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Cardiometabolic Risk

High Intestinal Cholesterol Absorption Is Associated With Cardiovascular Disease and Risk Alleles in *ABCG8* and *ABO*

Evidence From the LURIC and YFS Cohorts and From a Meta-Analysis

Günther Silbernagel, MD,* M. John Chapman, PhD, DSc,† Bernd Genser, PhD,‡§ Marcus E. Kleber, PhD,*‡‖ Günter Fauler, PhD,¶ Hubert Scharnagl, PhD,¶ Tanja B. Grammer, MD,‡ Bernhard O. Boehm, MD,# Kari-Matti Mäkelä, BM,** Mika Kähönen, MD,†† Rafael Carmena, MD,‡‡ Ernst R. Rietzschel, MD, PhD,§§‖‖ Eric Bruckert, MD,¶¶ John E. Deanfield, MD, PhD,## Tatu A. Miettinen, MD, PhD,*** Olli T. Raitakari, MD, PhD,†††‡‡‡ Terho Lehtimäki, MD, PhD,** Winfried März, MD‡¶§§§

Bern, Switzerland; Heidelberg, Ulm, and Mannheim, Germany; Paris, France; Salvador, Brazil; Graz, Austria; Tampere, Helsinki, and Turku, Finland; Valencia, Spain; Ghent, Belgium; and London, United Kingdom

Objectives This study sought to determine whether high intestinal cholesterol absorption represents a cardiovascular risk factor and to link *ABCG8* and *ABO* variants to cardiovascular disease (CVD).

Background Plant sterol–enriched functional foods are widely used for cholesterol lowering. Their regular intake yields a 2-fold increase in circulating plant sterol levels that equally represent markers of cholesterol absorption. Variants in *ABCG8* and *ABO* have been associated with circulating plant sterol levels and CVD, thereby suggesting atherogenic effects of plant sterols or of cholesterol uptake.

Methods The cholestanol-to-cholesterol ratio (CR) was used as an estimate of cholesterol absorption because it is independent of plant sterols. First, we investigated the associations of 6 single nucleotide polymorphisms in *ABCG8* and *ABO* with CR in the LURIC (LUdwisghafen RIsk and Cardiovascular health study) and the YFS (Young Finns Study) cohorts. Second, we conducted a systematic review and meta-analysis to investigate whether CR might be related to CVD.

Results In LURIC, the minor alleles of *rs4245791* and *rs4299376* and the major alleles of *rs41360247*, *rs6576629*, and *rs4953023* of the *ABCG8* gene and the minor allele of *rs657152* of the *ABO* gene were significantly associated with higher CR. Consistent results were obtained for *rs4245791*, *rs4299376*, *rs6576629*, and *rs4953023* in YFS. The meta-analysis, including 6 studies and 4,362 individuals, found that CR was significantly increased in individuals with CVD.

Conclusions High cholesterol absorption is associated with risk alleles in *ABCG8* and *ABO* and with CVD. Harm caused by elevated cholesterol absorption rather than by plant sterols may therefore mediate the relationships of *ABCG8* and *ABO* variants with CVD. (J Am Coll Cardiol 2013;62:291–9) © 2013 by the American College of Cardiology Foundation

From the *Department of Angiology, Swiss Cardiovascular Center, University of Bern, Bern, Switzerland; †Dyslipidemia, Inflammation and Atherosclerosis Research Unit, National Institute for Health and Medical Research, Hôpital de la Pitié-Salpetriere, Paris, France; ‡Mannheim Institute of Public Health, Social and Preventive Medicine, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany; §Instituto de Saúde Coletiva, Federal University of Bahia, Salvador, Brazil; ‖Division of Cardiology, Department of Internal Medicine, Ulm University, Ulm, Germany; ¶Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria; #Division of Endocrinology, Department of Internal Medicine, Ulm University, Ulm, Germany; **Department of Clinical Chemistry, Fimlab Laboratories, Tampere University Hospital and University of Tampere, Tampere, Finland; ††Department of Clinical Physiology, Tampere University Hospital and University of Tampere, Tampere, Finland; ‡‡Division of Endocrinology and Nutrition, Department of Medicine, University of Valencia, Valencia, Spain; §§Department of Cardiovascular Diseases, Ghent University Hospital, Ghent, Belgium; ‖‖Department of Public Health, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium; ¶¶Division of Endocrinology and Metabolism, Hôpital de la Pitié-Salpétrière, Paris, France; ##Vascular Physiology

