Phytosterol plasma concentrations and coronary heart disease in the prospective Spanish EPIC cohort

Verónica Escurriol,[*,†] Montserrat Cofán,[*,†] Concepción Moreno-Iribas,[§,] Nerea Larrañaga,[**,††] Carmen Martínez,[**,§§] Carmen Navarro,[**,***] Laudina Rodríguez,[†††] Carlos A. González,[§§§,****] Dolores Corella,[†,††††] and Emilio Ros[1,*,†]**

Lipid Clinic, Endocrinology and Nutrition Service,* Institut d'Investigacions Biomèdiques August Pi i Sunyer, Hospital Clínic, Barcelona, Spain; CIBER Fisiopatología de la Obesidad y Nutrición,† CIBER Epidemiología y Salud Pública,** and RTIC RD06/0020,**** Instituto de Salud Carlos III, Spain; Public Health Institute of Navarra,§ Pamplona, Spain; Public Health Department of Gipuzkoa,†† Gipuzkoa, Spain; Andalusian School of Public Health,§§ Granada, Spain; Department of Epidemiology,*** Regional Health Council, Murcia, Spain; Health Council and Health Services,††† Asturias, Spain; Unit of Nutrition,§§§ Environment, and Cancer, Catalan Institute of Oncology, Barcelona; and Department of Preventive Medicine and Public Health,†††† School of Medicine, University of Valencia, Valencia, Spain

Abstract Phytosterol intake with natural foods, a measure of healthy dietary choices, increases plasma levels, but increased plasma phytosterols are believed to be a coronary heart disease (CHD) risk factor. To address this paradox, we evaluated baseline risk factors, phytosterol intake, and plasma noncholesterol sterol levels in participants of a case control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) Spanish cohort who developed CHD (n = 299) and matched controls (n = 584) who remained free of CHD after a 10 year follow-up. Sitosterol-to-cholesterol ratios increased across tertiles of phytosterol intake (P = 0.026). HDL-cholesterol level increased, and adiposity measures, cholesterol/HDL ratios, and levels of glucose, triglycerides, and lathosterol, a cholesterol synthesis marker, decreased across plasma sitosterol tertiles (P < 0.02; all). Compared with controls, cases had nonsignificantly lower median levels of phytosterol intake and plasma sitosterol. The multivariable-adjusted odds ratio for CHD across the lowest to highest plasma sitosterol tertile was 0.59 (95% confidence interval, 0.36–0.97). Associations were weaker for plasma campesterol. The apolipoprotein E genotype was unrelated to CHD risk or plasma phytosterols. The data suggest that plasma sitosterol levels are associated with a lower CHD risk while being markers of a lower cardiometabolic risk in the EPIC-Spain cohort, a population with a high phytosterol intake.—Escurriol, V., M. Cofán, C. Moreno-Iribas, N. Larrañaga, C. Martínez, C. Navarro, L. Rodríguez, C. A. González, D. Corella, and E. Ros. **Phytosterol plasma concentrations and coronary heart disease in the prospective Spanish EPIC cohort. *J. Lipid Res.* 2010.** 51: **618–624.**

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Dietary sterols consist of animal-derived cholesterol and plant-derived noncholesterol sterols or phytosterols. Phytosterols are important components of a vegetable-based diet and are particularly abundant in whole grains, nuts, seeds, and oils derived from them. The principal molecular forms are sitosterol and campesterol (1). These compounds are structurally related to cholesterol but have bulkier and more hydrophobic molecules, which confer them a higher affinity for intestinal micelles than has cholesterol. Consequently, cholesterol is displaced from micelles, and the amount available for absorption is limited. The phytosterol content of usual diets is similar to that of cholesterol (150 to 450 mg/day), but their intestinal absorption is much less efficient (see review in Ref. 2). Because of low absorption and rapid biliary elimination, physiological plasma concentrations of phytosterols are in the order of 10^{-3} those of cholesterol. Their ratios to cholesterol are accepted as surrogate markers for the efficiency of cholesterol absorption, while those of the

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Abbreviations: apoE, apolipoprotein E; BMI, body mass index; CHD, coronary heart disease; EPIC, European Prospective Investigation into Cancer and Nutrition; OR, odds ratio.

