Cholesterol Absorption, Synthesis, and Fecal Output in Postmenopausal Women With and Without Coronary Artery Disease

Radhakrishnan A. Rajaratnam, Helena Gylling, Tatu A. Miettinen

Abstract—Hypercholesterolemia is a prominent risk factor for coronary artery disease (CAD), yet cholesterol metabolism has not been evaluated in women with CAD. The objective of this study was to determine the interrelations of CAD, serum squalene and sterols, and cholesterol metabolism with each other in postmenopausal women. For this purpose, we measured serum squalene and sterols and fecal steroids (cholesterol and bile acids) and squalene by gas-liquid chromatography and evaluated cholesterol absorption and synthesis in postmenopausal women with CAD (n = 29) and age-matched controls (n = 20). On similar dietary lipid intake, the cholesterol absorption efficiency and mean serum cholesterol level were comparable, but the squalene-to-cholesterol ratio was higher in cases than in controls. The presence of CAD was inversely associated with fecal total steroids (logistic regression coefficient \( \beta/SE = -2.11, P = 0.04 \)) and cholesterol synthesis (\( \beta/SE = -2.14, P = 0.04 \)) and turnover (\( \beta/SE = -2.19, P = 0.03 \)) after adjustment for dietary cholesterol, family history of CAD, smoking, low and high density lipoprotein cholesterol, and serum triglyceride levels. A high serum squalene ratio was not related to cholesterol synthesis but was inversely related to fecal squalene excretion, which was lower in cases than in controls. In conclusion, the presence of CAD in postmenopausal women is independently associated with altered cholesterol metabolism, as reflected by low synthesis and inefficient elimination of cholesterol. (Arterioscler Thromb Vasc Biol. 2001;21:1650-1655.)

Key Words: cholesterol synthesis ■ bile acids ■ squalene ■ atherosclerosis ■ women

From among the etiological factors for coronary artery disease (CAD), an increased serum cholesterol concentration is most prominent. Intestinal cholesterol is absorbed and transported to the liver, where it is mixed with hepatic cholesterol, followed by secretion into the circulating lipoproteins, conversion to bile acids, or elimination in the feces by biliary secretion as cholesterol and bile acids. According to cholesterol homeostasis, cholesterol absorption, elimination, and synthesis are interrelated, regulate LDL receptor activity, and contribute accordingly to the regulation of serum cholesterol level. Absorption, elimination, and synthesis of cholesterol have been elucidated in patients with familial hypercholesterolemia, obesity, and diabetes, who are susceptible to the development of CAD.\(^2\)\(^-\)\(^5\) However, only a limited number of studies have evaluated cholesterol metabolic variables in patients with CAD in relation to non-CAD controls. Accordingly, compared with controls, cholesterol synthesis seems to be low in a limited number of CAD patients with primary hypercholesterolemia and less consistently so in those with mild hypercholesterolemia.\(^6\) In subjects with familial hypercholesterolemia, low bile acid synthesis is associated with the prevalence of CAD in male subjects\(^7\) and also with a poor prognosis after a 15-year follow-up.\(^8\) Coronary patients have been claimed to eliminate subnormal fecal bile acids.\(^9\) In addition, type 2 diabetic subjects with CAD have a higher cholesterol absorption efficiency than diabetics without CAD.\(^10\) Our recent study has demonstrated that from among serum noncholesterol sterols, women with CAD had higher serum ratios of plant sterols to cholesterol and lower respective lathosterol (cholesterol precursor) values compared with controls, suggesting high absorption and low synthesis of cholesterol in CAD.\(^11\) However, these patients had high serum levels of squalene, a nonsterol intermediate in cholesterol biosynthesis, and of desmosterol, the last intermediate of the cholesterol synthesis pathway through the unsaturated side chain. These findings suggest, in contrast to other noncholesterol sterols, enhanced cholesterol synthesis in women with CAD. To resolve the discrepant interpretation of serum squalene and desmosterol versus other noncholesterol sterols and to relate the variables of cholesterol metabolism to CAD, we investigated the absorption, fecal elimination, and synthesis of cholesterol in postmenopausal women with angiographically verified CAD and in age-matched healthy controls. Fecal output of squalene...
and precursor sterols was also measured and related to serum squalene and biliary cholesterol secretion.

