The relationships of cholesterol metabolism and plasma plant sterols with the severity of coronary artery disease[1,S]

Guenther Silbernagel,* Guenter Fauler,† Wilfried Renner,† Eva M. Landl,† Michael M. Hoffmann,§ Bernhard R. Winkelmann, Bernhard O. Boehm,†† and Winfried März[2,†,§§,***]**

Division of Endocrinology,* Diabetology, Nephrology, Vascular Disease, and Clinical Chemistry, Department of Internal Medicine, University of Tübingen, Tübingen, Germany; Clinical Institute of Medical and Chemical Laboratory Diagnostics,† Medical University of Graz, Graz, Austria; Division of Clinical Chemistry,§ Department of Internal Medicine, University of Freiburg, Freiburg, Germany; Cardiology Group Frankfurt-Sachsenhausen,** Frankfurt, Germany; Division of Endocrinology,†† Department of Internal Medicine, University of Ulm, Ulm, Germany; Department of Public Health,§§ Social and Preventive Medicine, Mannheim Medical Faculty, University of Heidelberg, Mannheim, Germany; and Synlab Center of Laboratory Diagnostics,*** Heidelberg, Germany

Abstract Changes in the balance of cholesterol absorption and synthesis and moderately elevated plasma plant sterols have been suggested to be atherogenic. Measuring cholestanol, lathosterol, campesterol, and sitosterol, we investigated the relationships of cholesterol metabolism and plasma plant sterols with the severity of coronary artery disease (CAD) in 2,440 participants of the Ludwigshafen Risk and Cardiovascular health (LURIC) study. The coronary status was determined by angiography, and the severity of CAD was assessed by the Friesinger Score (FS). An increase in the ratio of cholestanol to cholesterol was associated with high FS ($P = 0.006$). In contrast, a high ratio of lathosterol to cholesterol went in parallel with low FS ($P < 0.001$). Whereas the campesterol to cholesterol ratio significantly correlated with the FS ($P = 0.026$), the relationship of the sitosterol to cholesterol ratio with the FS did not reach statistical significance in the whole group. Increased campesterol, sitosterol, and cholestanol to lathosterol ratios were associated high FS ($P < 0.001$). To conclude, there is a modest association of high cholesterol absorption and low cholesterol synthesis with an increased severity of CAD. An atherogenic role of plasma plant sterols themselves, however, seems unlikely in subjects without sitosterolaemia.—Silbernagel, G., G. Fauler, W. Renner, E. M. Landl, M. M. Hoffmann, B. R. Winkelmann, B. O. Boehm, and W. März. **The relationships of cholesterol metabolism and plasma plant sterols with the severity of coronary artery disease. *J. Lipid Res.* 2009.** 50: **334–341.**

Supplementary key words cholesterol absorption • cholesterol synthesis • phytosterols • campesterol • sitosterol • cholestanol • lathosterol

Elevated plasma total and LDL cholesterol represent major cardiovascular risk factors (1). Plant sterols are structurally highly similar to cholesterol (2). However, the physiological plasma plant sterol concentration is less than 1 mg per decilitre (3).

Sitosterolaemia, a rare genetic disorder, is characterized by up to 100-fold elevated plasma plant sterols (4, 5). Special excretion proteins, the ATP-binding cassette transporter G5 (ABCG5) and the ATP-binding cassette transporter G8 (ABCG8), which are expressed in the intestine and in the liver, are defective in patients with sitosterolaemia. These transporters eliminate plant sterols and thus protect against the accumulation of plant sterols in the body (6, 7). Due to the fact that patients suffering from sitosterolaemia develop severe premature atherosclerosis,plant sterols have been suspected to be atherogenic (5). This raises the question if moderately elevated plasma plant sterols are harmful in the general population as well. Clinical studies concerning this issue have been controversial (3, 8–17).

Efforts to examine a possible link between plant sterols and coronary artery disease (CAD) have to consider that plasma plant sterols are surrogate markers for cholesterol absorption (18, 19). Plasma cholesterol is either derived from uptake in the intestine or from endogenous synthesis (20). The individual balance of cholesterol absorption and synthesis is an heritable trait with the *ABCG5* and *ABCG8* as well as the *Niemann-Pick Type C1 Like 1* genes being

Manuscript received 12 March 2008 and in revised form 29 July 2008 and in re-revised form 2 September 2008.

Published, JLR Papers in Press, September 3, 2008.
DOI 10.1194/jlr.P800013-JLR200

Abbreviations: ABCG5, ATP-binding cassette transporter G5; ABCG8, ATP-binding cassette transporter G8; ANOVA, analysis of variance; APOE, apolipoprotein E; BMI, body mass index; CAD, coronary artery disease; FS, Friesinger Score; GCMS, gas chromatography and mass spectrometry; GLM, general linear model; LURIC, Ludwigshafen risk and cardiovascular health.

[1] Declaration of Helsinki: The study complies with the Declaration of Helsinki and to Title 45, US Code of Federal Regulations, Part 46, Protection of Human Subjects, Revised November 13, 2001, effective December 13, 2001.

[2] To whom correspondence should be addressed.
e-mail: winfried.maerz@synlab.de

[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of a table.

involved (6, 7, 21–24). Importantly, changes in cholesterol homeostasis have also been suggested to be associated with CAD and mortality (8–10, 14–16).

To investigate the relationships of cholesterol metabolism and plasma plant sterols with CAD, we measured cholestanol (not a plant sterol, but a marker of cholesterol absorption) (19), campesterol and sitosterol (plant sterols and markers of cholesterol absorption), and lathosterol (marker of cholesterol synthesis) (18, 19, 25) in 2,440 well-characterized patients in whom the coronary status was assessed by angiography (26).