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Abbreviations and Acronyms

CI = confidence interval

CVD = cardiovascular disease

GWA = genome-wide association

LDL = low-density lipoprotein

RR = risk ratio

SMD = standardized mean difference

SNP = single nucleotide polymorphism

The American Heart Association has recommended plant sterol–enriched foods (e.g., margarines) for cholesterol lowering (1). Moreover, the European Heart Association and the European Atherosclerosis Society have mentioned plant sterol-enriched functional foods as cholesterol lowering agents in their guidelines for the management of dyslipidemia (2). The regular intake of plant sterols reduces low-density lipoprotein (LDL) cholesterol by about 13 mg/dl (3) but also raises circulating plant sterols from about 1 mg/dl (4) by approximately 2-fold (5). Patients with sitosterolemia, a rare genetic disorder caused by mutations in the ATP-binding cassette transporters G5 and G8 (ABCG5 and ABCG8) (6), have up to 50-fold increased circulating plant sterols and may develop early onset cardiovascular disease (CVD) (7). Hence, it has been suggested that plant sterols are atherogenic (8–10). These concerns have been reinforced by the detection of plant sterols in carotid atherosclerotic plaques (11). In addition, plant sterol intake was related to increased plant sterol content in aortic valve cusps (12). Two recent studies have confirmed that consumption of plant sterols as part of a dietary portfolio and as an adjunct to treatment with ezetimibe has favorable effects on the lipid profile (13,14). Nevertheless, in continuance of the long-lasting safety discussion, these studies also garnered critical comments (15,16). The debate was further fueled by a genome-wide association (GWA) study showing that common variants in the *ABCG8* (major allele of *rs41360247* and minor allele of *rs4245791*) and *ABO* (minor allele of *rs657152*) genes increased both circulating plant sterols and cardiovascular risk (17). Correlations of variants in *ABCG8* (minor allele of *rs4299376*) and *ABO* with CVD subsequently have been replicated in other large-scale GWA studies (18–20). However, because circulating plant sterols are markers for cholesterol uptake (21), the genetic data may also indicate adverse vascular effects of high cholesterol absorption.

The current study consisted of a genetic analysis and a meta-analysis; its purpose was to investigate whether high intestinal cholesterol absorption represents a cardiovascular risk factor and to link *ABCG8* and *AB0* variants to CVD. We used the ratio of circulating cholestanol-to-cholesterol to estimate levels of intestinal cholesterol absorption independently of plant sterol concentrations (22).

Methods

Genetic analyses. STUDY DESIGN AND PARTICIPANTS. Genetic association studies were performed in the LURIC (LUdwigshafen RIsk and Cardiovascular health study) and the YFS (Young Finns Study) (23,24).

LURIC is a cross-sectional and prospective German cohort study designed to investigate biochemical and genetic cardiovascular risk factors. A total of 3,316 participants referred for coronary angiography were recruited between July 1997 and January 2000 at the Ludwigshafen Heart Center (23). Measurements of lathosterol, cholestanol, campesterol, and sitosterol were completed in 1,257 LURIC participants who did not receive statins and did not have type 1 diabetes (25). Individuals in this subgroup with available data on *ABCG8* or *ABO* single nucleotide polymorphisms (SNPs) were included in the current analyses.

YFS is a Finnish population-based, 27-year follow-up study on the evolution of cardiovascular risk factors from childhood to adulthood (24). The first cross-sectional study was conducted in 1980 at 5 centers and included 3,596 participants in the age groups of 3, 6, 9, 12, 15, and 18 years who were randomly chosen from the national population registry. In 2001, a total of 2,620 individuals, who were then aged 24 to 39 years, were studied. The sterol and lipid determinations used in the current analysis were taken from the year 2001 participants. Sterol and genetic data were available in 434 subjects.

Both studies were approved by the local ethical committees and performed according to the Declaration of Helsinki. Informed written consent was obtained from all participants (23,24). Diabetes mellitus was categorized according to the 2009 criteria of the American Diabetes Association (26).

LABORATORY ANALYSES. All laboratory measurements were performed on fasting blood samples. In LURIC, cholesterol was measured with enzymatic reagents from WAKO (Neuss, Germany) on a WAKO 30 R or Olympus AU640 analyzer (Tokyo, Japan) (23). Lipoproteins were separated by a combined ultracentrifugation precipitation method (beta-quantification).

Unit, Institute of Cardiovascular Sciences, University College London, London, United Kingdom; ***Division of Internal Medicine, Department of Medicine, University of Helsinki, Biomedicum Helsinki, Helsinki, Finland; †††Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland; ‡‡‡Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital, Turku, Finland; and the §§§Synlab Academy, Mannheim, Germany. Dr. Miettinen passed away before the completion of the work. LURIC has received funding through the 6th Framework Program (integrated project Bloodomics, grant LSHM-CT-2004-503485) and 7th of Framework Program (integrated project AtheroRemo, Grant Agreement number 201668) of the European Union. YFS has been financially supported by the Academy of Finland grants 126925, 121584, 124282, 129378 (Salve), 117787 (Gendi), and 41071 (Skidi); the Social Insurance Institution of Finland; the Kuopio, Tampere and Turku University Hospital Medical Funds (grant 9M048 and 9N035) (Dr. Lehtimäki); the Juho Vainio Foundation; the Paavo Nurmi Foundation; the Finnish Foundation of Cardiovascular Research; the Finnish Cultural Foundation; the Tampere Tuberculosis Foundation; the Emil Aaltonen Foundation (Dr. Lehtimäki); and the 7th Framework Program (integrated project AtheroRemo, Grant Agreement number 201668) of the European Union. This study has been supported by grants from Danone Research. Dr. Silbernagel received a research grant from Unilever. Dr. Chapman has received research funding from Pfizer; Kowa, and Merck and has served on the advisory boards of Merck, Kowa, Amgen, Danone, and Roche. Dr. Genser has received a research grant and lecture fees from Danone Research. Dr. Bruckert has received honoraria for meetings or participation on advisory boards from Merck, Pfizer, Astra Zeneca, Unilever, Danone, Kraft, sanofi-avantis, MSD-Schering Plough, Servier, Genfit, and Aegerion. Dr. März has received lecture fees, grants, and consulting honoraria from Danone Research and a research grant from Unilever. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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Apolipoproteins B and E were measured by turbidimetry with reagents from Greiner (Flacht, Germany). Cholestanol (not a plant sterol; an intestinal absorption marker), campesterol and sitosterol (the 2 most abundant plant sterols; absorption markers), and lathosterol (cholesterol precursor; synthesis marker) were measured by using gas chromatography and mass spectrometry (25).