**Methods**

**Subjects**
The study was performed in a subgroup of the whole study population participating in our recent study but excluding those on hormone replacement therapy and with obesity (body mass index [BMI] >30 kg/m²). Baseline data of 29 cases and 20 controls described in the previous study were analyzed, and additional studies were performed. In brief, the patients were postmenopausal and aged 50 to 55 years. The cases were referred to the Department of Medicine, University of Helsinki, for diagnostic coronary angiography. Each had at least 50% occlusion in 2 major coronary vessels, and 20 had suffered an acute myocardial infarction at least 6 months earlier. The control group comprised postmenopausal women of the same age chosen randomly from the Helsinki population registry. They were free of chest pain, and their electrocardiograms were normal. All subjects were free of severe heart failure; liver, intestinal, and malignant diseases; thyroid dysfunction; and diabetes mellitus and were not taking hypolipidemic medication. β-Blocking agents were used by 21 cases, and calcium channel blockers were used by 6 patients and 2 controls (P < 0.02). Twenty-seven CAD patients received acetylsalicylic acid, and 1 patient received an angiotensin-converting enzyme inhibitor. The study was approved by the Ethics Committee of our hospital.

**Analytical Methods**
The subjects were advised to continue their normal diet, and they kept a 7-day dietary record. After a 12-hour overnight fast, 2 blood samples 1 week apart were drawn for baseline measurements, and their mean values were recorded. Serum cholesterol and triglycerides were determined by the Friedewald formula because serum triglyceride levels were <3 mmol/L. Apo E phenotypes were determined by isoelectric focusing. Dietary energy, cholesterol, fat, fatty acids, carbohydrates, and fiber were calculated from 7-day dietary records by use of Micro-Nutrica software. The global risk for CAD was calculated according to the Framingham risk assessment. Serum squalene, noncholesterol sterols, and cholesterol were measured by gas-liquid chromatography (GLC). In the text, the squalene and noncholesterol sterols to cholesterol ratios are expressed as squalene and noncholesterol sterols. Concentrations are noted specifically when the cholesterol turnover was the sum of cholesterol synthesis and absorption.

**Cholesterol Metabolism**
Each subject consumed a capsule containing 200 mg of CrO₃, 0.1 μCi of [¹⁴C]cholesterol, and 0.2 μCi of [³H]sitostanol 3 times daily with the main meals during the 7-day dietary recording. CrO₃ was added for evaluation of fecal flow. Stools were collected during the final 3 days. Fecal neutral steroids, bile acids, squalene, cholesterol precursors, and plant sterols were measured by GLC as described earlier. Fecal neutral steroids of cholesterol origin, campesterol, and sitosterol include their coprostanol and coprostanone derivatives.

Cholesterol absorption efficiency was calculated from the difference between the dietary intake and fecal output of the [¹⁴C]cholesterol/[³H]sitostanol ratio. Cholesterol synthesis is the difference between dietary cholesterol and the fecal output of steroids (neutral steroids and bile acids). The total intestinal cholesterol pool was calculated with an equation for the calculation of cholesterol absorption and assuming that the percent absorption of endogenous and exogenous cholesterol was equal. Biliary cholesterol secretion was the difference between the intestinal cholesterol pool and dietary cholesterol intake. The estimated values were similar to those for biliary cholesterol secretion as measured with the constant-infusion technique. The intestinal pools of dietary and total cholesterol were multiplied by fractional cholesterol absorption to derive the absorbed mass of dietary and total cholesterol, respectively.