MATERIALS AND METHODS

Study design and participants

We studied participants of the Ludwigshafen Risk and Cardiovascular health (LURIC) study (26). LURIC is a cohort study designed to investigate cardiovascular risk factors. A total of 3,316 patients scheduled for coronary angiography were recruited between July 1997 and January 2000 at the Heart Center Ludwigshafen, Germany. All of them were Caucasian, of German ancestry, and living in the southwest of Germany. Except for acute coronary syndromes, individuals had to be in a stable clinical condition (i.e., no concomitant acute illness such as infection or recent accident/surgery). The availability of a coronary angiogram was postulated. Indications for angiography in individuals in clinically stable condition were chest pain and/or noninvasive test results consistent with myocardial ischemia. Patients with and without statin treatment were included in the study. Subjects suffering from any acute illness other than acute coronary syndrome (other acute cardiac disease, such as decompensated heart failure or decompensated valvular disease, or acute noncardiac disease, such as infection, endocrine disease or any type of surgery within the previous 3 months) were excluded from the study. Furthermore, patients with chronic polymorbid disease in which the noncardiac disease predominated (i.e., chronic renal failure and hemodialysis, severe rheumatoid arthritis, persistent incapacitation after accident/trauma) or with a history of malignant disease within the previous 5 years and those subjects incapable of understanding the purpose of the study were ruled out. Seven subjects suffering from type 1 diabetes were additionally eliminated from the present analysis. The study was approved by the ethics committee at the "Ärztekammer Rheinland-Pfalz" and was conducted in accordance with the "Declaration of Helsinki." Informed written consent was obtained from all participants.

CAD was assessed by angiography with maximum luminal narrowing estimated by visual analysis. The severity of CAD was quantified with the Friesinger Score (FS) (27). Diabetes mellitus was diagnosed according to the criteria of the American Diabetes Association (28). Furthermore, individuals with a history of diabetes or treatment with oral antidiabetics or insulin were considered diabetic. Hypertension was diagnosed if the systolic and/or diastolic blood pressure exceeded 140 and/or 90 mmHg or if there was a history of hypertension, also evident through the use of antihypertensive drugs.

Subjects included in the study were numbered by the order of recruitment. Measurement of cholestanol, campesterol, sitosterol, and lathosterol was performed in a total of 2,440 LURIC participants that were randomly selected.

Laboratory procedures

The standard laboratory methods have been described (26). Fasting blood samples collected before angiography were kept frozen at −80°C between the day of blood draw and the day of analysis. Plasma total cholesterol and triglycerides were determined enzymatically (26). Cholestanol, campesterol, sitosterol, and lathosterol were quantified in plasma using a recently published gas chromatography and mass spectrometry (GCMS)-based method (29).

Instruments and analytical method. A Thermo Trace 2000 gas chromatograph coupled to a Fisons MD 800 quadrupole mass spectrometer was used with Helium as carrier gas. Noncholesterol sterols were analyzed applying electron ionization GCMS with single-ion-monitoring mode.

Sample preparation. A 100 μl portion of a solution containing epicoprostanol as internal standard and butylated hydroxytoluene as antioxidant was admixed to 100 μl of plasma. Kept at 75°C, the samples were hydrolyzed in ethanolic KOH-solution. After liquid-phase extraction, solid-phase purification, and derivatisation to trimethylsilylethers, the samples were subjected to GCMS-analysis.

Apolipoprotein E (*APOE*) genotyping was performed by allele-specific restriction analysis with AflIII and HaeII as described (30). Three groups comprising carriers of at least one *APOE* 2 allele (*APOE* 2/2, *APOE* 2/3, *APOE* 2/4), *APOE* 3/3 homozygotes, and the remaining individuals (*APOE* 3/4 or *APOE* 4/4) were formed. Data on the *APOE* genotype were available in 2,437 of the 2,440 subjects.

Statistical analysis

The FS was broken down to four (A–D) categories of severity of CAD (A = FS 0–1, B = FS 2–4, C = FS 5–8, and D = FS 9–15). The clinical and biochemical characteristics of the study participants were expressed as numbers and percentages of subjects in the cases of categorical variables and as means ± SD or means with interquartile ranges in the cases of continuous variables. Comparisons among the four groups of the FS were analyzed using the χ^2-test or univariate analysis of variance (ANOVA). Adjusted *P* values were calculated with logistic regression or general linear models (GLMs) with covariates as indicated (**Table 1**). Ratios of noncholesterol sterols to cholesterol were calculated to standardize for the variation of plasma cholesterol. Additionally, ratios of absorption sterols to lathosterol were formed. Crude noncholesterol sterols, their ratios to cholesterol, and absorption sterol to lathosterol ratios were transformed logarithmically. Pearson correlations between noncholesterol sterols and cholesterol were computed. In addition, Pearson correlations among noncholesterol sterol to cholesterol ratios were calculated. Using GLMs in which those factors not under examination were included as covariates, the effects of sex, age, body mass index (BMI), diabetes, hypertension, and statin use on noncholesterol sterol to cholesterol ratios were studied (**Table 2**). Multivariate GLMs were generated to examine the relationships of the FS with uncorrected noncholesterol sterols, noncholesterol sterol to cholesterol ratios, and absorption marker to lathosterol ratios. Furthermore, the impact of the *APOE* genotype on the associations of noncholesterol sterol to cholesterol ratios and absorption sterol to lathosterol ratios with the FS was examined (data not shown). Analyses were performed in the whole group (**Table 3**), in the subgroup of patients who did not receive statins (**Table 4**), in the subgroup of statin users (see supplementary table), and in the subgroup of nondiabetics (data not shown). All statistical tests were two-sided, and $P < 0.05$ was considered significant. The SPSS 15.0 statistical package (SPSS Inc.) was used.