In YFS, total and high-density lipoprotein cholesterol were measured with enzymatic reagents from Olympus and Roche Diagnostics (Mannheim, Germany), respectively, on an Olympus AU400 analyzer. LDL cholesterol was calculated according to the Friedewald formula (27). Apolipoprotein B was analyzed by using turbidimetry with reagents for Orion Diagnostica (Espoo, Finland). Noncholesterol sterols were measured with gas-liquid chromatography (28).

GENOTYPING. Genomic DNA was prepared from peripheral blood in both cohorts. In LURIC, microarrays (Affymetrix 500k, Affymetrix 6.0, Illumina IBC 50k Cardiochip, and Illumina 200k Metabochip) were used to genotype the *ABCG8* and *ABO* SNPs. In YFS, genotyping was performed by using the custom-built Illumina Human 670k BeadChip. SNPs were excluded in case of a low genotyping call rate (<0.95), Hardy-Weinberg-Equilibrium p value $<10^{-6}$, minor allele frequency <0.01, heterozygosity, Sequenom fingerprint discrepancy, duplicated samples, or possible relatedness (pi-hat > 0.2). *Rs4245791* (n = 1,212), *rs4299376* (n = 1,251), *rs41360247* (n = 1,157), *rs6576629* (n = 1,151), and *rs4953023* (n = 1,251) in the *ABCG8* gene and *rs657152* (n = 1,202) in the *ABO* gene were available in LURIC. *Rs4245791* (n = 433), *rs4299376* (n = 434), *rs6576629* (n = 434), *rs4953023* (n = 434), and *rs657152* (n = 434) were available in YFS.

STATISTICAL ANALYSIS. Hardy-Weinberg equilibrium was examined with the chi-square test. R^2 was calculated by using the Web-tool according to Gaunt et al. (29) in both cohorts. We examined the distribution of the baseline clinical and biochemical characteristics across variants of the *ABCG8* and *ABO* genes. Categorical data are presented as counts and percentages of subjects in each genotype group. Continuous data are presented as means ± SDs or medians with interquartile ranges. We used analysis of variance for continuous variables and chi-square tests for categorical variables to compare the distributions of variables across the genotypes. Additive genetic models were used for *rs4245791*, *rs4299376*, and *rs657152*. Because minor allele frequencies were <10% (resulting in very few study participants who were homozygous for the minor allele), we used dominant models for *rs41360247*, *rs6576629*, and *rs4953023*. We calculated ratios of the noncholesterol sterols to cholesterol to standardize for variation in cholesterol. Moreover, ratios of the absorption markers to lathosterol were computed to compare cholesterol absorption with de novo cholesterol synthesis. Data that were not normally distributed were transformed logarithmically. Analyzing data on 3 nonlinked groups of SNPs, we applied Dunn-Šidák correction for 3 independent tests. We did not correct for the tested traits of interest because they were not independent.

All statistical tests were 2-sided. Thus, p values <0.01695 were considered statistically significant. SPSS version 19.0 (IBM SPSS Statistics, IBM Corporation, Armonk, New York) and R version 2.11.1 (R Foundation for Statistical Computing, Vienna, Austria) statistical software packages were used in LURIC and YFS, respectively.

Meta-analysis of cholestanol and CVD. DATA SOURCES, SEARCH STRATEGY, AND SELECTION CRITERIA. We systematically reviewed the published literature according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) statement for the conduct of meta-analyses of epidemiological studies (30). Relevant studies were identified by searching in MEDLINE via PubMed (from 1950 to January 2012). Search terms including key words and headings were ("*cholestanol*") and ("*angiography*" or "*atherosclerosis*" or "*atherosclerotic plaque*" or "*CVD*" or "*cardiovascular mortality*" or "*cardiovascular event*" or "*cardiovascular risk*" or "*cerebrovascular disease*" or "*coronary artery disease*" or "*coronary calcium*" or "*coronary heart disease*" or "mortality" or "*myocardial infarction*" or "*peripheral arterial disease*" or "*stenosis*" or "*stroke*", or "*vascular*"). We included studies with different research designs that reported on the association of absolute cholestanol concentration and/or cholestanol-to-cholesterol ratio with CVD. Because few studies were eligible and because the authors used heterogeneous endpoints related to CVD, we defined a composite primary endpoint. The endpoint comprised different definitions of CVD, including angiographically verified CVD, myocardial infarction, ≥50% carotid stenosis (verified by using Doppler analysis), coronary insufficiency, angina pectoris, cerebrovascular accident, transient ischemic attack, major cardiovascular events, and cardiovascular mortality.

DATA EXTRACTION. For each study identified, we obtained the original publications and extracted the following data into a spreadsheet: author, year of publication, country of origin, research design, major characteristics of the study population, descriptive statistics of cholestanol concentrations and/or cholestanol-to-cholesterol ratios (if provided for CVD cases and controls separately) or risk ratios (RRs) with respect to CVD based on cholestanol concentrations and/or cholestanol-to-cholesterol ratios (odds ratios, incidence rate ratios, or hazard ratios), the statistical method used for analysis, and the covariates adjusted included in multivariate modeling. We transformed absolute sterol concentrations into micrograms per deciliter and ratios to cholesterol into micrograms per milligram. For studies that reported circulating cholestanol and/or cholestanol to cholesterol ratio in CVD cases and controls separately, we calculated standardized mean differences (SMDs). For studies that reported RRs, we transformed the RRs to compare individuals in the top one-third versus those in the bottom one-third of the sterol distribution, as previously described (31). If authors

reported several estimates of RRs derived from multiple models using different sets of adjusting variables, we considered the estimate adjusted for most potential confounding variables (that have been collected in the particular study) as the gold standard estimate.

