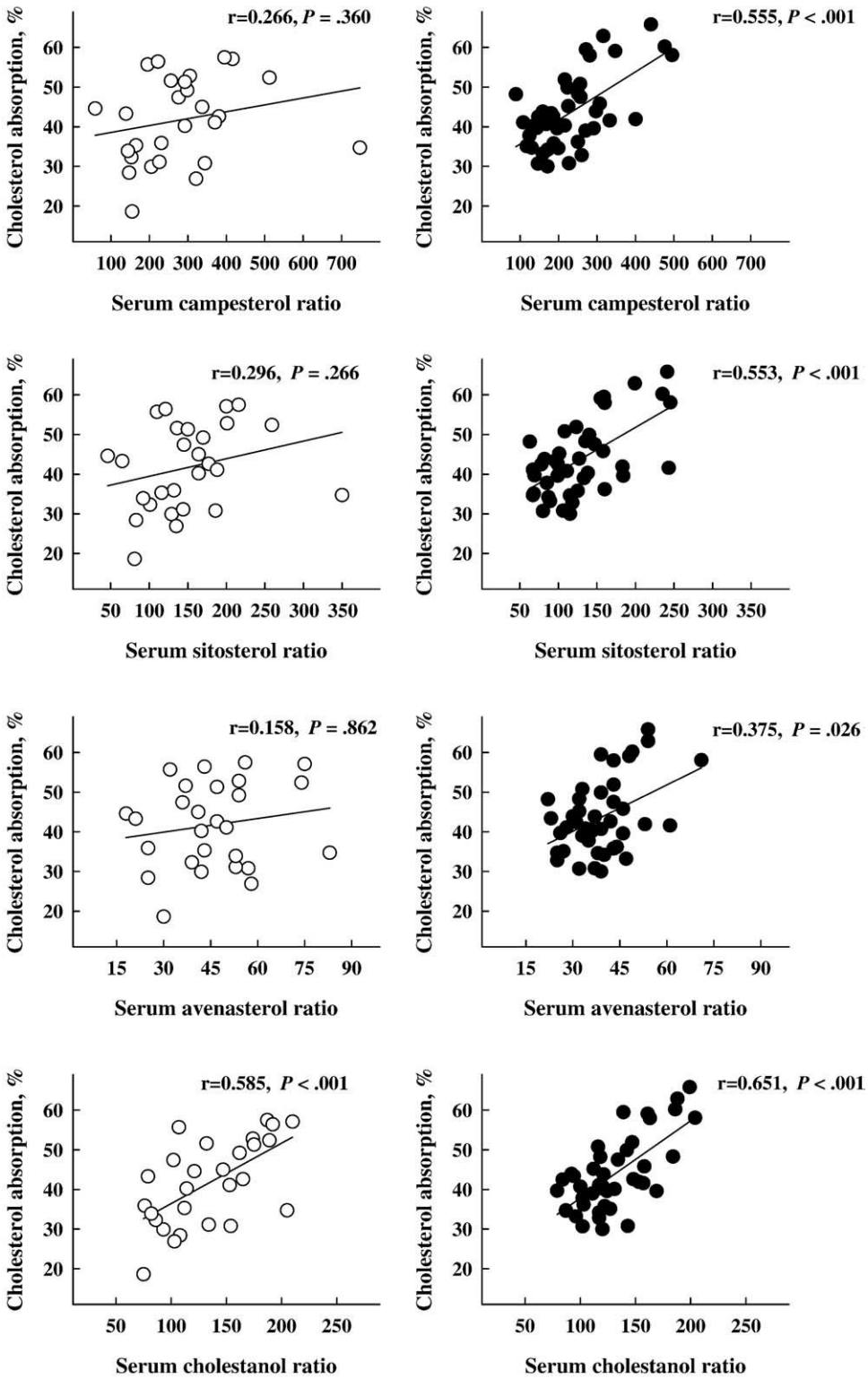

Fig. 1. Correlations between cholesterol absorption efficiency and serum plant sterol to cholesterol ratios (10^2 × micromoles per millimole of cholesterol) in women with CAD (left panel) and in control women (right panel).

