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Independent Association of Serum Squalene and Noncholesterol Sterols With Coronary Artery Disease in Postmenopausal Women

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OBJECTIVES The purpose of the study was to investigate whether cholesterol metabolism is associated with coronary artery disease (CAD) in postmenopausal women.

BACKGROUND Although hypercholesterolemia, a predominant risk factor of CAD, is related to cholesterol metabolism, the association between cholesterol metabolism and CAD is not well known.

METHODS In addition to conventional coronary risk factors, fasting serum squalene, Δ^8-cholestenol, desmosterol, lathosterol (indicators of cholesterol synthesis), cholestanol, campesterol and sitosterol (indicators of cholesterol absorption) were measured in 48 50- to 55-year-old consecutive women with angiographically verified CAD and in 61 age-matched healthy controls.

RESULTS The coronary patients had elevated ratios of squalene ($p < 0.001$), desmosterol ($p = 0.005$), campesterol ($p = 0.028$) and sitosterol ($p = 0.022$) to cholesterol, but had lower respective lathosterol value ($p = 0.041$) compared with the controls, despite similar serum cholesterol levels. Adjusted for age, body mass index, family history of CAD, smoking, hypertension, serum triglycerides, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol level and glycosylated hemoglobin A1c% (GHbA1c), the ratios of squalene (odds ratio, 1.36; 95% confidence interval, 1.17 to 1.57), lathosterol (0.98; 0.97 to 0.99), campesterol (1.01; 1.00 to 1.01) and sitosterol (1.01; 1.00 to 1.03) were significantly associated with the risk of CAD. In addition, family history of CAD and GHbA1c% were also independently related to the presence of CAD.

CONCLUSIONS The results suggest that women with elevated ratios of serum squalene, campesterol and sitosterol to cholesterol and low respective lathosterol values have enhanced risk for CAD. Thus, enhanced absorption and reduced synthesis of cholesterol may be related to coronary atherosclerosis. (J Am Coll Cardiol 2000;35:1185–91) © 2000 by the American College of Cardiology

During the past few decades, serum total cholesterol has been established as a strong independent risk factor for atherosclerosis (1). Consequently, effective lowering of serum cholesterol level results in a marked reduction of different clinical manifestations of coronary heart disease both in primary and secondary prevention (2,3). Serum cholesterol concentration is also regulated by cholesterol metabolism (i.e., synthesis, absorption and elimination) (4,5), which in turn are influenced by dietary fat and cholesterol intake (6). Cholesterol synthesis rate and absorption efficiency can be reflected by measuring serum noncholesterol sterols with gas–liquid chromatography (GLC) (7–11). Serum cholesterol precursors, including squalene, lanosterol and other methylated sterols, desmosterol and lathosterols, are positively related to cholesterol synthesis rate (7,8), even though the association with squalene is controversial (9–12).

Plant sterols, solely of dietary origin, such as campesterol and sitosterol, and cholestanol, a metabolite of cholesterol, are related to cholesterol absorption efficiency (10,11). Even though hypercholesterolemia has been well defined as a major risk factor in coronary patients, association of cholesterol metabolism and serum noncholesterol sterols with coronary artery disease (CAD) and its lipid and nonlipid risk factors is not clear. In recent studies, reduction of coronary events by simvastatin treatment was positively associated with basal ratios of cholestanol and plant sterols to cholesterol (13). In addition to cholesteryl esters, squalene has also been found in human atherosclerotic plaques (14), but its significance as a risk factor for CAD has not been studied. Accordingly, in addition to conventional

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Abbreviations and Acronyms

BMDP = Biomedical Data Program
CAD = coronary artery disease
ECG = electrocardiogram
FSH = follicle-stimulating hormone
GHbA1c = glycosylated hemoglobin A1c
GLC = gas–liquid chromatography
HDL = high-density lipoprotein
LDL = low-density lipoprotein
Q_ = quartiles
ROC = receiver operating characteristic
SHBG = sex hormone binding globulin

risk factors of CAD, squalene and noncholesterol sterols in fasting sera were assessed in postmenopausal women with angiographically documented CAD and were compared to postmenopausal controls.