TABLE 1. Clinical and biochemical characteristics of the study participants

Friesinger Score (4 categories)	A (0–1)	B (2–4)	C (5–8)	D (9–15)	P[a]
N	502	553	793	592	
Age (years)	58.1 ± 11.6	64.1 ± 9.8	63.6 ± 10.1	64.9 ± 9.3	<0.001[b]
Male sex	255 (50.8)	340 (61.5)	590 (74.4)	491 (82.9)	<0.001[c]
Statin use	97 (19.3)	212 (38.3)	483 (60.9)	391 (66.0)	<0.001
BMI (kg/m^2)	27.1 ± 4.2	27.8 ± 4.5	27.5 ± 4.2	27.3 ± 3.5	0.014
Type 2 diabetes					<0.001
No	418 (83.3)	392 (70.9)	518 (65.3)	358 (60.5)	
Yes, no insulin	77 (15.3)	135 (24.4)	236 (29.8)	181 (30.6)	
Yes, insulin	7 (1.4)	26 (4.7)	39 (4.9)	53 (9.0)	
Hypertension	310 (61.8)	402 (72.8)	606 (76.4)	452 (76.4)	0.001
Smoking					<0.001
Never	267 (53.2)	234 (42.3)	250 (31.5)	154 (26.0)	
Past	152 (30.3)	208 (37.6)	388 (48.9)	334 (56.4)	
Current	83 (16.5)	111 (20.1)	155 (19.5)	104 (17.6)	
Total Cholesterol (mg/dl)	199 ± 37	195 ± 36	192 ± 41	186 ± 38	0.132[d]
LDL Cholesterol (mg/dl)	118 ± 32	117 ± 32	114 ± 37	111 ± 33	0.028[d]
HDL Cholesterol (mg/dl)	43 ± 12	40 ± 11	38 ± 10	36 ± 10	<0.001[d]
Triglycerides, mg/dl)[e]	142	151	157	157	0.001[d,e]
Percentile 25 (mg/dl)	98	107	115	114	
Percentile 75 (mg/dl)	194	208	202	203	
C-reactive protein (mg/l)[e]	2.40	3.50	4.09	4.36	<0.001[d,e]
Percentile 25 (mg/l)	1.00	1.49	1.46	1.58	
Percentile 75 (mg/l)	5.87	8.21	9.54	9.93	
ApoE genotype					
E2/2, E2/3, E2/4	100 (19.9)	82 (14.9)	100 (12.6)	81 (13.7)	0.001
E3/3	314 (62.5)	337 (61.1)	498 (62.8)	398 (67.5)	0.328
E3/4, E4/4	88 (17.5)	133 (24.1)	195 (24.6)	111 (18.8)	0.117

ApoE, apolipoprotein E; BMI, body mass index. Values are means ± SD or means with interquartile ranges in the cases of continuous variables.

Values represent numbers (percentages) of subjects in the cases of categorical variables.

[a] General linear model (GLM) or logistic regression, adjusted for age and gender.

[b] GLM, adjusted for gender only.

[c] Logistic regression, adjusted for age only.

[d] Additionally adjusted for the use of lipid-lowering agents.

[e] GLM of logarithmically transformed values.

RESULTS

The clinical and biochemical characteristics of the study participants

Study participants with FS > 1 were significantly older and rather male than female than those with FS 0–1. The percentage of subjects receiving statins increased in parallel with the FS. Current or past smoking, type 2 diabetes, and hypertension were more prevalent in subjects with high FS. Elevated C-reactive protein and triglycerides, and decreased HDL cholesterol were associated with increased severity of CAD. Due to more frequent use of statins subjects with high FS had lower total and LDL cholesterol compared with individuals without CAD. There were modest differences in BMI among the four FS categories. The presence of the *APOE 2*× genotype was associated with low FS (Table 1).

Plasma noncholesterol sterol concentrations

The absolute mean plasma concentrations ± SD of campesterol, sitosterol, cholestanol, and lathosterol were 7.57 ± 4.50 μMol/l, 3.87 ± 2.33 μMol/l, 7.22 ± 3.01 μMol/l, and 7.00 ± 4.81 μMol/l, respectively. All noncholesterol sterols were positively correlated with total cholesterol ($r = 0.360$, $P < 0.001$; $r = 0.350$, $P < 0.001$; $r = 0.507$, $P < 0.001$; and $r = 0.436$, $P < 0.001$ for campesterol, sitosterol, cholestanol, and lathosterol, respectively). There was a strong positive correlation between the ratios of campesterol and sitosterol to cholesterol ($r = 0.879$, $P < 0.001$). An increase in the ratio of cholestanol to cholesterol ratio went in parallel with high plant sterol to cholesterol ratios ($r = 0.475$, $P < 0.001$ and $r = 0.454$, $P < 0.001$ for campesterol and sitosterol, respectively). The lathosterol to cholesterol ratio was inversely correlated with the ratios of campesterol, sitosterol, and cholestanol to cholesterol ($r = -0.306$, $P < 0.001$; $r = -0.247$, $P < 0.001$; $r = -0.350$, $P < 0.001$, respectively), revealing the expected reciprocal relationship between cholesterol absorption and synthesis.

The relationships of noncholesterol sterol to cholesterol ratios with clinical parameters

We examined the relationships of noncholesterol sterol to cholesterol ratios with sex, age, components of the metabolic syndrome, and the use of statins. BMI and statin use were strongly correlated with the noncholesterol sterol to cholesterol ratios. In the subgroup with high BMI (>26 kg/m^2 and >27 kg/m^2 in males and females, respectively), absorption marker ratios were decreased ($P < 0.001$), and the lathosterol to cholesterol ratio was increased ($P < 0.001$). Statin users had higher plant sterol to cholesterol ratios than nonusers ($P < 0.001$), whereas the cholesterol biosynthesis was suppressed, as expected ($P < 0.001$). The cholestanol to cholesterol ratio was slightly

TABLE 2. Associations of noncholesterol sterol to cholesterol ratios with distinct parameters

		Campesterol: Cholesterol			Sitosterol: Cholesterol			Cholestanol: Cholesterol			Lathosterol: Cholesterol		
	N	EMM	Diff	P	EMM	Diff	P	EMM	Diff	P	EMM	Diff	P
Gender													
Male	1,676	1.36			0.68			1.37			1.17		
Female	764	1.28	−5.8	0.006	0.67	−1.6	0.462	1.37	−0.2	0.889	1.14	−1.9	0.412
Age (years)													
<60	874	1.41			0.70			1.39			1.25		
60–70	890	1.30	−8.1	<0.001	0.68	−2.6	0.284	1.34	−3.9	0.014	1.15	−7.8	0.002
>70	676	1.27	−10.1	<0.001	0.67	−4.1	0.120	1.38	−1.0	0.566	1.06	−14.9	<0.001
BMI (kg/m^2)													
<26 ♂ or 27 ♀	1,161	1.49			0.76			1.45			1.05		
>26 ♂ or 27 ♀	1,279	1.20	−19.7	<0.001	0.62	−17.9	<0.001	1.30	−10.6	<0.001	1.27	20.4	<0.001
Type 2 diabetes													
No	1,686	1.36			0.70			1.38			1.15		
Yes, no insulin	629	1.24	−9.1	<0.001	0.64	−9.1	<0.001	1.32	−4.3	0.006	1.20	4.6	0.092
Yes, insulin	125	1.40	3.1	0.502	0.68	−2.7	0.566	1.43	3.1	0.321	1.01	−12.6	0.006
Hypertension													
No	670	1.36			0.71			1.39			1.09		
Yes	1,770	1.32	−3.3	0.159	0.67	−5.2	0.028	1.36	−2.2	0.175	1.19	8.8	0.001
Statin use													
No	1,257	1.23			0.64			1.36			1.50		
Yes	1,183	1.44	17.0	<0.001	0.73	15.0	<0.001	1.39	2.2	0.105	0.88	−41.6	<0.001

GLMs, adjusted for each of the other variables; EMM are estimated marginal means (mmol/mol); Diff indicates the percent change between the respective categories.

elevated in subjects taking statins ($P = 0.105$). Provided that patients did not receive insulin treatment, type 2 diabetes was significantly associated with high synthesis and low absorption of cholesterol. Individuals older than 60 years had decreased noncholesterol sterol to cholesterol ratios compared with those younger than 60. Hypertension was associated with increased lathosterol to cholesterol ratio ($P < 0.01$), and male gender was correlated with elevated campesterol to cholesterol ratio ($P = 0.006$) (Table 2).