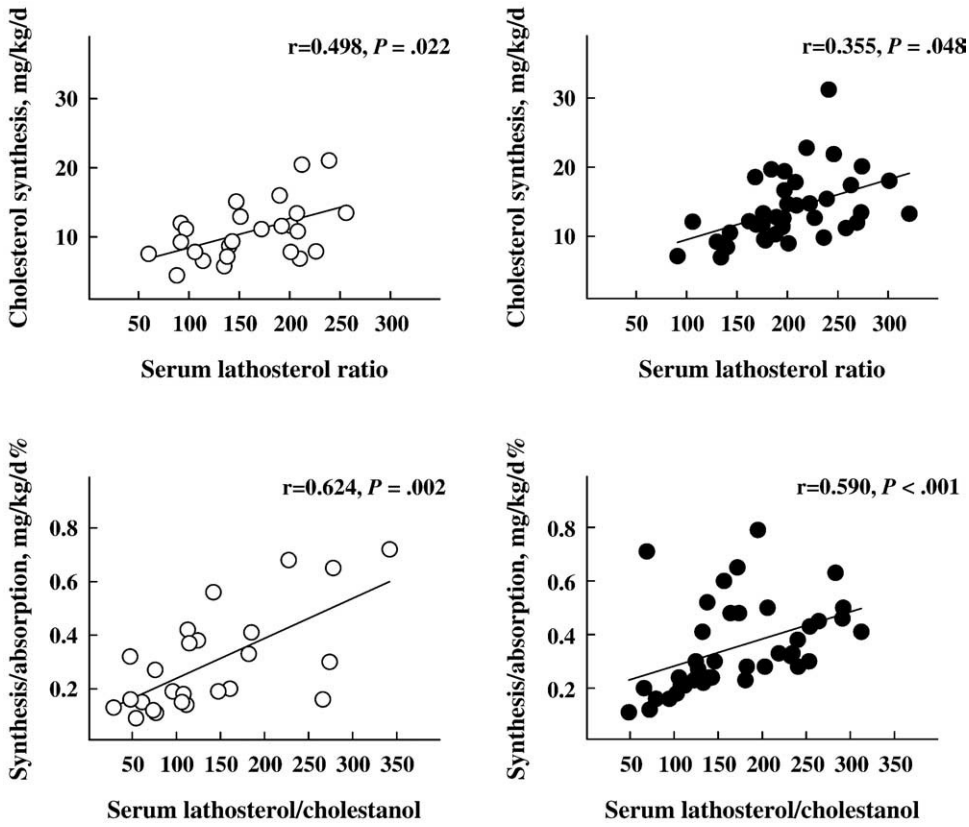

Fig. 2. Correlations between absolute cholesterol synthesis and serum lathosterol to cholesterol ratio (10^2 × micromoles per millimole of cholesterol) (upper panel), and between absolute cholesterol synthesis-absorption and serum lathosterol-cholestanol ratios (micrograms per microgram) (lower panel) in women with CAD and in control women.

−0.635, P = .002 in CAD and r = −0.419, P = .014 in controls, respectively). Fecal bile acids and neutral sterols were interrelated in control women (r = 0.432, P = .01), but not in CAD women (r = 0.073, P = 1). In CAD women, serum plant sterol ratios (and concentrations) only tended to associate with cholesterol absorption percentage (Fig. 1, left panel), whereas serum cholestanol ratio was significantly related to cholesterol absorption also in CAD women similarly to control women (Fig. 1, right panel). Serum lathosterol ratio was associated with absolute cholesterol synthesis (Fig. 2, upper panels) and negatively with cholesterol absorption percentage in both groups (Table 4). The lathosterol-cholestanol (Table 4, Fig. 2) and even the lathosterol-sitosterol ratios (Table 4) were good

Table 4
Correlations between relative (serum noncholesterol sterols) and absolute markers of cholesterol absorption and synthesis in postmenopausal women with and without CAD

Relative markers of cholesterol metabolism	Absolute markers of cholesterol metabolism					
	Cholesterol absorption efficiency, %		Cholesterol synthesis, mg/(kg d)		Cholesterol synthesis/ absorption, mg/kg/d/%	
	CAD (n = 27)	Control (n = 43)	CAD (n = 27)	Control (n = 43)	CAD (n = 27)	Control (n = 43)
Lathosterol[a]	−0.580†	−0.469†	0.498*	0.355*	0.598†	0.469†
Sitosterol[a]	0.296	0.553‡	−0.244	−0.429*	−0.284	−0.538‡
Cholestanol[a]	0.585†	0.651‡	−0.449*	−0.406*	−0.554†	−0.567‡
Lathosterol-sitosterol[b]	−0.529†	−0.633‡	0.455*	0.475†	0.530*	0.617‡
Lathosterol-cholestanol[b]	−0.636‡	−0.639‡	0.517*	0.432†	0.624†	0.590‡

[a] 10^2 × micromoles per millimole of cholesterol.
[b] Micrograms per microgram.
* P < .05.
† P < .01.
‡ P < .001.

indicators of absolute cholesterol absorption and synthesis in both groups. In CAD women, serum squalene was associated neither with serum lathosterol level nor with absolute cholesterol synthesis. Furthermore, in CAD women, there was only 1 significant association between serum plant sterols and the absolute metabolic variables; and this was with fecal neutral sterols ($r = -0.430$, $P = .03$ in CAD; $r = -0.558$, $P < .001$ in controls).

The genotype frequencies of the different polymorphisms were in Hardy-Weinberg equilibrium in the study population. Frequencies of the different polymorphisms did not differ between the groups (data not shown).