STATISTICAL ANALYSIS. We conducted two meta-analyses based on: 1) SMDs between CVD cases and controls; and 2) RRs. We examined for potential publication bias by using Egger's tests and Begg's tests as well as funnel plots. I^2 statistics and chi-square tests were used to investigate whether there was heterogeneity of estimates among studies. We calculated pooled summary estimates by using fixed effects (Mantel-Haenszel method) and random effects (DerSimonian and Laird model) meta-analysis techniques (32,33). Forest plots were used for visualizing study-specific estimates and the pooled fixed and random effects estimators. Statistical hypothesis testing was conducted 2-sided, and p values <0.05 were considered significant. Stata version 12.0 (Stata Corp., College Station, Texas) was used for analysis.

Results

Genetic studies. All SNPs were in Hardy-Weinberg-Equilibrium in LURIC and YFS (all $p > 0.15$). The minor allele frequencies were 0.33 and 0.22 for *rs4245791*, 0.33 and 0.22 for *rs4299376*, 0.06 for *rs41360247* (not available in YFS), 0.06 and 0.08 for *rs6576629*, 0.06 and 0.08 for *rs4953023*, and 0.40 and 0.44 for *rs657152* in LURIC and YFS, respectively. *Rs4245791* and *rs4299376* showed high r^2 in both cohorts. Furthermore, *rs41360247*, *rs6576629,* and *rs4953023* were strongly correlated (Online Tables 1 and 2).

Sex, age, body mass index, and type 2 diabetes were not significantly related to the *ABCG8* and *ABO* variants in either the LURIC or the YFS cohort (Tables 1 to 3; Online Tables 3 to 5).

In LURIC, the major alleles of *rs41360247* (Online Table 3), *rs6576629* (Table 2), and *rs4953023* (Online Table 5) were associated with increased total cholesterol. There was also a trend toward an association between the minor allele of *rs4245791* (Table 1) and increased total cholesterol. Furthermore, we observed a tendency toward higher LDL cholesterol in carriers of the minor alleles of *rs4245791* (Table 1) and *rs4299376* (Online Table 4) and of the major allele of *rs6576629* (Table 2). The major alleles of *rs41360247* (Online Table 3), *rs6576629* (Table 2), and *rs4953023* (Online Table 5) were associated with increased apolipoprotein B and E. A trend toward an association with apolipoprotein B was observed for *rs4245791* (Table 1).

The minor alleles of *rs4245791* (Table 1) and *rs4299376* (Online Table 4) and the major alleles of *rs41360247* (Online Table 3), *rs6576629* (Table 2), and *rs4953023* (Online Table 5) were related to elevated levels of cholestanol, campesterol, and sitosterol; high absorption marker to cholesterol ratios; and high absorption marker to lathosterol ratios. The minor allele of *rs657152* (Table 3) was associated with high circulating campesterol and a high ratio of cholestanol-to-cholesterol. A decrease in circulating lathosterol and the lathosterol to cholesterol ratio was observed for the minor alleles of *rs4245791* (Table 1) and *rs4299376* (Online Table 4). There was a trend toward a lower lathosterol to cholesterol ratio in carriers of the major alleles of *rs6576629* (Table 2) and *rs4953023* (Online Table 5).

In YFS, there was a trend for an association between the minor allele of *rs657152* (Table 3) and increased LDL cholesterol concentration. The minor alleles of *rs4245791* (Table 1) and *rs4299376* (Online Table 4) and the major alleles of *rs6576629* (Table 2) and *rs4953023* (Online Table 5) were associated with elevated cholestanol, campesterol, and sitosterol levels and high absorption marker to cholesterol ratios. In addition, *rs4245791* (Table 1) and *rs4299376* (Online Table 4) were associated with all ratios of the absorption markers to lathosterol. Likewise, *rs6576629* (Table 2) and *rs4953023* (Online Table 5) were associated with the campesterol and sitosterol to lathosterol ratios.

Meta-analysis. Based on our search terms, we found 84 abstracts in MEDLINE; 9 publications were selected to be reviewed in full text (4,25,34–40). Among these, 2 studies were excluded because of imprecise definitions of CVD (e.g., family history of coronary artery disease [35] and carotid intima media thickness [39]), 1 study was excluded because the cross-sectional data on the relationship between cholestanol and CVD had been retrieved from another publication in the same cohort (34,38), and 1 study was excluded because neither data for calculating SMDs nor RRs were presented (40). We included another study that did not show up in MEDLINE using our search term but was added based on personal knowledge (41). Thus, 6 studies (1 case–control study, 3 cohort studies, 1 cross sectional study, and 1 nested case–control study) with a total of 4,362 participants were included in the analyses. The studies had a sample size ranging from 109 to 2,440, and the participants ranged in age from 53.4 to 80.1 years. We used the fixed effects estimator because there was no significant heterogeneity among the studies.