The relationships of uncorrected noncholesterol sterols, noncholesterol sterol to cholesterol ratios, and absorption sterol to lathosterol ratios with the severity of CAD

The relationships of cholesterol homeostasis with the FS were similar in users of statins (see supplementary table) and subjects who did not take lipid-lowering drugs (Table 4). In the entire cohort, high FS was significantly associated with increased cholestanol and campesterol to cholesterol ratios ($P = 0.006$ and $P = 0.026$, respectively), and decreased lathosterol to cholesterol ratio ($P < 0.001$). The association of the sitosterol to cholesterol ratio with the severity of CAD did not reach statistical significance in the whole group (Table 3). In agreement, uncorrected cholestanol, lathosterol, and campesterol were significantly associated with the FS, and the relationship of uncorrected sitosterol with the severity of CAD did not reach statistical significance in the entire cohort (data not shown). Increased ratios of absorption sterols to lathosterol were correlated with high FS ($P < 0.001$) (Table 3). After exclusion of statin users, the relationship of the FS with cholestanol and lathosterol to cholesterol ratios ($P = 0.014$ and $P = 0.001$, respectively), and cholestanol, campesterol, and sitosterol to lathosterol ratios ($P < 0.001$, $P = 0.002$, and $P = 0.003$, respectively) remained significant. Due to lower sample size compared with the whole group, the association of the campesterol to cholesterol ratio with the FS was not significant in individuals who did not take statins (Table 4). In statin users, the FS was inversely correlated with the lathosterol to cholesterol ratio ($P = 0.010$) and positively related to the ratios of cholestanol, campesterol, and sitosterol to lathosterol ($P = 0.021$, $P = 0.025$, and $P = 0.033$, respectively). The relationships of absorption marker to cholesterol ratios with the FS did not reach statistical significance in the subgroup of statin users (data not shown). When subjects with type 2 diabetes were exluded from the entire cohort, high absorption and low synthesis of cholesterol were consistently associated with an increased severity of CAD and even the relationship of the sitosterol to cholesterol with the FS reached statistical significance (data not shown). The *APOE* genotype had no influence on the associations of the FS with noncholesterol sterol to cholesterol ratios and absorption sterol to lathosterol ratios in the whole group and in the subgroups of statin users, of subjects not receiving lipid-lowering drugs, and of nondiabetics (data not shown).

DISCUSSION

There is a modest association of high cholesterol absorption and low cholesterol synthesis with an increased severity of coronary atherosclerosis in participants of the LURIC study. As such a relationship can also be shown for cholestanol, a specific atherogenic role of plant sterols themselves, however, seems unlikely in subjects without sitosterolaemia.

Up to now, no large study has been published investigating the relationship of cholesterol homeostasis with CAD diagnosed by angiography. Nonetheless, the matter seems of interest because not only the synthesis but also the

TABLE 3. Non-cholesterol sterol to cholesterol ratios, absorption sterol to lathosterol ratios, and the severity of coronary artery disease (CAD) (whole group)

Friesinger Score (4 categories)	N	EMM (CI 95%)	Difference (%)	P
Campesterol: Cholesterol				
A (0–1)	502	1.28 (1.22–1.34)		
B (2–4)	553	1.32 (1.27–1.38)	3.6	0.260
C (5–8)	793	1.34 (1.30 –1.39)	5.4	0.087
D (9–15)	592	1.37 (1.32–1.43)	7.8	0.026
Sitosterol: Cholesterol				
A (0–1)	502	0.66 (0.63–0.69)		
B (2–4)	553	0.68 (0.65–0.71)	3.7	0.260
C (5–8)	793	0.69 (0.66–0.71)	3.9	0.232
D (9–15)	592	0.70 (0.67–0.73)	5.7	0.110
Cholestanol: Cholesterol				
A (0–1)	502	1.31 (1.26–1.35)		
B (2–4)	553	1.37 (1.34–1.41)	5.2	0.018
C (5–8)	793	1.39 (1.36–1.42)	6.4	0.004
D (9–15)	592	1.39 (1.35–1.43)	6.6	0.006
Lathosterol: Cholesterol				
A (0–1)	502	1.28 (1.22–1.35)		
B (2–4)	553	1.15 (1.10–1.20)	−10.5	0.001
C (5–8)	793	1.13 (1.09–1.17)	−12.0	<0.001
D (9–15)	592	1.11 (1.06–1.16)	−13.6	<0.001
Campesterol: Lathosterol				
A (0–1)	502	0.99 (0.92–1.07)		
B (2–4)	553	1.15 (1.07–1.23)	15.8	0.004
C (5–8)	793	1.19 (1.12–1.26)	20.0	<0.001
D (9–15)	592	1.24 (1.16–1.33)	25.0	<0.001
Sitosterol: Lathosterol				
A (0–1)	502	0.51 (0.48–0.55)		
B (2–4)	553	0.59 (0.56–0.63)	15.8	0.003
C (5–8)	793	0.61 (0.57–0.64)	18.1	0.001
D (9–15)	592	0.63 (0.59–0.67)	22.4	<0.001
Cholestanol: Lathosterol				
A (0–1)	502	1.02 (0.95–1.09)		
B (2–4)	553	1.20 (1.13–1.27)	17.6	<0.001
C (5–8)	793	1.23 (1.17–1.29)	20.9	<0.001
D (9–15)	592	1.26 (1.18–1.33)	23.5	<0.001

GLMs, adjusted for gender, age, BMI, type 2 diabetes with and without insulin treatment, hypertension, smoking, and the use of statins; A–D, Friesinger Score categories; N, number of patients; EMM, estimated marginal means for the respective noncholesterol sterol to cholesterol ratios (mmol/mol) and absorption sterol to lathosterol ratios (dimensionless); CI, confidence interval; difference indicates the percent change of noncholesterol sterol to cholesterol ratios or absorption marker to lathosterol ratios with increasing Friesinger Score categories.