METHODS

Subjects. The study recruited 50–55-year-old consecutive postmenopausal coronary patients who had been successively treated for CAD in the University Central Hospital of Helsinki between 1988 and 1996. Twenty-nine coronary women had suffered from myocardial infarction, 26 had been treated with angioplasty and 21 with bypass surgery, all at least six months earlier. Coronary angiography was interpreted to be pathological with at least 50% occlusion in two coronary vessels. Age-matched postmenopausal women were randomly chosen from the population registry of Helsinki as controls. The controls were free of chest pain and dyspnea, and their electrocardiograms (ECGs) were normal. Postmenopause was defined by amenorrhea and elevated serum follicle-stimulating hormone level (FSH > 30 U/liter). Hypolipidemic drug users and subjects with severe heart, liver or digestive tract or malignant diseases were excluded.

All subjects underwent physical examinations before collection of blood samples following a 12-h fast. History of CAD in family members, and smoking history and medications, including hormone replacement therapy, were recorded and blood pressure was measured. Beta-blocker medication was more common among the cases than among healthy control women (Table 1). Calcium blockers were

Table 1. Baseline Characteristics

Characteristic	Cases (n = 48)	Controls (n = 61)	p Value
Age (yrs)	53.1 ± 0.5	53.6 ± 0.4	> 0.1
Body mass index (kg/m^2)	26.0 ± 0.6	26.7 ± 0.6	> 0.1
Apolipoprotein E2/E3/E4 (n)	1/25/22	2/34/25	> 0.1
Dietary fat intake (g·kg^{-1}·d^{-1})*	1.10 ± 0.08	1.11 ± 0.07	> 0.1
Dietary cholesterol intake (mg·kg^{-1}·d^{-1})*	4.09 ± 0.37	4.13 ± 0.25	> 0.1
Dietary plant sterol intake (mg·kg^{-1}·d^{-1})*	4.15 ± 0.23	4.64 ± 0.23	0.092
Family history of CAD (n)	39	20	< 0.001
Hypertension (n)	22	15	0.020
Systolic blood pressure (mm Hg)	133.7 ± 4.1	132.7 ± 2.9	> 0.1
Diastolic blood pressure (mm Hg)	83.4 ± 1.5	84.7 ± 1.6	> 0.1
Beta-blocker users (n)	34	6	< 0.001
Exogenous hormone users (n)	18	28	> 0.1
Smokers (n)	26	18	0.013
Serum cholesterol (mmol/liter)			
Total	5.94 ± 0.14	5.71 ± 0.12	> 0.1
Low density lipoprotein	3.99 ± 0.12	3.66 ± 0.12	0.024
High density lipoprotein	1.31 ± 0.03	1.50 ± 0.05	0.003
Serum triglycerides (mmol/liter)	1.39 ± 0.09	1.21 ± 0.09	0.055
Blood hemoglobin (g/liter)	143.8 ± 1.4	138.2 ± 1.2	0.002
Blood glucose (mmol/liter)	4.61 ± 0.11	4.57 ± 0.08	> 0.1
Blood GHb A1c (%)	5.61 ± 0.06	5.30 ± 0.05	< 0.001
Serum insulin (mU/liter)	8.47 ± 0.60	8.71 ± 0.81	> 0.1
Serum SHBG (nmol/liter)	68.9 ± 5.2	67.4 ± 4.8	> 0.1
Serum FSH (U/liter)	40.9 ± 4.1	40.5 ± 3.6	> 0.1
Serum thyrotropin (mU/liter)	2.87 ± 0.32	2.87 ± 0.35	> 0.1
Blood leukocyte (xE9/liter)	6.15 ± 0.21	5.70 ± 0.29	0.016
Erythrocyte sedimentation rate (mm/h)	13.5 ± 1.7	10.9 ± 1.0	> 0.1

CAD = coronary artery disease; GHbA1c = glycosylated hemoglobin A1c; FSH = follicle-stimulating hormone; SHBG = sex hormone binding globulin; apolipoprotein E2 = phenotype 2/3; apolipoprotein E3 = phenotype 3/3; apolipoprotein E4 = phenotypes 2/4, 3/4 and 4/4. Differences between continuous variables were analyzed by Mann-Whitney rank-sum test and categorical values by chi-square test.