**Table 1. Baseline Characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n = 29)</th>
<th>Controls (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>52.5 ± 2.9</td>
<td>53.1 ± 3.1</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>67.4 ± 10.1</td>
<td>71.8 ± 11.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.7 ± 3.7</td>
<td>26.5 ± 4.2</td>
</tr>
<tr>
<td>Apo E 2/3, 2/4, 3/3, 3/4, 4/4; n</td>
<td>1, 1, 12, 12, 3</td>
<td>0, 1, 10, 7, 2</td>
</tr>
</tbody>
</table>

**Home diet**
- Cholesterol, mg/d: 265 ± 103 vs 268 ± 85
- Fat, g/d: 71.7 ± 23.1 vs 73.9 ± 23.3
- Saturated fatty acids, E%: 15.0 ± 3.7 vs 15.5 ± 2.8
- Monounsaturated fatty acids, E%: 13.2 ± 2.9 vs 12.6 ± 2.3
- Polyunsaturated fatty acids, E%: 6.34 ± 2.14 vs 5.78 ± 1.83
- Carbohydrates, g/d: 170 ± 46 vs 192 ± 52
- Fiber, g/d: 18.1 ± 6.7 vs 20.1 ± 6.4
- Serum total cholesterol, mmol/L: 6.13 ± 0.90 vs 5.85 ± 1.34
- LDL cholesterol, mmol/L: 4.23 ± 0.85 vs 3.82 ± 1.15
- HDL cholesterol, mmol/L: 1.29 ± 0.23 vs 1.52 ± 0.31
- LDL/HDL: 3.38 ± 0.91 vs 2.67 ± 1.21
- Serum triglycerides, mmol/L: 1.35 ± 0.52 vs 1.11 ± 0.86
- Serum squalene*: 39.8 ± 8.1 vs 28.3 ± 6.4
- Serum desmosterol*: 94.2 ± 43.2 vs 73.5 ± 12.7
- Serum lathosterol*: 168 ± 57 vs 204 ± 53
- Serum cholesterol*: 133 ± 42 vs 131 ± 34
- Serum campesterol*: 281 ± 136 vs 240 ± 99
- Serum sitosterol*: 150 ± 61 vs 132 ± 51

Apo E indicates apolipoprotein E phenotypes; E%, energy percentage. Values are mean ± SD.

Values are 10⁻³ mmol/mol of cholesterol.

‡ P < 0.05, † P < 0.01 vs controls.

**Data Analysis**
Continuous variables are presented as mean ± SE. The data were analyzed with the BMDP computer software package (BMDP Statistical Software, Inc.). Group differences were analyzed by Student’s t test and the Mann-Whitney rank-sum test. Relationships between continuous variables were tested by computing Spearman rank correlation coefficients and further by stepwise regression or nonlinear regression analyses. Associations between CAD and all other parameters were analyzed by logistic regression analysis, based on maximum-likelihood ratios. The presence of CAD was included into the model as the dependent variable, and family history of CAD and smoking (each yes or no) was the independent categorical variable, with the others as independent continuous variables. Goodness of fit to the prediction was examined with the Hosmer-Lemeshow test and the logistic function with the C.C. Brown test (BMDP Statistical Software). A P value < 0.05 was considered significant.

**Results**

**Baseline Characteristics**
The dietary intake of cholesterol and fat; energy percentage of saturated, monounsaturated, and polyunsaturated fatty acids; BMI; and the frequency of apo E phenotypes in the cases and controls were similar (Table 1). Compared with controls, the cases smoked more frequently (17 vs 6, P = 0.04), and more frequently they had a family history of
TABLE 2.  Cholesterol Metabolism