Only 1 study reported on the relationship between uncorrected circulating cholestanol levels in CVD cases and controls, and it showed no association. The SMD in circulating cholestanol between cases and controls was 25 (95% confidence interval [CI]: −0.353 to 0.404; $p = 0.895$) (34). No study was retrieved that reported on RRs with regard to CVD based on uncorrected circulating cholestanol. Four studies were retrieved reporting on cholestanol-to-cholesterol ratios in CVD cases and controls separately (4,34,37,40). Among these, 3 reported higher cholestanol-to-cholesterol ratios in cases than in controls (4,37,40), and 1 showed no difference (34). There was neither publication bias ($p = 0.774$) (Online Fig. 1) nor study heterogeneity ($p = 0.535$). The pooled SMD between CVD cases and controls was 0.17 (95% CI: 0.09 to 0.25; $p < 0.001$) (Fig. 1). Two studies reported on RRs with regard to CVD based on

Table 1 ***Rs4245791*** **Frequency in the** ***ABCG8*** **Gene and Anthropometric Parameters and Plasma Lipids and Sterol Levels in the LURIC and YFS Cohorts**

Alleles	TT	TC	CC	p Value*
LURIC				
No. of subjects	549	527	136	–
Male	366 ± 66.7	351 ± 66.6	88 ± 64.7	0.904
Age (yrs)	62.2 ± 11.1	63.0 ± 11.0	63.7 ± 10.8	0.293
Body mass index (kg/m^2)	27.3 ± 3.9	27.2 ± 4.3	27.3 ± 4.2	0.929
Type 2 diabetes	167 ± 30.4	152 ± 28.8	41 ± 30.1	0.846
Total cholesterol (mg/dl)	199 ± 37	203 ± 35	206 ± 38	0.046
LDL cholesterol (mg/dl)	121 ± 31	124 ± 32	129 ± 32	0.020
HDL cholesterol (mg/dl)	40 ± 11	40 ± 11	42 ± 12	0.144
Apolipoprotein B (mg/dl)	106 ± 22	110 ± 24	109 ± 23	0.018
Apolipoprotein E (mg/dl)	9.2 ± 3.4	9.4 ± 3.1	9.1 ± 2.8	0.629
Campesterol (μg/dl)	223 (152–309)	277 (189–394)	348 (243–483)	<0.001
Sitosterol (μg/dl)	122 (86–167)	145 (103–212)	176 (124–251)	<0.001
Cholestanol (μg/dl)	256 (205–325)	281 (226–355)	314 (246–390)	<0.001
Lathosterol (μg/dl)	315 (214–456)	300 (210–432)	267 (195–414)	0.034
Campesterol/cholesterol (μg/mg)	1.13 (0.81–1.52)	1.36 (0.98–1.90)	1.66 (1.25–2.29)	<0.001
Sitosterol/cholesterol (μg/mg)	0.63 (0.46–0.82)	0.73 (0.52–1.02)	0.87 (0.67–1.19)	<0.001
Cholestanol/cholesterol (μg/mg)	1.31 (1.06–1.58)	1.40 (1.16–1.71)	1.54 (1.28–1.89)	<0.001
Lathosterol/cholesterol (μg/mg)	1.59 (1.10–2.29)	1.53 (1.04–2.09)	1.33 (0.96–2.09)	0.001
Campesterol/lathosterol	0.67 (0.38–1.28)	0.92 (0.55–1.56)	1.26 (0.71–2.14)	<0.001
Sitosterol/lathosterol	0.37 (0.21–0.67)	0.48 (0.30–0.82)	0.62 (0.63–1.12)	<0.001
Cholestanol/lathosterol	0.80 (0.49–1.34)	0.93 (0.58–1.56)	1.14 (0.67–1.79)	<0.001
YFS				
No. of subjects	266	146	21	–
Male	266 ± 100	146 ± 100	21 ± 100	1.000
Age (yrs)	36.1 ± 2.4	35.4 ± 2.5	36.0 ± 2.3	0.029
Body mass index (kg/m^2)	26.5 ± 3.4	26.5 ± 3.9	26.7 ± 3.5	0.846
Type 2 diabetes	0 ± 0	0 ± 0	0 ± 0	1.000
Total cholesterol (mg/dl)	213 ± 39	213 ± 38	220 ± 38	0.658
LDL cholesterol (mg/dl)	142 ± 35	140 ± 35	148 ± 33	0.867
HDL cholesterol (mg/dl)	45 ± 11	44 ± 11	48 ± 11	0.850
Apolipoprotein B (mg/dl)	121 ± 27	121 ± 27	122 ± 26	0.867
Campesterol (μg/dl)	478 (372–618)	551 (438–677)	759 (548–895)	<0.001
Sitosterol (μg/dl)	208 (160–260)	241 (196–304)	336 (260–380)	<0.001
Cholestanol (μg/dl)	260 (221–307)	278 (233–315)	294 (251–324)	0.010
Lathosterol (μg/dl)	272 (200–347)	245 (190–322)	213 (186–278)	0.113
Campesterol/cholesterol (μg/mg)	2.63 (2.13–3.41)	3.22 (2.52–3.77)	4.20 (2.95–4.83)	<0.001
Sitosterol/cholesterol (μg/mg)	1.17 (0.97–1.50)	1.43 (1.13–1.67)	1.80 (1.41–2.09)	<0.001
Cholestanol/cholesterol (μg/mg)	1.40 (1.24–1.58)	1.48 (1.35–1.68)	1.51 (1.45–1.66)	0.001
Lathosterol/cholesterol (μg/mg)	1.48 (1.11–1.79)	1.38 (1.09–1.73)	1.17 (0.92–1.75)	0.077
Campesterol/lathosterol	1.76 (1.17–2.84)	2.26 (1.57–3.22)	3.00 (2.27–4.01)	<0.001
Sitosterol/lathosterol	0.75 (0.51–1.18)	0.98 (0.65–1.36)	1.31 (0.91–1.93)	<0.001
Cholestanol/lathosterol	0.93 (0.71–1.36)	1.09 (0.81–1.51)	1.25 (0.85–1.84)	0.007

Values are n (%) in cases of categorical data and means ± SDs or medians (interquartile ranges) in cases of continuous data. *Calculated with chi-square test for categorical data and with analysis of variance for continuous data (additive model).