*Randomly measured in 29 cases and 41 controls.

being taken by 16 of the cases and by 4 control women, diuretics, respectively, by 3 and 5, whereas angiotensin-converting enzyme inhibitors were used by 3 and 5, respectively. Eighteen coronary patients and 28 healthy women used exogenous hormone substitution. Nine women in the case group and 10 in the control group used estrogens, and 2 women in each group used progestin only, whereas 7 coronary patients and 16 healthy women used both estrogen and progestin. The study was approved by the ethics committee of our hospital. All subjects volunteered for the study and gave informed consent.

Baseline measurements. Subjects were advised to continue their normal diets, and dietary fat and cholesterol were calculated from seven-day dietary recalls (15). Cholesterol and triglycerides were measured enzymatically by commercial kits (Boehringer Diagnostica, Germany). Low-density lipoprotein (LDL) cholesterol was calculated by Friedewald formula (16) as triglyceride values were below 3 mmol/liter in every subject. Cholesterol, squalene and noncholesterol sterols were measured by GLC from the same run as described earlier (12,17), so that the ratios to cholesterol could be adequately calculated. Briefly, 5 α-cholestane was added to serum samples as an internal standard after which the samples were saponified with 95% ethanol and KOH. Nonsaponified lipids were extracted by hexane, and squalene and noncholesterol sterols were quantitated on a 50-m-long Hewlett-Packard Ultra I column, using commercial squalene and noncholesterol sterols to localize their peaks. An average of two baseline squalene and noncholesterol sterol concentrations were used in the following. Serum squalene and noncholesterol sterol concentration were both positively and significantly correlated with serum total cholesterol concentration, as squalene and sterols are carried in serum by lipoproteins, mainly by LDL. Therefore, squalene and noncholesterol sterol values were expressed in ratio to cholesterol to eliminate the interindividual variation of the lipoprotein levels.

Blood hemoglobin was measured by electronic blood cell counter; leukocytes were measured by microscopic evaluation with May-Grünwald-Giemsa stain; erythrocyte sedimentation rate by Westergren method; serum thyrotropin by immunoluminometric assay (18); serum FSH by immunoluminometric assay (19); serum sex hormone binding globulin (SHBG) by immunofluorometric assay (20); serum insulin by radioimmunoassay (21); blood glycosylated hemoglobin A1c (GHbA1c) by high-performance liquid chromatography (22); and blood glucose was measured enzymatically (hexokinase).

Data analysis. Continuous variables were presented as mean value ± SE. Group differences were analyzed by the Mann-Whitney rank-sum test. Relationships between continuous variables were assessed by the Spearman rank correlation test and between categorical and continuous variables by the chi-square test. For the latter test, continuous variables were divided at the median point into two categories. Associations between CAD and all other parameters were first analyzed by univariate logistic regression analysis and then by multivariate analysis, based on maximum likelihood ratio. The presence of CAD was included into the model as the dependent variable, and family history of CAD (yes or no), smoking (yes or no) and hypertension (yes or no) as independent categorical variables and others as independent continuous variables. Goodness of fit to the prediction was examined by Hosmer-Lemeshow and logistic function by the C.C. Brown test.

To compare the probability of the correct risk evaluation by squalene to cholesterol ratio, LDL cholesterol and serum triglycerides, receiver operating characteristic (ROC) curves were drawn by plotting the proportions of true positive results (sensitivity) versus the proportions of false positive results (1-specificity). Study subjects were rated on their risk for CAD based on quartiles (Q) of the factors (i.e., gradually increasing risk from Q1 to Q4). The ROC curves were described by area under the curve. Statistical analysis was performed with Biomedical Data Program (BMDP) statistical software package. A p value less than 0.05 was considered significant.