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n=29)</th>
<th>Controls (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol absorption, %</td>
<td>41.9 ± 1.9</td>
<td>43.0 ± 1.7</td>
</tr>
<tr>
<td>Absorbed total cholesterol, mg · kg⁻¹ · d⁻¹</td>
<td>7.59 ± 0.49†</td>
<td>9.57 ± 0.81</td>
</tr>
<tr>
<td>Absorbed dietary cholesterol, mg · kg⁻¹ · d⁻¹</td>
<td>1.76 ± 0.19</td>
<td>1.65 ± 0.15</td>
</tr>
<tr>
<td>Fecal steroids, mg · kg⁻¹ · d⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bile acids</td>
<td>4.96 ± 0.39</td>
<td>6.19 ± 0.62</td>
</tr>
<tr>
<td>Neutral steroids†</td>
<td>10.5 ± 0.6</td>
<td>12.6 ± 0.9</td>
</tr>
<tr>
<td>Endogenous neutral steroids†</td>
<td>8.18 ± 0.57†</td>
<td>10.5 ± 0.9</td>
</tr>
<tr>
<td>Total steroids†</td>
<td>15.5 ± 0.7†</td>
<td>18.8 ± 1.3</td>
</tr>
<tr>
<td>Campesterol†</td>
<td>0.96 ± 0.07</td>
<td>1.06 ± 0.15</td>
</tr>
<tr>
<td>Sitosterol†</td>
<td>4.15 ± 0.23</td>
<td>3.03 ± 0.37</td>
</tr>
<tr>
<td>Cholesterol, μg · kg⁻¹ · d⁻¹</td>
<td>183 ± 9</td>
<td>235 ± 27</td>
</tr>
<tr>
<td>Squalene, μg · kg⁻¹ · d⁻¹</td>
<td>82.1 ± 9.4†</td>
<td>129 ± 22</td>
</tr>
<tr>
<td>Lanosterol, μg · kg⁻¹ · d⁻¹</td>
<td>99.7 ± 6.8</td>
<td>121 ± 11</td>
</tr>
<tr>
<td>Dihydrolanosterol, μg · kg⁻¹ · d⁻¹</td>
<td>47.0 ± 4.1‡</td>
<td>84.1 ± 10.0</td>
</tr>
<tr>
<td>Lathosterol, μg · kg⁻¹ · d⁻¹</td>
<td>249 ± 16</td>
<td>308 ± 30</td>
</tr>
<tr>
<td>Cholesterol synthesis, mg · kg⁻¹ · d⁻¹</td>
<td>11.4 ± 0.8‡</td>
<td>15.0 ± 1.3</td>
</tr>
<tr>
<td>Cholesterol turnover, mg · kg⁻¹ · d⁻¹</td>
<td>13.1 ± 0.7‡</td>
<td>16.6 ± 1.3</td>
</tr>
<tr>
<td>Intestinal cholesterol pool, mg · kg⁻¹ · d⁻¹</td>
<td>18.1 ± 0.8‡</td>
<td>22.2 ± 1.5</td>
</tr>
<tr>
<td>Dietary cholesterol intake</td>
<td>4.09 ± 0.23</td>
<td>3.81 ± 0.29</td>
</tr>
<tr>
<td>Biliary cholesterol secretion</td>
<td>14.0 ± 0.8‡</td>
<td>18.4 ± 1.5</td>
</tr>
</tbody>
</table>

Values are mean±SE.

*Includes coprostanol and coprostanone derivatives.
†P<0.05, ‡P<0.001 vs controls.

CAD (23 vs 6, P<0.000). The global risk for CAD within 10 years was significantly higher in cases than controls (9.3±0.6% vs 6.3±0.8%, P=0.005). Serum lathosterol values were lower, those of plant sterols tended to be increased, and those of squalene and desmosterol were higher in cases than controls. Alanine aminotransferase activity in cases and controls was similar (29.2±3.1 vs 24.6±2.7 U/L). Even though serum total and LDL cholesterol levels only tended to be higher, those of HDL cholesterol were lower in cases than controls, and women with CAD had significantly elevated LDL-to-HDL ratios and serum triglyceride levels.

CAD and Cholesterol Metabolism

Cholesterol absorption efficiency was comparable between the 2 groups, but fecal excretion of squalene and dihydrolanosterol, endogenous neutral and total steroids, intestinal pool of biliary and total cholesterol, absorbed mass of total cholesterol, and cholesterol synthesis and turnover were lower in cases than in controls (Table 2). Multivariate logistic regression analysis, adjusted for dietary intake of cholesterol, family history of CAD, smoking, LDL (or total cholesterol) and HDL cholesterol, and serum triglyceride levels showed that the presence of CAD was positively associated with serum squalene (β/SE=2.49, P<0.05) and inversely with serum lathosterol (β/SE=−2.10, P<0.05), fecal total steroids, intestinal pool of biliary and total cholesterol, and cholesterol synthesis and turnover (Table 3). In addition, independent associations of CAD with a family history of CAD and smoking were also found in all models (P<0.05 for both).