TABLE 4. Noncholesterol sterol to cholesterol ratios, absorption sterol to lathosterol ratios, and the severity of CAD (subgroup without statins)

Friesinger Score (4 categories)	N	EMM (CI 95%)	Difference (%)	P
Campesterol: Cholesterol				
A (0–1)	405	1.19 (1.00–1.25)		
B (2–4)	341	1.25 (1.19–1.32)	5.2	0.191
C (5–8)	310	1.26 (1.19–1.34)	6.3	0.141
D (9–15)	201	1.28 (1.19–1.38)	7.6	0.121
Sitosterol: Cholesterol				
A (0–1)	405	0.62 (0.59–0.66)		
B (2–4)	341	0.65 (0.62–0.69)	4.5	0.267
C (5–8)	310	0.64 (0.61–0.68)	3.5	0.422
D (9–15)	201	0.66 (0.61–0.71)	5.9	0.234
Cholestanol: Cholesterol				
A (0–1)	405	1.30 (1.25–1.34)		
B (2–4)	341	1.40 (1.35–1.45)	7.9	0.004
C (5–8)	310	1.37 (1.32–1.43)	6.0	0.037
D (9–15)	201	1.40 (1.34–1.47)	8.2	0.014
Lathosterol: Cholesterol				
A (0–1)	405	1.63 (1.54–1.72)		
B (2–4)	341	1.45 (1.38–1.53)	−10.9	0.004
C (5–8)	310	1.46 (1.38–1.55)	−10.3	0.009
D (9–15)	201	1.38 (1.28–1.48)	−15.4	0.001
Campesterol: Lathosterol				
A (0–1)	405	0.73 (0.67–0.80)		
B (2–4)	341	0.86 (0.79–0.94)	18.1	0.008
C (5–8)	310	0.87 (0.79–0.95)	18.5	0.011
D (9–15)	201	0.93 (0.83–1.04)	27.1	0.002
Sitosterol: Lathosterol				
A (0–1)	405	0.38 (0.35–0.41)		
B (2–4)	341	0.45 (0.41–0.49)	17.1	0.010
C (5–8)	310	0.44 (0.40–0.48)	15.3	0.029
D (9–15)	201	0.48 (0.43–0.53)	25.0	0.003
Cholestanol: Lathosterol				
A (0–1)	405	0.79 (0.74–0.86)		
B (2–4)	341	0.96 (0.89–1.04)	21.0	0.001
C (5–8)	310	0.94 (0.87–1.02)	18.2	0.004
D (9–15)	201	1.02 (0.92–1.13)	27.9	<0.001

GLMs, adjusted for gender, age, BMI, type 2 diabetes with and without insulin treatment, hypertension, and smoking; A–D, Friesinger Score categories; N, number of patients; EMM, estimated marginal means for the respective noncholesterol sterol to cholesterol ratios (mmol/mol) and absorption sterol to lathosterol ratios (dimensionless); CI, confidence interval; difference indicates the percent change of noncholesterol sterol to cholesterol ratios or absorption marker to lathosterol ratios with increasing Friesinger Score categories.

absorption of cholesterol is amenable to pharmacological manipulation. Furthermore, there is an ongoing discussion if moderately increased plasma plant sterols represent a cardiovascular risk factor.

Most clinical trials conducted so far report positive associations between plasma plant sterols and CAD (8–11, 14–17). However, there has been no consensus on the interpretation of data. Several authors have proposed proatherogenic effects of enhanced cholesterol absorption, whereas others have suggested special perils of elevated plasma plant sterols. In two well-performed studies, the Dallas Heart Study (3) and the European Prospective Investigation of Cancer–Norfolk study (13), plasma plant sterols were not adversely related to CAD. The results of the Longitudinal Aging Study Amsterdam suggesting beneficial effects of high plasma plant sterols were not adjusted for BMI (12).

Our study provides a large and reliable body of data on the relationships of cholesterol homeostasis and plasma plant sterols with CAD. In contrast to previous work, we measured noncholesterol sterols in 2,440 persons who all underwent coronary angiography (26). Hence, an exact classification of the coronary status was available.

The plasma concentrations of noncholesterol sterols were in agreement with data published previously (3, 13). Confounding due to the consumption of plant sterol or stanol ester margarines can be ruled out because patients were recruited before those functional foods were brought onto the market in Germany. We were able to confirm the reported associations of noncholesterol ratios with the use of statins (31), BMI (32), and type 2 diabetes (33). In opposition to the plant sterol ratios, the cholestanol ratio was only slightly elevated in users of statins compared with subjects who did not take lipid-lowering drugs. However, this observation has also been made in a subgroup of the Scandinavian Simvastatin Survival Study (34). Male gender was correlated with increased campesterol to cholesterol ratio in our study and a previous work (13).

In the entire cohort, high ratios of cholestanol and campesterol to cholesterol, and of absorption sterols to lathosterol were associated with an increased severity of coronary atherosclerosis. By contrast, the lathosterol to cholesterol ratio was decreased in subjects with high FS compared with individuals without CAD. The sitosterol to cholesterol ratio did not significantly correlate with the FS in the whole group but reached statistical significance in the subgroup of nondiabetics. These results were also confirmed by subgroup analysis in individuals with statins and in those without lipid-lowering drugs.

Data on the relationships of the *APOE* genotype with cholesterol absorption and synthesis are controversial (35, 36). Additional adjustment for *APOE* variants did not affect the associations of cholesterol homeostasis with the severity of CAD.

Our findings are in support of previous studies reporting high absorption and low synthesis of cholesterol to be associated with CAD. We think the repeatedly observed positive correlation of plasma plant sterols with CAD reflects atherogenic effects of increased intestinal cholesterol uptake.

The fact that plant sterols have been detected in atherosclerotic plaques does not conflict with the interpretation of our results. Of note, plant sterols obviously do not accumulate in plaques disproportionately to cholesterol (37, 38). Indeed, plant sterols may exert harmful effects when they are up to 100-fold elevated (5) but this apparently does not apply to variation within or close to their physiological range. The existence of very effective secretion pumps for plant sterols in the intestine and the liver underlines that these compounds are unwanted by the human body (6, 7). However, because of this efficient protective mechanism plant sterols are probably not able to promote atherogenesis in nonsitosterolaemic subjects.