RESULTS

Baseline characteristics. The mean age, body mass index, frequency of apolipoprotein E phenotypes and exogenous hormone users and dietary intakes of fat, cholesterol and plant sterols were similar among the women without and with CAD (Table 1). Eighty-one percent of the coronary patients and 33% of the healthy women had family history of CAD. The ratio was 2.5 times larger in the case group than in the control group ($p < 0.000$). The coronary patients had significantly higher frequency of smokers and hypertensive subjects, elevated concentrations of LDL cholesterol, GHbA1c, hemoglobin and leukocytes and lower levels of high density lipoprotein (HDL) cholesterol compared with the healthy women controls. In concentration and ratio to cholesterol, squalene, desmosterol, campesterol and sitosterol were significantly higher and those of lathosterol were lower in the case group than in the control group (Table 2). Among nonsmokers, non–beta-blocker users and nonhormone users, the cases still had significantly higher ratios of squalene and plant sterols and lower respective lathosterol values than did the controls. The women with and without exogenous hormones and beta- and calcium-blocking agents had similar concentrations of baseline parameters in both groups.

Relationship between the factors associated with CAD. The ratios of squalene were positively associated with those of desmosterol and GHbA1c%, and negatively with HDL cholesterol level (Table 3). An inverse association between serum total cholesterol concentrations and squalene ratios was found in the coronary patients and less consistently in the controls (Fig. 1), even though the squalene values below the median showed a significant respective correlation (r =

Table 2. Fasting Serum Squalene and Noncholesterol Sterols

Variable	Concentration, μg/dl		Ratio, 10^2 × mmol/mol of Cholesterol	
	Cases (n = 48)	Controls (n = 61)	Cases (n = 48)	Controls (n = 61)
Squalene	91.3 ± 2.4‡	60.8 ± 2.1	42.1 ± 1.2‡	28.2 ± 0.9
Δ^8-Cholestenol	33.2 ± 1.9	34.5 ± 2.1	15.1 ± 0.1	15.9 ± 0.9
Desmosterol	212.4 ± 17.9*	163.3 ± 4.8	97.6 ± 8.8†	75.6 ± 2.0
Lathosterol	373.8 ± 20.0*	425.5 ± 16.5	170.6 ± 8.2*	196.2 ± 6.7
Cholestanol	284.6 ± 13.8	282.4 ± 10.8	129.8 ± 5.7	129.5 ± 3.8
Campesterol	610.9 ± 44.3*	493.4 ± 24.8	278.3 ± 18.6*	227.9 ± 10.9
Sitosterol	332.3 ± 21.7*	271.0 ± 13.2	151.5 ± 9.1*	124.9 ± 5.6

Mean ± SE. *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$, cases vs. controls.

−0.30, p = 0.033). The squalene content of serum cholesterol was lower in the controls than in the cases, especially in lower and higher cholesterol levels. None of the ratios of noncholesterol sterols to cholesterol were associated with serum total cholesterol level.

The ratios of the precursor sterols were inversely related to those of plant sterols ($p < 0.01$) and HDL cholesterol, and positively to serum triglyceride concentration and body mass index (Table 3). The two latter variables were inversely associated with the plant sterols and cholestanol ratios. The GHbA1c% was positively associated with the concentrations of LDL cholesterol and serum triglycerides, and inversely with HDL cholesterol and serum SHBG levels, whereas blood glucose and insulin values were inversely correlated with the plant sterol ratios. Family history of CAD was correlated with the ratios of squalene ($p < 0.001$), desmosterol (p = 0.030) and sitosterol (p = 0.040). The correlations cited here were not different between the women with and without hormone replacement therapy.

Association between CAD and risk factors. In univariate analysis, the presence of CAD was positively associated with family history of CAD, hypertension, smoking, LDL cholesterol level, GHbA1c% and the ratios of squalene, desmosterol, campesterol and sitosterol and inversely with HDL cholesterol level and lathosterol ratio (Table 4). Adjustment for age, body mass index, family history of CAD, smoking, hypertension, LDL and HDL cholesterol levels, serum triglycerides and GHbA1c% eliminated the association of CAD with desmosterol, attenuated the association with lathosterol and did not affect the associations with squalene and plant sterols. In addition to the sterols, only GHbA1c% was significantly associated with CAD in all models. The presence of CAD was associated with LDL only in the squalene model (Model 1, Table 4), with smoking in the lathosterol model (Model 3, Table 4) and with family history of CAD in all except the squalene model. Adding SHBG, insulin and blood glucose to the models separately and substituting LDL cholesterol with serum total cholesterol in each model did not materially alter the results.