TABLE 3.  Association Between Cholesterol Metabolic Variables and Coronary Artery Disease by Multivariate Logistic Regression Analysis (n=49)

<table>
<thead>
<tr>
<th>Variable</th>
<th>β†</th>
<th>β/SE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol absorption efficiency</td>
<td>0.00</td>
<td>0.06</td>
<td>NS</td>
</tr>
<tr>
<td>Absorbed dietary cholesterol</td>
<td>0.78</td>
<td>0.88</td>
<td>NS</td>
</tr>
<tr>
<td>Absorbed total cholesterol</td>
<td>−0.39</td>
<td>−1.81</td>
<td>0.08</td>
</tr>
<tr>
<td>Fecal bile acids</td>
<td>−0.22</td>
<td>−1.03</td>
<td>NS</td>
</tr>
<tr>
<td>Fecal neutral steroids</td>
<td>−0.31</td>
<td>−1.76</td>
<td>0.09</td>
</tr>
<tr>
<td>Fecal total steroids</td>
<td>−0.33</td>
<td>−2.11</td>
<td>0.04</td>
</tr>
<tr>
<td>Fecal squalene</td>
<td>−0.76</td>
<td>−0.85</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol synthesis</td>
<td>−0.32</td>
<td>−2.14</td>
<td>0.04</td>
</tr>
<tr>
<td>Cholesterol turnover</td>
<td>−0.36</td>
<td>−2.19</td>
<td>0.04</td>
</tr>
<tr>
<td>Intestinal cholesterol pool</td>
<td>−0.31</td>
<td>−2.12</td>
<td>0.04</td>
</tr>
<tr>
<td>Biliary cholesterol secretion</td>
<td>−0.32</td>
<td>−2.18</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*Adjusted for dietary cholesterol intake, smoking, family history of coronary artery disease, serum triglycerides, LDL cholesterol, and HDL cholesterol.

Effects of β-Blocker Medication

The cases with and without β-blocker medication use had comparable fasting serum cholesterol (6.0±0.2 vs 6.4±0.3 mmol/L) and squalene (40.0±1.9 vs 38.1±2.2 10⁻⁵ mmol/mol cholesterol), dietary cholesterol intake (3.8±0.4 vs 4.6±1.0 mg · kg⁻¹ · d⁻¹), fecal bile acids (5.0±0.5 vs 4.7±0.2 mg · kg⁻¹ · d⁻¹), endogenous neutral steroids (8.3±0.6 vs 7.0±1.3 mg · kg⁻¹ · d⁻¹), and squalene (83.8±11.9 vs 86.8±13.5 μg · kg⁻¹ · d⁻¹) and cholesterol synthesis (13.3±0.8 vs 11.8±1.3 μg · kg⁻¹ · d⁻¹). In addition, the cases not taking such medication still had significantly higher serum squalene ratios and a lower fecal output of squalene and endogenous neutral and total steroids and cholesterol synthesis than did controls.

Serum Squalene, Sterols, and Cholesterol Metabolism

Serum lathosterol values, in contrast to serum squalene and desmosterol, were significantly associated with cholesterol synthesis in all subjects (r=0.53, P<0.001). Serum squalene values were negatively correlated with biliary cholesterol secretion and fecal squalene excretion in subjects with fecal squalene output, biliary secretion of cholesterol, and cholesterol synthesis that were below the median (Table 4, Figure 1). Fecal squalene output was significantly positively correlated with cholesterol synthesis and biliary secretion of cholesterol, especially in subjects with above-median levels of fecal squalene, biliary cholesterol secretion, and cholesterol synthesis. Cholesterol absorption efficiency was positively correlated with serum campesterol (r=0.36, P=0.01), sitosterol (r=0.32, P<0.05), and HDL cholesterol (r=0.43, P<0.01) levels and inversely with serum triglyceride level (r=−0.45, P<0.01), which in turn was related to cholesterol synthesis and turnover (both P<0.05). The 2 latter variables were significantly correlated with BMI (r=0.42 and 0.52, respectively). Serum total (r=−0.47, P<0.05) and LDL cholesterol levels were significantly related to fecal bile acids in the cases (Figure 2). Use of logarithmic transformation, square roots, or fitting to the exponential function did not improve these correlations. In stepwise regression analysis, with cholesterol absorption efficiency and fecal bile acids as
independent variables and LDL or HDL cholesterol as dependent variables, 20% and 24% of the respective variabilities in LDL and HDL cholesterol concentrations were explained by fecal bile acids in the study population.