HDL = high-density lipoprotein; LDL = low-density lipoprotein; LURIC = Ludwisghafen Risk and Cardiovascular Health; YFS = Young Finns Study.

cholestanol-to-cholesterol ratios (25,41). Both studies showed that high cholestanol-to-cholesterol ratios were associated with increased cardiovascular risk. There was neither publication bias ($p = 0.317$) (Online Fig. 2) nor study heterogeneity ($p = 0.483$). The pooled RR for CVD comparing the highest versus the lowest tertile of the cholestanol-to-cholesterol ratio was 1.72 (95% CI: 1.28 to 2.32; $p < 0.001$) (Fig. 2). In short, a high cholestanol-to-cholesterol ratio was significantly related to increased risk for CVD.

Discussion

This study found that high rates of cholesterol absorption, as reflected by an elevated cholestanol-to-cholesterol ratio, are associated with risk alleles in *ABCG8* and *ABO* and with present and future CVD. The LURIC and YFS data confirm previous GWA studies showing that risk alleles in *ABCG8* and *ABO* are related to increased total and LDL cholesterol concentrations and the major protein components of LDL

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Table 2 ***Rs6576629*** **Frequency in the** ***ABCG8*** **Gene and Anthropometric Parameters and Plasma Lipids and Sterol Levels in the LURIC and YFS Cohorts**

Alleles	GG	GA + AA	p Value*
LURIC			
Number	1,010	138 + 3	–
Male	669 ± 66.2	94 ± 66.7	0.920
Age (yrs)	63.8 ± 11.1	62.9 ± 10.1	0.966
Body mass index (kg/m^2)	27.3 ± 4.2	26.9 ± 3.8	0.392
Type 2 diabetes	298 ± 29.5	43 ± 30.5	0.809
Total cholesterol (mg/dl)	202 ± 36	193 ± 36	0.007
LDL cholesterol (mg/dl)	124 ± 32	118 ± 31	0.041
HDL cholesterol (mg/dl)	40 ± 11	40 ± 10	0.591
Apolipoprotein B (mg/dl)	109 ± 23	103 ± 22	0.003
Apolipoprotein E (mg/dl)	9.4 ± 3.2	8.6 ± 3.0	0.012
Campesterol (μg/dl)	262 (180–386)	202 (135–289)	<0.001
Sitosterol (μg/dl)	141 (99–201)	108 (81–154)	<0.001
Cholestanol (μg/dl)	277 (220–353)	235 (176–292)	<0.001
Lathosterol (μg/dl)	300 (204–433)	316 (237–439)	0.234
Campesterol/cholesterol (μg/mg)	1.31 (0.94–1.85)	1.07 (0.75–1.39)	<0.001
Sitosterol/cholesterol (μg/mg)	0.70 (0.51–0.97)	0.59 (0.43–0.77)	<0.001
Cholestanol/cholesterol (μg/mg)	1.39 (1.13–1.69)	1.24 (0.98–1.47)	<0.001
Lathosterol/cholesterol (μg/mg)	1.50 (1.03–2.12)	1.65 (1.23–2.29)	0.022
Campesterol/lathosterol	0.86 (0.49–1.57)	0.64 (0.38–1.13)	<0.001
Sitosterol/lathosterol	0.47 (0.27–0.82)	0.36 (0.20–0.59)	<0.001
Cholestanol/lathosterol	0.93 (0.57–1.52)	0.68 (0.48–1.24)	<0.001
YFS			
No. of subjects	367	62 + 5	–
Male	367 ± 100	67 ± 100	1.000
Age (yrs)	35.8 ± 2.5	36.4 ± 2.3	0.057
Body mass index (kg/m^2)	26.6 ± 3.9	25.9 ± 3.2	0.154
Type 2 diabetes	0 ± 0	0 ± 0	1.000
Total cholesterol (mg/dl)	214 ± 36	211 ± 52	0.542
LDL cholesterol (mg/dl)	142 ± 33	137 ± 45	0.287
HDL cholesterol (mg/dl)	45 ± 11	46 ± 10	0.570
Apolipoprotein B (mg/dl)	121 ± 25	118 ± 35	0.450
Campesterol (μg/dl)	530 (413–677)	444 (343–545)	<0.001
Sitosterol (μg/dl)	229 (180–286)	193 (148–224)	<0.001
Cholestanol (μg/dl)	275 (232–316)	236 (206–282)	<0.001
Lathosterol (μg/dl)	258 (194–333)	267 (192–350)	0.433
Campesterol/cholesterol (μg/mg)	2.89 (2.36–3.75)	2.56 (2.01–3.05)	0.001
Sitosterol/cholesterol (μg/mg)	1.31 (1.06–1.62)	1.07 (0.94–1.33)	<0.001
Cholestanol/cholesterol (μg/mg)	1.46 (1.33–1.63)	1.33 (1.19–1.46)	<0.001
Lathosterol/cholesterol (μg/mg)	1.41 (1.09–1.77)	1.51 (1.12–1.78)	0.322
Campesterol/lathosterol	1.99 (1.32–3.10)	1.61 (1.07–2.54)	0.015
Sitosterol/lathosterol	0.89 (0.58–1.34)	0.72 (0.47–1.01)	0.007
Cholestanol/lathosterol	1.02 (0.77–1.47)	0.87 (0.68–1.25)	0.021

Values are n (%) in cases of categorical data and means ± SDs or medians (inter-quartile ranges) in cases of continuous data. *Calculated with chi-square test for categorical data and with analysis of variance for continuous data (dominant model).

Abbreviations as in Table 1.