The presence of CAD was predicted by the squalene to cholesterol ratio stronger than was LDL cholesterol and serum triglycerides (Fig. 2). Thus, the area under ROC curves for the squalene ratio, LDL cholesterol and serum triglycerides were 0.885, 0.651 and 0.617, respectively,

Table 3. Correlations Between Factors Related to CAD

Variable	Serum Squalene	Serum Δ^8-Cholestenol	Serum Desmosterol	Serum Lathosterol	Serum Campesterol	Serum Sitosterol	Serum Cholestanol	Blood GHbA1c
Body mass index	−0.05	0.40‡	0.42‡	0.47‡	−0.22*	−0.27†	−0.39‡	0.11
Serum squalene	1.00	−0.03	0.21*	−0.10	0.19*	0.20*	0.16	0.28†
Serum cholesterol	−0.17	−0.01	−0.14	−0.05	−0.07	−0.05	−0.01	0.12
LDL cholesterol	−0.05	−0.05	−0.13	−0.13	0.08	0.12	0.11	0.20*
HDL cholesterol	−0.27†	−0.27†	−0.35‡	−0.19*	0.00	−0.06	0.07	−0.25†
Serum triglycerides	0.17	0.47‡	0.44‡	0.35‡	−0.33‡	−0.30†	−0.39‡	0.25†
Blood GHbA1c	0.28†	0.05	0.22*	0.00	−0.01	0.01	−0.09	1.00
Blood glucose	−0.09	0.08	0.15	0.17	−0.34‡	−0.40‡	−0.30†	0.17
Serum insulin	0.05	0.28†	0.30†	0.35‡	−0.24*	−0.23*	−0.28†	0.21*
Serum SHBG	0.11	−0.27†	−0.24*	−0.32‡	0.09	0.05	0.14	−0.28†

Values are Spearman's rank correlation coefficients (n = 109).
GHbA1c = glycosylated hemoglobin A1c; SHBG = sex hormone binding globulin.
Squalene and noncholesterol sterol values are in terms of 10^2 × mmol/mol of cholesterol.
*$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$.

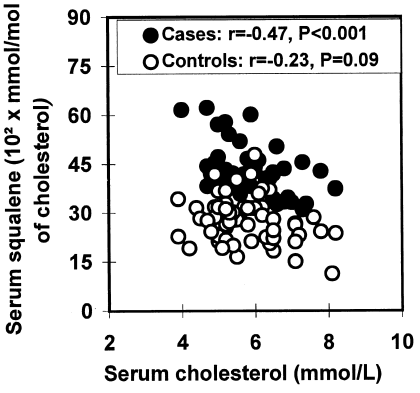

Figure 1. Association between serum squalene to cholesterol ratio and serum total cholesterol. **Closed circles** are cases and **open circles** are controls.

indicating that the squalene quartiles correctly predict cases and controls with a probability of 88.5%, LDL with 65.1% and serum triglycerides with 61.7%.

DISCUSSION

Cholesterol metabolism reflected by noncholesterol sterols. The present study revealed the independent associations of serum squalene, lathosterol, campesterol and sitosterol with the presence of angiographically documented CAD in postmenopausal women. High serum squalene may be a sign for enhanced risk of CAD, and it was related to serum cholesterol concentration. It has been shown that low lathosterol and high plant sterol ratios reflect low cholesterol synthesis rate and high cholesterol absorption efficiency in normal subjects (11). Thus, independent inverse association of lathosterol and positive associations of campesterol and sitosterol with CAD suggest that low synthesis and high absorption of cholesterol may be related to atherosclerosis in women independently of other lipid and nonlipid risk factors. Low cholesterol synthesis in the CAD women could be a consequence of high cholesterol absorption, but whether it is associated with low cholesterol elimination has to be studied further by sterol-balance technique studies. Even though contraceptive pills increase cholesterol precursor levels in platelets (23), in the present population the women with exogenous hormone substitution had similar serum squalene and noncholesterol sterol concentrations as those without the substitution.