### Discussion

**Perturbed Baseline Cholesterol Metabolism in Women With CAD**

The new observations of the present study show that diminished biliary cholesterol secretion and fecal elimination of cholesterol as bile acids and neutral steroids, as well as reduced cholesterol synthesis and turnover, were associated with an increased risk for CAD in women who had a nonsignificant elevation (+11%) of LDL cholesterol levels but significantly low HDL cholesterol levels. Thus, the findings show for the first time that CAD is associated with decreased, not increased, cholesterol synthesis and that cholesterol turnover is also reduced. These findings could have resulted primarily from impaired hepatic secretion of cholesterol subsequent to low endogenous cholesterol synthesis, which was also reflected by reduced serum lathosterol and cholesterol precursor levels in the feces of women with CAD. In fact, statins, which inhibit cholesterol synthesis, also reduce biliary secretion, fecal elimination and turnover of cholesterol,25–27 and fecal excretion of cholesterol precursors,25 analogous to our present observations, but in contrast to them, statins markedly reduce serum cholesterol levels. Serum cholestanol levels, which reflect cholestasis in different clinical conditions,28,29 were similar in cases and controls. This result and the liver function tests could not show any “cholestasis-like” mechanism for reduced biliary secretion of lipids and subsequent reduction in cholesterol synthesis, yet there may have been some retention of cholesterol or altered lipoprotein metabolism, which tended to increase LDL cholesterol.

Although cholesterol absorption efficiencies in cases and controls were similar, cholesterol mass absorption was lower in the cases owing to diminished biliary cholesterol secretion into the intestine. Thus, in contrast to the normal situation, low intestinal cholesterol flow to the liver was unable to increase cholesterol synthesis in the women with CAD. Serum plant sterol values tended to be increased in the cases and were associated with cholesterol absorption efficiency, in concordance with earlier studies.30,31 A reason for the trend of

### Table 4

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Serum Squalene vs Fecal Squalene</th>
<th>Serum Squalene vs BCS</th>
<th>Fecal Squalene vs BCS</th>
<th>Fecal Squalene vs CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects (N=49)</td>
<td>0.13 (NS)</td>
<td>0.19 (NS)</td>
<td>0.57 (&lt;0.001)</td>
<td>0.46 (&lt;0.001)</td>
</tr>
<tr>
<td>Cases (n=29)</td>
<td>0.20 (NS)</td>
<td>0.20 (NS)</td>
<td>0.36 (0.05)</td>
<td>0.25 (NS)</td>
</tr>
<tr>
<td>Controls (n=20)</td>
<td>0.37 (NS)</td>
<td>0.35 (NS)</td>
<td>0.59 (0.006)</td>
<td>0.47 (0.03)</td>
</tr>
<tr>
<td>Subjects with fecal squalene ≤5.60 mg/d (n=25)</td>
<td>0.46 (0.02)</td>
<td>0.46 (0.02)</td>
<td>0.49 (0.01)</td>
<td>0.24 (NS)</td>
</tr>
<tr>
<td>&gt;5.60 mg/d (n=24)</td>
<td>0.10 (NS)</td>
<td>0.01 (NS)</td>
<td>0.53 (0.009)</td>
<td>0.41 (0.05)</td>
</tr>
<tr>
<td>Subjects with BCS ≤14.85 mg/kg·d (n=25)</td>
<td>0.40 (0.047)</td>
<td>0.25 (NS)</td>
<td>0.23 (NS)</td>
<td>0.01 (NS)</td>
</tr>
<tr>
<td>&gt;14.85 mg/kg·d (n=24)</td>
<td>0.09 (NS)</td>
<td>0.01 (NS)</td>
<td>0.63 (0.001)</td>
<td>0.51 (0.01)</td>
</tr>
<tr>
<td>Subjects with CS ≤13.67 mg/kg·d (n=25)</td>
<td>0.52 (0.008)</td>
<td>0.41 (0.04)</td>
<td>0.17 (NS)</td>
<td>0.04 (NS)</td>
</tr>
<tr>
<td>&gt;13.67 mg/kg·d (n=24)</td>
<td>0.06 (NS)</td>
<td>0.10 (NS)</td>
<td>0.61 (0.002)</td>
<td>0.46 (0.03)</td>
</tr>
</tbody>
</table>