(namely, apolipoproteins B and E) (42–44). Our results for lathosterol suggest that high absorbers of cholesterol down-regulate endogenous cholesterol synthesis. However, this protective mechanism against hypercholesterolemia is obviously not strong enough. Elevated cholesterol absorption may thus result in a higher lifetime cholesterol burden and consequently increased plaque formation.

High rates of cholesterol absorption, rather than elevated circulating plant sterol levels, seem to mediate the relationship of risk alleles in *ABCG8* and *ABO* with CVD. An animal study also found that moderately decreased cholesterol absorption rates were associated with atheroprotection (45). Moreover, a recent meta-analysis found no evidence of an association between circulating plant sterols and their ratios to cholesterol and CVD (46). The discrepancy between the meta-analysis findings for cholestanol and for plant sterols may reflect the fact that plant sterols, unlike cholestanol, are also surrogate markers for dietary vegetable and fruit intake (47).

Table 3 ***Rs657152*** **Frequency in the** ***ABO*** **Gene and Anthropometric Parameters and Plasma Lipids and Sterol Levels in the LURIC and YFS Cohorts**

Alleles	GG	GT	TT	p Value*
LURIC				
No. of subjects	432	570	200	–
Male	285 ± 66.0	380 ± 66.7	134 ± 67.0	0.959
Age (yrs)	63.2 ± 10.7	62.6 ± 11.5	62.7 ± 10.5	0.673
Body mass index (kg/m^2)	27.4 ± 4.3	27.4 ± 4.2	27.0 ± 4.0	0.525
Type 2 diabetes	124 ± 28.7	168 ± 29.5	57 ± 28.5	0.949
Total cholesterol (mg/dl)	198 ± 37	202 ± 35	203 ± 36	0.219
LDL cholesterol (mg/dl)	198 ± 37	124 ± 35	123 ± 31	0.426
HDL cholesterol (mg/dl)	40 ± 11	40 ± 11	41 ± 11	0.358
Apolipoprotein B (mg/dl)	107 ± 23	108 ± 23	107 ± 23	0.770
Apolipoprotein E (mg/dl)	9.2 ± 2.8	9.2 ± 3.2	9.6 ± 4.0	0.319
Campesterol (μg/dl)	232 (164–353)	257 (180–376)	282 (176–415)	0.011
Sitosterol (μg/dl)	130 (91–192)	137 (96–192)	150 (103–217)	0.088
Cholestanol (μg/dl)	273 (211–337)	264 (215–338)	285 (225–369)	0.020
Lathosterol (μg/dl)	301 (208–437)	307 (211–439)	297 (191–442)	0.894
Campesterol/cholesterol (μg/mg)	1.22 (0.87–1.77)	1.29 (0.94–1.84)	1.39 (0.92–2.02)	0.031
Sitosterol/cholesterol (μg/mg)	0.67 (0.49–0.92)	0.69 (0.50–0.95)	0.73 (0.51–1.01)	0.152
Cholestanol/cholesterol (μg/mg)	1.38 (1.12–1.64)	1.35 (1.10–1.66)	1.46 (1.18–1.79)	0.0169
Lathosterol/cholesterol (μg/mg)	1.51 (1.04–2.22)	1.54 (1.10–2.12)	1.46 (0.97–2.17)	0.273
Campesterol/lathosterol	0.79 (0.44–1.51)	0.77 (0.49–1.46)	0.97 (0.48–1.73)	0.067
Sitosterol/lathosterol	0.44 (0.24–0.79)	0.42 (0.25–0.75)	051 (0.25–0.88)	0.085
Cholestanol/lathosterol	0.90 (0.54–1.50)	0.87 (0.54–1.39)	1.01 (0.59–1.62)	0.050
YFS				
No. of subjects	140	210	84	–
Male	140 ± 100	210 ± 100	84 ± 100	1.000
Age (yrs)	35.9 ± 2.4	35.8 ± 2.4	36.0 ± 2.5	0.689
Body mass index (kg/m^2)	26.8 ± 3.9	26.4 ± 3.8	26.3 ± 3.5	0.265
Type 2 diabetes	0 ± 0	0 ± 0	0 ± 0	1.000
Total cholesterol (mg/dl)	209 ± 41	214 ± 37	220 ± 39	0.054
LDL cholesterol (mg/dl)	136 ± 35	143 ± 34	147 ± 37	0.026
HDL cholesterol (mg/dl)	44 ± 12	46 ± 11	49 ± 10	0.733
Apolipoprotein B (mg/dl)	120 ± 28	120 ± 27	125 ± 26	0.244
Campesterol (μg/dl)	473 (369–634)	530 (424–673)	528 (407–647)	0.646
Sitosterol (μg/dl)	206 (160–274)	227 (187–288)	224 (174–268)	0.747
Cholestanol (μg/dl)	252 (220–308)	275 (234–310)	268 (220–316)	0.142
Lathosterol (μg/dl)	256 (203–330)	252 (186–338)	264 (207–341)	0.900
Campesterol/cholesterol (μg/mg)	2.70 (2.23–3.51)	2.93 (2.38–3.68)	2.78 (2.20–3.63)	0.844
Sitosterol/cholesterol (μg/mg)	1.21 (1.00–1.59)	1.32 (1.06–1.62)	1.21 (1.03–1.56)	0.708
Cholestanol/cholesterol (μg/mg)	140 (1.26–1.57)	1.47 (1.32–1.66)	1.43 (1.28–1.58)	0.960
Lathosterol/cholesterol (μg/mg)	1.50 (1.16–1.78)	1.39 (1.05–1.75)	1.39 (1.08–1.80)	0.279
Campesterol/lathosterol	1.76 (1.26–2.75)	2.05 (1.34–3.20)	1.93 (1.20–3.00)	0.404
Sitosterol/lathosterol	0.76 (0.56–1.22)	0.94 (0.60–1.34)	0.84 (0.53–1.26)	0.504
Cholestanol/lathosterol	0.94 (0.72–1.30)	1.05 (0.78–1.53)	1.02 (0.76–1.38)	0.240

Values are numbers (percentages) in cases of categorical data and means ± SDs or medians (inter-quartile ranges) in cases of continuous data. *Calculated with chi-square test for categorical data and with analysis of variance for continuous data (dominant model).