The pathophysiological mechanisms underlying the associations between cholesterol homeostasis and CAD are not fully understood. With the use of statins, a reduction in coronary events can be achieved in subjects with hypercholesterolaemia (39). The well-established effectiveness of statin treatment in lowering plasma total and LDL cholesterol depends on baseline cholesterol metabolism. Statins suppress the synthesis of cholesterol considerably less efficiently in subjects with low synthesis and high absorption of cholesterol (31). In consequence, coronary events have not been reduced by the use of statins in those patients with high cholesterol absorption (40).

However, baseline cholesterol metabolism not only predicts the risk of future coronary events in patients under statin regimen, but also in those who do not take lipid-lowering drugs. It seems improbable that an increased absorption in particular of plant sterols promotes atherogenesis. More likely, a higher cholesterol lifetime burden, as suggested previously (16), might link detrimental changes in cholesterol homeostasis to CAD. In support of this hypothesis, polymorphisms in the *ABCG5* gene, which plays an important role in intestinal cholesterol uptake, were associated with the response to dietary cholesterol (41).

Evidence suggests that high intestinal cholesterol uptake might increase the risk of coronary atherosclerosis. Thus, subjects with increased cholesterol absorption, particularly those in whom total and LDL cholesterol goals are not reached by the use of statins alone, might have benefit from cholesterol absorption inhibitors. These are ezetimibe (42, 43) and plant stanol or sterol margarines (44–47). However, definite recommendations for the use of cholesterol absorption inhibitors in addition to statins will require large prospective studies with hard cardiovascular end points. To this date, these data are not available but clinical trials are ongoing. In a recently published study, however, carotid intima-media thickness surprisingly was not reduced by an ezetimibe plus simvastatin regimen compared with simvastatin alone (43). Yet, ezetimibe was found to inhibit the development of atherosclerosis in mice (48). Concerns about the safety of plant sterol margarines with regard to atherogenesis are not warranted (49). The physiological plasma plant sterol concentration is usually less than 1 mg per decilitre (3, 13). Due to the regular intake of plant sterol containing functional foods plasma plant sterol concentration is forced up by about the factor two only (50). However, patients with sitosterolaemia, who develop severe premature atherosclerosis, have up to 100-fold elevated plasma plant sterols (4, 5). In addition, the administration of plant sterols and stanols has been shown to be effective in the prevention of coronary atherosclerosis in animal models (51, 52) and to be associated with increased carotid artery compliance in a recent clinical study (53). Of note, a reduction in LDL cholesterol of approximately 10% can be achieved by the regular intake of plant sterol or stanol containing functional foods (44–47).

It is a limitation of our study that systematic information on the dietary habits of our subjects has not been available. To compensate for this drawback, we have additionally measured cholestanol, which is an absorption marker less dependent on vegetable food intake than plant sterols. In agreement with the campesterol to cholesterol ratio, increased cholestanol to cholesterol ratio was associated with high FS. Moreover, previous work has supported the view that even vegetarian lifestyle does not have major effects on the plasma plant sterol concentration (54). Furthermore, we cannot exclude the possibility that sample storage for up to 10 years may have affected plasma sterol concentrations. However, sterols stored at −80 degrees were proven stable in a set of samples over 10 years in a study published previously (12).

In conclusion, we provide evidence that high absorption and low synthesis of cholesterol is associated with a more severe CAD. Prospective clinical studies investigating if the use of cholesterol absorption inhibitors will reduce cardiovascular risk are needed.

The authors extend appreciation to the participants of the LURIC study; without their collaboration this article would not have been written. We thank the LURIC study team either temporarily or permanently involved in patient recruitment and sample and data handling; the laboratory staff at the Ludwigshafen General Hospital; and the Universities of Freiburg, Ulm, Tübingen, and Graz. Furthermore, we are grateful for expert advice by Prof. Dr. Brigitte M. Winklhofer-Roob and

for the support by the "GEN-AU project GOLD–Genomics Of Lipid-associated Disorders."