Values are correlation coefficients and (P values).

---

**Figure 1.** Correlation of serum squalene with fecal squalene output. For values below the median fecal squalene output (5.6 mg/d), r = 0.46, P < 0.05; for those above the median fecal squalene output, r = 0.10, P = NS.

**Figure 2.** Association between LDL cholesterol and fecal bile acids in cases (●) and controls (○). Regression line for cases (–●–): y = 5.42 – 0.23x; r = 0.57, P < 0.01; for controls (–○–), y = 4.64 – 0.12x; r = 0.17, P = NS.
increased plant sterols could be increased absorption; namely, dietary plant sterols are concentrated in the reduced intestinal cholesterol pool of the cases. This situation should favor micellar incorporation and absorption of plant sterols.

**Squalene and Cholesterol Metabolism**

Serum squalene was increased in all serum lipoprotein fractions, including the chylomicrons from women with CAD,

32 but it was not related to cholesterol synthesis, a finding seen in some earlier studies.33,34 Like levels of noncholesterol sterols, those of squalene are higher in human bile than in serum,35 suggesting that hepatic squalene is partly secreted into the bile. Approximately 85% of intestinal squalene can be absorbed,36 and unabsorbed squalene is detected in the feces of patients on squalene-free diets.35 Effective absorption of squalene from the reduced intestinal cholesterol pool of the cases could have contributed to their low fecal excretion of squalene. The positive relation between fecal excretion of squalene and hepatic cholesterol synthesis and biliary secretion of cholesterol suggests that biliary squalene secretion was also low in the women with CAD. The negative correlation of serum squalene levels with fecal squalene excretion in subjects with low fecal squalene output (see Figure 1 and Table 4) suggests that high serum squalene in the cases is caused in part by reduced biliary lipid secretion. Dietary squalene intake was minimal; thus, it was not considered to be associated with the serum concentrations in these patients. The high serum squalene and desmosterol values, despite low cholesterol synthesis, suggest that either hepatic uptake of circulatory lipoproteins is impaired or that an extrahepatic contribution of squalene and desmosterol is enhanced in women with CAD. Squalene is present predominantly in skin and adipose tissues,35 and a substantial amount of squalene is synthesized in extrahepatic tissues.37 However, newly synthesized squalene, in contrast to cholesterol, is virtually not released into the circulatory lipoproteins from adipocytes.38 Thus, the extrahepatic origin of serum squalene remains unknown. A high serum desmosterol level in women with CAD was related to squalene, suggesting their similar sites of origin by conversion of squalene through the unsaturated side-chain pathway of cholesterol synthesis to desmosterol. Under physiological conditions, cholesterol synthesis from lanosterol occurs mainly through the saturated side-chain pathway, and the major cholesterol precursor found in fasting serum is lathosterol. The reason for preferable cholesterol synthesis by way of the unsaturated side-chain pathway in the cases is unknown, but reduced sterol Δ24- reductase activity could be a contributing factor.