High serum squalene concentrations in CAD women. Squalene in human atherosclerotic plaques (14) could originate from de novo synthesis (24) or from the circulation. In fact, 70% of basal serum squalene is carried in LDL (25), so that squalene could accumulate in atheroma via LDL infiltration. Why then is serum squalene concentration elevated in CAD? It could originate from diet and from endogenous synthesis. Dietary intake levels of fat and cholesterol were similar in the two present groups, suggest-

Table 4. Associations Between Risk Factors and Coronary Artery Disease

Variable	Univariate Analysis	Multivariate Analysis				
		Model 1	Model 2	Model 3	Model 4	Model 5
Age	0.95 (0.85–1.07)	1.04 (0.84–1.29)	0.97 (0.83–1.13)	0.98 (0.84–1.15)	1.01 (0.86–1.17)	1.00 (0.86–1.17)
Body mass index	0.96 (0.87–1.06)	0.90 (0.72–1.13)	0.85 (0.73–0.99)	0.96 (0.82–1.12)	0.89 (0.78–1.04)	0.90 (0.78–1.05)
Family history of CAD	8.88 (3.57–22.1)‡	3.87 (0.78–19.2)	6.98 (2.23–21.8)‡	6.72 (2.10–21.4)‡	6.02 (1.93–18.7)‡	6.08 (1.95–19.0)‡
Smoking	2.49 (1.11–5.60)*	2.31 (0.56–9.48)	2.32 (0.79–6.84)	3.84 (1.22–12.1)*	2.71 (0.94–7.84)	2.89 (0.99–8.39)
Hypertension	2.37 (1.03–5.42)*	2.32 (0.48–11.1)	1.51 (0.51–4.47)	2.02 (0.64–6.42)	1.91 (0.62–5.88)	2.02 (0.64–6.39)
LDL cholesterol	1.56 (1.02–2.41)*	3.94 (1.28–12.2)*	1.53 (0.85–2.77)	1.21 (0.66–2.23)	1.46 (0.80–2.65)	1.48 (0.81–2.71)
HDL cholesterol	0.13 (0.03–0.52)†	1.84 (0.09–34.7)	0.54 (0.07–4.01)	0.22 (0.03–1.77)	0.39 (0.05–2.92)	0.45 (0.06–3.21)
Serum triglycerides	1.47 (0.82–2.63)	1.19 (0.44–3.20)	1.05 (0.44–2.51)	1.65 (0.68–3.99)	1.75 (0.73–4.24)	1.67 (0.69–4.05)
GHbA1c	3.41 (2.21–20.2)‡	9.84 (1.28–75.9)*	5.59 (1.40–22.3)*	8.45 (2.00–35.8)†	7.94 (1.97–32.0)†	8.42 (2.07–34.2)†
Squalene§	1.30 (1.18–1.43)‡	1.36 (1.17–1.57)‡	—	—	—	—
Desmosterol§	1.02 (1.00–1.05)†	—	1.03 (0.99–1.06)	—	—	—
Lathosterol§	0.99 (0.98–0.99)*	—	—	0.98 (0.97–0.99)†	—	—
Campesterol§	1.00 (1.00–1.01)*	—	—	—	1.01 (1.00–1.01)*	—
Sitosterol§	1.01 (1.00–1.02)*	—	—	—	—	1.01 (1.00–1.03)*

Values are odds ratio (95% CI).
CAD = coronary artery disease; GHbA1c = glycosylated hemoglobin A1c.
*p < 0.05; †p < 0.01; ‡p < 0.001. §Variables are in terms of 10^2 × mmol/mol of cholesterol.