REFERENCES

1. Wilson, P. W. F., R. B. D'Agostino, D. Levy, A. M. Belanger, H. Silbershatz, and W. B. Kannel. 1998. Prediction of coronary heart disease using risk factor categories. *Circulation.* **97:** 1837–1847.
2. Ostlund, R., Jr. 2002. Phytosterols in human nutrition. *Annu. Rev. Nutr.* **22:** 533–549.
3. Wilund, K. R., L. Yu, F. Xu, G. Vega, S. Grundy, J. C. Cohen, and H. H. Hobbs. 2004. Plant sterol levels are not associated with atherosclerosis in mice and men. *Arterioscler. Thromb. Vasc. Biol.* **24:** 2326–2332.
4. Bhattacharyya, A., and W. E. Connor. 1974. β-Sitosterolaemia and xanthomatosis. A newly described lipid storage disease in two sisters. *J. Clin. Invest.* **53:** 1033–1043.
5. Salen, G., I. Horak, M. Rothkopf, J. L. Cohen, J. Speck, G. S. Tint, V. Shore, B. Dayal, T. Chen, and S. Shefer. 1985. Lethal atherosclerosis associated with abnormal plasma and tissue sterol composition in sitosterolaemia with xanthomatosis. *J. Lipid Res.* **26:** 1126–1133.
6. Berge, K. E., H. Tian, G. A. Graf, L. YU, N. V. Grishin, J. Schultz, P. Kwiterovich, B. Shan, R. Barnes, and H. H. Hobbs. 2000. Accumulation of dietary cholesterol in sitosterolaemia caused by mutations in adjacent ABC transporters. *Science.* **290:** 1771–1775.
7. Lee, M. H., K. Lu, S. Hazard, H. Yu, S. Shulenin, H. Hidaka, H. Kojima, R. Allikmets, N. Sakuma, R. Pegoraro, et al. 2001. Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. *Nat. Genet.* **27:** 79–83.
8. Glueck, C. J., J. Speirs, T. Tracy, P. Streicher, E. Illig, and J. Vandegrift. 1991. Relationships of serum plant sterols (phytosterols) and cholesterol in 595 hypercholesterolemic subjects, and familial aggregation of phytosterols, cholesterol and premature coronary heart disease in hyperphytosterolemic probands and their first degree relatives. *Metabolism.* **40:** 842–848.
9. Sutherland, W. H. F., M. J. A. Williams, E. R. Nye, N. J. Restieaux, S. A. de Jong, and H. L. Walker. 1998. Associations of plasma noncholesterol sterol levels with severity of coronary artery disease. *Nutr. Metab. Cardiovasc. Dis.* **8:** 386–391.
10. Sudhop, T., B. M. Gottwald, and K. von Bergmann. 2002. Serum plant sterols as a potential risk factor for coronary heart disease. *Metabolism.* **51:** 1519–1521.
11. Assmann, G., P. Cullen, J. Erbey, D. R. Ramey, F. Kannenberg, and H. Schulte. 2006. Plasma sitosterol elevations are associated with an increased incidence of coronary events in men: results of a nested case-control analysis of the Prospective Cardiovascular Munster (PROCAM) study. *Nutr. Metab. Cardiovasc. Dis.* **16:** 13–21.
12. Fassbender, K., D. Lutjohann, M. G. Dik, M. Bremmer, J. Koenig, S. Walter, Y. Liu, M. Letiembre, K. von Bergmann, and C. Jonker. 2008. Moderately elevated plant sterol levels are associated with reduced cardiovascular risk-The LASA study. *Atherosclerosis.* **196:** 283–288.
13. Pinedo, S., M. N. Vissers, K. von Bergmann, M. D. Elharchaoui, D. Lutjohann, R. Luben, N. J. Wareham, J. J. P. Kastelein, K. T. Khaw, and S. M. Boeckholdt. 2007. Plasma levels of plant sterols and the risk of future coronary artery disease in apparently healthy men and women: The Prospective Epic-Norfolk Population Study. *J. Lipid Res.* **48:** 139–144.
14. Rajaratnam, R. A., H. Gylling, and T. A. Miettinen. 2000. Independent association of serum squalene and noncholesterol sterols with coronary artery disease in postmenopausal women. *J. Am. Coll. Cardiol.* **35:** 1185–1191.
15. Matthan, N. R., J. M. LaRoque, M. Pencina, R. B. D'Agostino, E. J. Schaefer, and A. H. Lichtenstein. 2005. Increased cholesterol absorption and decreased cholesterol synthesis characterize Framingham Offspring study participants with coronary heart disease. *Circulation.* **112:** II-816–II-817 (abstract).
16. Strandberg, T. E., R. S. Tilvis, K. H. Pitkala, and T. A. Miettinen. 2006. Cholesterol and glucose metabolism and recurrent cardiovascular events among elderly. *J. Am. Coll. Cardiol.* **48:** 708–714.
17. Weingärtner, O., D. Lütjohann, S. Ji, N. Weisshoff, F. List, T. Sudhop, K. von Bergmann, K. Gertz, J. König, H. J. Schäfers, et al. 2008. Vascular effects of diet supplementation with plant sterols. *J. Am. Coll. Cardiol.* **51:** 1553–1561.
18. Miettinen, T. A., R. S. Tilvis, and Y. A. Kesämiemi. 1990. Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. *Am. J. Epidemiol.* **13:** 20–31.
19. Miettinen, T. A., R. S. Tilvis, and Y. A. Kesaniemi. 1989. Serum cholestanol and plant sterol levels in relation to cholesterol metabolism in middle-aged men. *Metabolism.* **38:** 136–140.
20. Gylling, H. 2004. Cholesterol metabolism and its implications for therapeutic interventions in patients with hypercholesterolaemia. *Int. J. Clin. Pract.* **58:** 859–866.
21. Altmann, S. W., H. R. Davis, Jr., L. J. Zhu, X. Yao, L. M. Hoos, G. Tetzloff, S. P. Iyer, M. Maguire, A. Golovko, M. Zeng, et al. 2004. Niemann-Pick C1 like 1 protein is critical for intestinal cholesterol absorption. *Science.* **303:** 1424–1431.
22. Gylling, H., M. Hallikainen, J. Pihlajamäki, J. Agren, M. Laakso, R. A. Rajaratnam, R. Rauramaa, and T. A. Miettinen. 2004. Polymorphisms in the ABCG5 and ABCG8 genes associate with cholesterol absorption and insulin sensitivity. *J. Lipid Res.* **45:** 1660–1665.
23. Berge, K. E., K. von Bergmann, D. Lutjohann, R. Guerra, S. M. Grundy, H. H. Hobbs, and J. C. Cohen. 2002. Heritability of plasma noncholesterol sterols and relationship to DNA sequence polymorphism in ABCG5 and ABCG8. *J. Lipid Res.* **43:** 486–494.
24. Cohen, J. C., A. Pertsemlidis, S. Fahmi, S. Esmail, G. L. Vega, S. M. Grundy, and H. Hobbs. 2006. Multiple rare variants in NPC1L1 associated with reduced sterol absorption and plasma low-density lipoprotein levels. *Proc. Natl. Acad. Sci. USA.* **103:** 1810–1815.
25. Matthan, N. R., and A. H. Lichtenstein. 2004. Approaches to measuring cholesterol absorption in humans. *Atherosclerosis.* **174:** 197–205.
26. Winkelmann, B. R., W. Marz, B. O. Boehm, R. Zotz, J. Hager, P. Hellstern, and J. Senges. LURIC Study Group (Ludwigshafen Risk and Cardiovascular Health). 2000. Rationale and design of the LURIC study - a resource for functional genomics, pharmacogenomics and long-term prognosis of cardiovascular disease. *Pharmacogenomics.* **2 (1, Suppl 1):** S1–S73.
27. Friesinger, G. C., E. E. Page, and R. S. Ross. 1970. Prognostic significance of coronary arteriography. *Trans. Assoc. Am. Physicians.* **83:** 78–92.
28. American Diabetes Association. 2006. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* **29 (Suppl 1):** S43–S48.
29. Stojakovic, T., C. Putz-Bankuti, G. Fauler, H. Scharnagl, M. Wagner, V. Stadlbauer, G. Gurakuqi, R. E. Stauber, W. März, and M. Trauner. 2007. Atorvastatin in patients with primary biliary cirrhosis and incomplete biochemical response to ursodeoxycholic acid. *Hepatology.* **46:** 776–784.
30. Zivelin, A., N. Rosenberg, H. Peretz, Y. Amit, N. Kornbrot, and U. Seligsohn. 1997. Improved method for genotyping apolipoprotein E polymorphisms by a PCR-based assay simultaneously utilizing two distinct restriction enzymes. *Clin. Chem.* **43:** 1657–1659.
31. Miettinen, T. A., T. E. Strandberg, and H. Gylling. 2000. Noncholesterol sterols and cholesterol lowering by long-term simvastatin treatment in coronary patients. *Arterioscler. Thromb. Vasc. Biol.* **20:** 1340–1346.
32. Simonen, P., H. Gylling, A. N. Howard, and T. A. Miettinen. 2000. Introducing a new component of the metabolic syndrome: low cholesterol absorption. *Am. J. Clin. Nutr.* **72:** 82–88.
33. Gylling, H., and T. A. Miettinen. 1997. Cholesterol absorption, synthesis, and LDL metabolism in NIDDM. *Diabetes Care.* **20:** 90–95.
34. Miettinen, T. A., and H. Gylling. 2007. Blood glucose and metabolism of cholesterol in coronary patients with and without simvastatin treatment. *Clin. Chim. Acta.* **379:** 53–58.
35. Kesäniemi, Y. A., C. Ehnholm, and T. A. Miettinen. 1987. Intestinal cholesterol absorption efficiency in man is related to apoprotein E phenotype. *J. Clin. Invest.* **80:** 578–581.
36. von Bergmann, K., D. Lütjohann, B. Lindenthal, and A. Steinmetz. 2003. Efficiency of intestinal cholesterol absorption in humans is not related to apoE phenotype. *J. Lipid Res.* **44:** 193–197.
37. Mellies, M. J., T. T. Ishikawa, C. J. Glueck, K. Bove, and J. Morrison. 1976. Phytosterols in aortic tissue in adults and infants. *J. Lab. Clin. Med.* **88:** 914–921.
38. Miettinen, T. A., M. Railo, M. Lepäntalo, and H. Gylling. 2005. Plant sterols in serum and in atherosclerotic plaques of patients undergoing carotid endarterectomy. *J. Am. Coll. Cardiol.* **45:** 1794–1801.
39. Shepherd, J., S. M. Cobbe, I. Ford, C. G. Isles, A. R. Lorimer, P. W. MacFarlane, J. H. McKillop, and C. J. Packard. 1995. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N. Engl. J. Med.* **333:** 1301–1307.