Abbreviations as in Table 1.

We cannot rule out the possibility that high circulating cholestanol itself represents a cardiovascular risk factor. In cerebrotendinous xanthomatosis, a rare lipid storage disease caused by mutations in the gene encoding sterol 27-hydroxylase, circulating cholestanol is markedly increased (48). Patients with this defect often present with dementia, ataxia, cataracts, and xanthomas in the tendons and in the nervous system. CVD is prevalent in about 10% of the patients with cerebrotendinous xanthomatosis. It would stand to reason that cholestanol directly promotes atherogenesis by accumulation in the atherosclerotic plaque. However, in an autopsy study, cholestanol represented just 2.8% of all the sterols in aortic deposits. Another hypothesis suggests that an alternative pathway of reverse cholesterol transport may be impaired in cerebrotendinous xanthomatosis and that high cholestanol would be indicative of such problems. To sum up, the intrinsic role of cholestanol in CVD remains to be elucidated and definitely merits further research. Nevertheless, the "absorption theory" seems robust in at least partly explaining the observations made in the genetic studies and in the

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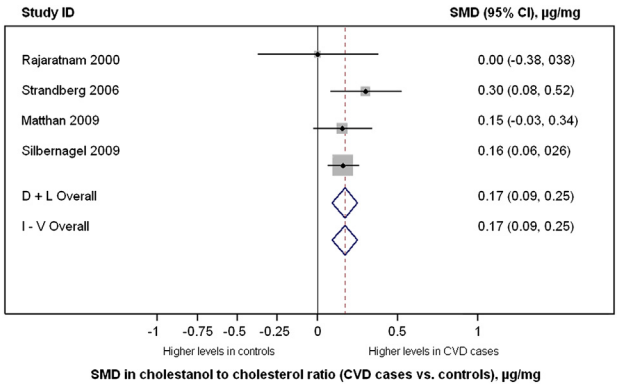

Figure 1 Forest Plot for the Association of Cholestanol-to-Cholesterol Ratio With CVD Based on SMDs

CVD = cardiovascular disease; ID = identifier; SMD = standardized mean difference.

meta-analysis. More information on sterol 27-hydroxylase, including original genetic data, are provided in the Online Text, Online Table 6, and Online Figure 3.

Because high cholesterol absorption is associated with increased cardiovascular risk, inhibition of cholesterol uptake represents a promising target in the prevention and treatment of CVD. However, prospective clinical studies clearly demonstrating that the use of the cholesterol absorption inhibitor ezetimibe prevents cardiovascular complications are lacking. Whether plant sterol–enriched functional foods will reduce not only cholesterol absorption but also hard endpoints has not yet been investigated. At the same time, our data may dispel concerns that the modest increase in circulating plant sterol levels associated with their regular dietary intake could result in an adverse outcome.

Study limitations. The major strength of the genetic analysis within LURIC is derived from the fact that it relies on 1 of the largest studies in which information on noncholesterol sterols and genetics have been collected simultaneously. Furthermore, we are able to replicate results obtained from LURIC in YFS, a cohort with contrasting patient characteristics. The meta-analysis is limited to a small number of observational studies, and these studies are heterogeneous with regard to their design and adjustment for potential confounding variables. Nevertheless, we have made the first attempt systematically to collate and analyze evidence from epidemiological studies that have investigated the relationships of cholestanol with CVD.

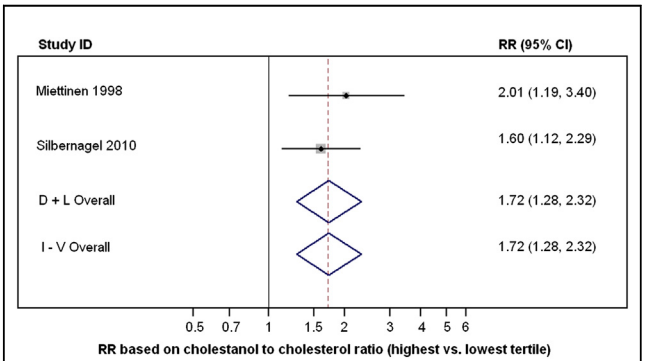

Figure 2 Forest Plot for the Association of Cholestanol-to-Cholesterol Ratio With CVD Based on RRs

RR = risk ratio; other abbreviations as in Figure 1.

Conclusions

Our data support an atherogenic role for high intestinal cholesterol absorption. Harm caused by elevated cholesterol absorption rather than by high circulating plant sterols may, therefore, mediate the relationships of *ABCG8* and *ABO* variants with CVD.

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Reprint requests and correspondence: Dr. Günther Silbernagel, Department of Angiology, Swiss Cardiovascular Center, Inselspital, University of Bern, 3010 Bern, Switzerland. E-mail: guenther.silbernagel@insel.ch.

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Key Words: *ABCG8* ■ *ABO* ■ cardiovascular disease ■ cholestanol ■ intestinal cholesterol absorption ■ plant sterols.

APPENDIX

For supplemental tables and figures, and text, please see the online version of this article.