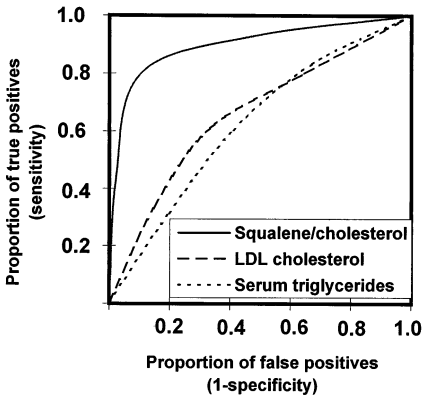

Figure 2. Receiver operating characteristic (ROC) curves for predicting the presence of coronary artery disease by squalene to cholesterol ratio, LDL cholesterol and serum triglycerides. Area under the curve for the squalene ratio was 0.885; LDL cholesterol was 0.651 and serum triglycerides was 0.617.

ing also that squalene intake was similar. Furthermore, the dietary diaries indicated that dietary squalene sources (e.g., olive oil) were poorly consumed by the present women. Considering minimal dietary intake of squalene, less than 0.5 g/day, which is known to have no effect on its serum level (26), differences in intestinal squalene absorption could hardly explain the differences of serum squalene in the two groups. Thus, the high squalene levels in the coronary patients were most likely of endogenous origin.

Cholesterol synthesis decreases with increasing serum cholesterol level (8,27). The inverse association of squalene to cholesterol ratio with serum cholesterol in the coronary patients suggests that the higher the serum cholesterol level the lower the squalene synthesis. This type of association was less consistent in the control women. Serum squalene concentrations most likely reflect endogenous presqualene cholesterol synthesis, but its association with overall cholesterol synthesis is controversial, especially in chronic situations (9). We sense that in acute metabolic changes, as in diurnal variation (25) and after apheresis (28), serum squalene ratios increase with increasing cholesterol synthesis. Squalene was not correlated either with body mass index or with the precursor sterols except with desmosterol, indicating cholesterol synthesis through the unsaturated side-chain pathway rather than the saturated one. Furthermore, low lathosterol ratios in the coronary women may also imply that high squalene ratio is not due to high cholesterol synthesis rate, but reflects difficulties in further conversion of squalene to cholesterol, some being metabolized through desmosterol.

Plant sterols. Coronary patients with high baseline cholestanol and plant sterol ratios to cholesterol appear to be clinically resistant to lowering of coronary recurrence by simvastatin, a finding partly contributed by further increase of plant sterol ratios during statin treatment (13). High serum plant sterols themselves are strongly atherogenic, especially in phytosterolemia (29,30). In fact, the plant sterol ratios were increased in the present cases, suggesting that absorption of cholesterol and plant sterols was increased because dietary plant sterol intakes were similar in the two groups. It remains to be shown what the lowest plant sterol levels are that contribute to enhanced CAD risk.

Glycosylated hemoglobin A1c%. Glycosylated hemoglobin A1c% was independently associated with the presence of CAD in the postmenopausal women, in agreement with a previous study (31). Its positive association with LDL cholesterol and serum triglycerides and negative association with HDL cholesterol level might partly explain its relation to CAD. In addition, its association with squalene and desmosterol indicates that high cholesterol synthesis through unsaturated side-chain pathway characterizes women with high normal blood GHbA1c%.

Study limitations and future plans. The control women had no clinical manifestation of CAD, and the rest ECG was normal, such that stress ECG was not considered to be necessary. In fact, this was a case-control study, in which the controls had significantly different CAD risk factors from the CAD patients with narrow selection criteria. Prospective studies of the healthy women and CAD patients on hypolipidemic treatments will show stability and possible drug-induced changes of the findings.

Conclusions. The prevalence of CAD in postmenopausal women was independently and positively associated with the ratios of squalene, campesterol and sitosterol to cholesterol and inversely with the respective lathosterol value, suggesting that high absorption and low synthesis of cholesterol might play a significant role in the development of atherosclerosis. Thus, altered cholesterol metabolism in postmenopausal women without or with CAD cannot be evaluated only from serum cholesterol level; rather, it needs quantification of basal serum squalene and noncholesterol sterols, which also would reveal information about the risk for CAD, especially in combination with family history of CAD and GHbA1c%. Serum cholesterol lowering is partially influenced by the pattern of cholesterol metabolism, indicating that combined malabsorption with inhibition of synthesis of cholesterol by stanol ester margarine and statins, respectively, would be an effective hypolipidemic measure for postmenopausal coronary patients (13,32).

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