40. Miettinen, T. A., H. Gylling, T. Strandberg, and S. Sarna. 1998. Baseline serum cholestanol as predictor of recurrent coronary events in subgroup of Scandinavian simvastatin survival study. *BMJ.* **316:** 1127–1130.
41. Herron, K. L., M. M. McGrane, D. Waters, I. E. Lofgren, R. M. Clark, J. M. Ordovas, and M. L. Fernandez. 2006. The ABCG5 polymorphism contributes to individual responses to dietary cholesterol and carotenoids in eggs. *J. Nutr.* **136:** 1161–1165.
42. Sudhop, T., D. Lutjohann, A. Kodal, M. Igel, D. L. Tribble, S. Shah, I. Perevozskaya, and K. von Bergmann. 2002. Inhibition of intestinal cholesterol absorption by ezetimibe in humans. *Circulation.* **106:** 1943–1948.
43. Kastelein, J. J., F. Akdim, E. S. Stroes, A. H. Zwinderman, M. L. Bots, A. F. Stalenhoef, F. L. Visseren, E. J. Sijbrands, M. D. Trip, E. A. Stein, et al. 2008. Simvastatin with or without ezetimibe in familial hypercholesterolemia. *N. Engl. J. Med.* **358:** 1431–1443.
44. Law, M. 2000. Plant sterol and stanol margarines and health. *BMJ.* **320:** 861–864.
45. Miettinen, T. A., P. Puska, H. Gylling, H. Vanhanen, and E. Vartiainen. 1995. Serum cholesterol lowering by sitostanol ester margarine in a mildly hypercholesterolemic random population. *N. Engl. J. Med.* **333:** 1308–1312.
46. Weststrate, J. A., and G. W. Meijer. 1998. Plant sterol-enriched margarines and reduction of plasma total- and LDL-cholesterol concentrations in normocholesterolaemic and mildly hypercholesterolaemic subjects. *Eur. J. Clin. Nutr.* **52:** 334–343.
47. Plat, J., and R. P. Mensink. 2005. Plant stanol and sterol esters in the control of blood cholesterol levels: mechanism and safety aspects. *Am. J. Cardiol.* **96 (1A):** 15D–22D.
48. Davis, H. R., Jr., D. S. Compton, L. Hoos, and G. Tetzloff. 2001. Ezetimibe, a potent cholesterol absorption inhibitor, inhibits the development of atherosclerosis in apoE knockout mice. *Arterioscler. Thromb. Vasc. Biol.* **21:** 2032–2038.
49. Jones, P. J. 2007. Ingestion of phytosterols is not potentially hazardous. *J. Nutr.* **137:** 2485.
50. Fransen, H. P., N. de Jong, M. Wolfs, H. Verhagen, W. M. Verschuren, D. Lütjohann, K. von Bergmann, J. Plat, and R. P. Mensink. 2007. Customary use of plant sterol and plant stanol enriched margarine is associated with changes in serum plant sterol and stanol concentrations in humans. *J. Nutr.* **137:** 1301–1306.
51. Moghadasian, M. H., B. M. Mc Manus, D. V. Godin, B. Rodrigues, and J. J. Frolich. 1999. Proatherogenic and antiatherogenic effects of probucol and phytosterols in apolipoprotein E-deficient mice: possible mechanisms of action. *Circulation.* **99:** 1733–1739.
52. Plat, J., I. Beugels, M. J. Gijbels, M. P. de Winter, and R. P. Mensink. 2006. Plant sterol or stanol esters retard lesion formation in LDL receptor-deficient mice independent of changes in serum plant sterols. *J. Lipid Res.* **47:** 2762–2771.
53. Raitakari, O. T., P. Salo, and M. Ahotupa. 2008. Carotid artery compliance in users of plant stanol ester margarine. *Eur. J. Clin. Nutr.* **62:** 218–224.
54. Clarenbach, J. J., M. Reber, D. Lütjohann, K. von Bergmann, and T. Sudhop. 2006. The lipid-lowering effect of ezetimibe in pure vegetarians. *J. Lipid Res.* **47:** 2820–2824.