4. Discussion

The novel observations of the present study were that the elevated concentrations and ratios to cholesterol of serum plant sterols in CAD women only tended to be associated with absolute measures of cholesterol absorption, suggesting that, in this population, they are not preferably used as surrogate markers of cholesterol absorption. In addition, their paradoxical serum levels suggest some abnormality in their own metabolism in CAD women. Obviously, the perturbed serum plant sterol levels are not regulated by their absorption. We have earlier shown that cholesterol synthesis and biliary cholesterol secretion are diminished in CAD women [1], but the present study revealed that also bile acid synthesis was diminished in CAD. The interrelation between bile acid synthesis and fecal neutral sterol excretion observed in controls was lacking in CAD women. Regarding the *ABCG5* and *ABCG8* genes, we did not find any differences in the sequence variations in any of the genes between the groups, suggesting that these polymorphisms are not associated with the risk of CAD.

Postmenopausal CAD women were selected from a consecutive series of women aged 50 to 55 years admitted to the Helsinki University Hospital. Their postmenopausal status was carefully checked. The study population was recruited in the mid-1990s; and by that time, statin treatment was infrequent even in coronary subjects and especially in women. Helsinki University Hospital serves an area of a population of 1 million in South Finland. The control subjects were randomly selected by age from a population register of the same area and had similar body mass index and postmenopausal status. Therefore, our study population is likely to be representative of 50- to 55-year–old postmenopausal women with CAD.

The role of avenasterol as a relative marker of cholesterol absorption has not been studied in detail earlier. In the present study, we related serum avenasterol level to cholesterol absorption percentage; and it seems to be a reliable marker of absolute cholesterol absorption in addition to the other plant sterols in control women. Regarding the paradoxically high serum ratios of squalene and desmosterol in CAD women, they were not correlated with cholesterol synthesis. We also calculated the ratios of squalene and desmosterol to the relative absorption marker cholestanol, and it turned out that the elevated squalene ratio was sustained but that of desmosterol was less constant in CAD women. This finding suggests that the metabolism of desmosterol was less disturbed in CAD women as compared with that of squalene. It is quite obvious that serum squalene in CAD does not reflect cholesterol synthesis. The reason for the elevated serum squalene levels in CAD women remains open. However, it might reflect impaired postprandial fat clearance observed earlier in CAD women [26]. In the present series, serum triglycerides were elevated, suggesting that triglyceride-rich lipoprotein particles including post-absorptive lipoproteins were present to some extent in serum despite fasting samples; and these triglyceride-rich lipoproteins are enriched with squalene.

Why are serum plant sterols elevated in CAD? The interesting observation was that it was not only the metabolism of plant sterols, but also that of cholesterol and bile acids, which was perturbed in CAD women. The homeostasis between cholesterol absorption and synthesis seemed, however, uninterrupted, so that increased cholesterol absorption could compensate for the downregulated cholesterol synthesis. Plant sterols are absorbed using the same intestinal transporter systems as cholesterol, that is, NPC1L1 and ABCG5 and ABCG8 intestinal proteins. NPC1L1 is responsible for the plant sterol absorption and intestinal uptake; so in NPC1L1 null mice, sitosterol absorption was reduced by 64%, and the plasma levels of sitosterol and campesterol were more than 90% lower compared with the (+/+) mice [27]. In addition, NPC1L1 is expressed in the canalicular membranes of human hepatocytes; and in transgenic mice expressing human NPC1L1 in hepatocytes, the overexpression resulted in 10- to 20-fold decrease in biliary cholesterol concentration [28]. Biliary bile acid concentrations were not affected, but the plant sterol excretion was not measured in this animal study. Mutations in the ABCG5 or ABCG8 proteins cause an opposite effect to sterol absorption and biliary excretion [15-17]; but in subjects heterozygous for the mutations, the sterol absorption and excretion are practically unaffected [29]. The similar cholesterol absorption percentage between CAD women and control women suggests that the intestinal absorption of plant sterols, too, might not be disturbed. The similar distribution of the sequence variations of the *ABCG5* and *ABCG8* genes between the groups also supports this assumption because some of the polymorphic sites are associated with cholesterol absorption and synthesis [18,19]. Preferentially, we assume that the aberrant serum plant sterol levels might refer to the deranged cholesterol and bile acid metabolism in CAD women. We had no possibility to measure the composition of bile; but we have shown earlier that the biliary cholesterol secretion is diminished in CAD women [1], and in the present study also the amount of bile acid synthesis was lower in CAD women than in control women. These changes in cholesterol and bile acid

metabolism might diminish the biliary excretion of plant sterols, which results in their increased serum concentrations. Some support to this assumption is provided by the observation that the amounts of fecal campesterol + sitosterol were associated with fecal neutral sterols ($r = 0.474$, $P = .004$) and fecal bile acids ($r = 0.476$, $P = .034$) in the control but not in CAD women. The amount of plant sterols excreted through bile is much smaller than the amount of nonabsorbed dietary plant sterols in feces, the reason why the possible differences in biliary plant sterol excretion could not be observed in fecal analyses.

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