**Factors Affecting the Synthesis and Elimination of Cholesterol**

Cholesterol feeding in humans can downregulate cholesterol synthesis and upregulate bile acid synthesis in long-term consumption39 but only occasionally in short-term studies.40,41 Polysaturated dietary fats can raise the fecal excretion of neutral sterols,42 and plant sterols and stanols reduce cholesterol absorption.43 However, the dietary records indicated that the dietary intake of cholesterol and the fatty acid composition in the present cases and controls were similar. Furthermore, fecal plant sterols were also similar, indicating similar plant sterol consumption. In obese subjects, cholesterol excretion and synthesis are high, whereas its absorption efficiency is low.3,4 Subjects with apo E4 phenotypes show higher absorption of cholesterol than do those with E2 or E3 phenotypes.66 Cholesterol synthesis is high and absorption is low in diabetic patients compared with those levels in normal controls.3 The present cases were well matched with controls for BMI and apo E phenotypes. In addition, none of the subjects had diabetes. β-Blockers may influence serum lipid levels and cholesterol metabolism;45,46 however, the present study showed no effect of β-blocker medication use. Accordingly, such confounding factors are unlikely to have contributed to the differences in cholesterol metabolism between the cases and controls. Increased intestinal bile acid absorption may also impair cholesterol metabolism to bile acids through a feedback mechanism. The mechanism for perturbed cholesterol metabolism in women with CAD needs further exploration.

**How Could Impaired Synthesis and Elimination of Cholesterol Be Atherogenic?**

In a previous study, fecal bile acids and neutral sterols tended to be lower in a limited number of CAD patients with hypercholesterolemia but less consistently so in those with mild hypercholesterolemia, suggesting that CAD may be related to impaired elimination and reduced synthesis of cholesterol in the presence of hypercholesterolemia.6,8 Cholesterol is secreted into the bile as free cholesterol and bile acids. Bile acids are very efficiently reabsorbed from the intestine, and the amount of bile acids excreted in feces is compensated for by synthesis of de novo bile acids in hepatocytes. In the CAD cases, low fecal excretion of neutral steroids plus bile acids indicated that not only was the direct biliary secretion of cholesterol impaired but also the conversion of cholesterol to bile acids tended to be reduced. The inefficient hepatic catabolism of cholesterol could, despite low cholesterol synthesis, overload hepatocytes with cholesterol and thus downregulate LDL receptor expression.1 This process could partly explain the 11% elevation in LDL cholesterol level in the circulation of our female cases with CAD (see Figure 2). However, in multivariate analysis adjusted for LDL or HDL cholesterol levels, reduced cholesterol synthesis and output of fecal total steroids were still associated with the risk for CAD, suggesting that LDL and HDL cholesterol could not solely explain the presence of CAD in these women. In a prospective study, a high dietary cholesterol intake increased the risk of CAD irrespective of serum cholesterol levels,47 suggesting that downregulated cholesterol synthesis induced by cholesterol feeding48 would have been implicated in the development of atherosclerosis.

**Limitations and Conclusions**

Postmenopausal women were chosen because etiological factors for CAD have been studied less extensively in women, and they are less likely to have hypolipidemic treatment than men. The methods used to measure cholesterol metabolic variables are laborious; therefore, only a limited subjects have been included. The results suggest that reduced synthesis and turnover of cholesterol and the subsequent low cholesterol elimination associated with high serum squalene and desmosterol may play a role in the development of CAD in only mildly hypercholesterolemic postmenopausal women. The high serum values of the 2 precursors of cholesterol

Downloaded from http://atvb.ahajournals.org/ by guest on January 14, 2018
synthesis, of which squalene is independently associated with the presence of CAD, are not related to cholesterol synthesis, but in the presence of excessive hepatic production, they may reflect some unknown metabolic events that are related to enhanced development of atherosclerosis. In conclusion, measuring serum squalene and sterols and fecal steroids by GLC would provide additional information in the evaluation of risk for CAD in postmenopausal women. Because consumption of stanol ester margarines not only reduces cholesterol absorption but also enhances synthesis and elimination of cholesterol, their use may be preferable alone or, in resistant cases, in combination with statins for hypolipidemic treatment of postmenopausal women.43

References
Cholesterol Absorption, Synthesis, and Fecal Output in Postmenopausal Women With and Without Coronary Artery Disease
Radhakrishnan A. Rajaratnam, Helena Gylling and Tatu A. Miettinen

doi: 10.1161/hq1001.097019
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/21/10/1650

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/