[1]To whom correspondence should be addressed.
e-mail: eros@clinic.ub.es

cholesterol precursor lathosterol are a reliable index of cholesterol synthesis (3).

The lower absorption of phytosterols compared with cholesterol is attributable to active resecretion back into the intestinal lumen, a process that is mediated by the half-transporters ABCG5 and ABCG8. Genetic defects in these transporters (4–6) cause sitosterolemia, a rare autosomal recessive disorder characterized by intestinal sterol hyperabsorption, raised plasma phytosterol levels, xanthomas, and accelerated atherosclerosis. Because of the presumed pathogenic role of elevated plasma phytosterols in sitosterolemia, the question whether high levels of circulating phytosterols might also be atherogenic in nonsitosterolemic individuals has been much debated (7, 8). This is a substantial concern, given that inhibition of cholesterol absorption by gram doses of phytosterols incorporated into various foods is widely used as a nonpharmacological strategy for cholesterol lowering but is associated with increased serum phytosterol concentrations (8). Results of epidemiological studies have suggested either a direct association between plasma phytosterols and coronary heart disease (CHD) risk (9–12) or a null (13, 14) and even inverse association (15).

A relevant yet unexplored issue is the effect on CHD risk of naturally occurring phytosterols in the usual diet. Two large cross-sectional studies have shown a weak inverse association between dietary phytosterol intake and plasma total and LDL-cholesterol levels (16, 17). Participants in the Spanish cohort of European Prospective Investigation into Cancer and Nutrition (EPIC), a large prospective study in Europe (18), have a higher consumption of phytosterol-rich vegetables and fruits than most European countries and the US (19), confirming their adherence to the Mediterranean dietary pattern. We hypothesized that plasma phytosterol concentrations are markers of a healthy diet and are associated with a decreased risk for CHD, instead of an increased risk, in the Spanish EPIC cohort. To address this issue, we examined the association among dietary phytosterol intake, plasma levels of phytosterols, cardiometabolic risk factors, and the risk of future CHD.

METHODS

Study design

We performed a nested case-control study among participants of the Spanish EPIC study (19). Subjects were recruited as part of a 10 country collaborative study designed to investigate dietary and other determinants of cancer (18). The population of the Spanish branch of EPIC included 41,440 individuals. Participants were healthy men and women volunteers, principally blood donors, aged between 30 and 69 years at enrolment between October 1992 and July 1996 in five regions, three in Northern Spain (Asturias, Navarra, and Gipuzkoa) and two in Southern Spain (Murcia and Granada). In face-to-face interviews, each participant was administered questionnaires to collect information on lifestyle factors, including food consumption and smoking, and a complete medical history, including a prior diagnosis of hypertension, hyperlipidemia, or diabetes, and medication use. Anthropometric measurements [height and weight, with calculation of body mass index (BMI) in kg/m^2, and waist circumference] were obtained by using standardized procedures, and a blood sample was taken. Close to 60% of blood samples were collected after an overnight fast. To ascertain the vital status, annual record linkages were carried out with the national databases of the Instituto Nacional de Estadística, Spain. For this analysis, the follow-up for vital status was complete until December 31, 2004; the mean follow-up period was ≈10 years. All subjects provided written informed consent to a protocol approved by local ethical review boards.

Case ascertainment

Cases were defined as participants who had a definite fatal or nonfatal myocardial infarction or angina requiring a revascularization procedure. Participants who at recruitment had a prior diagnosis of CHD that was validated thereafter were excluded from further analyses (n = 193). For the identification of potential cases, a record linkage between the EPIC database and local hospital discharge registers was performed. A Population Myocardial Infarction Register available in three participating regions (Navarra, Guipuzkoa, and Murcia) was also used. At censoring, 468 definite cases of incident fatal and nonfatal myocardial infarction and 141 cases of angina were identified.

Participants

Of the 609 confirmed CHD cases, approximately one-half (n = 315) were randomly selected for inclusion in this analysis. Using an incidence density method (20), two controls, randomly selected among subjects in the cohort still at risk for CHD at the time of diagnosis of each case (namely, subjects that had not suffered a CHD event at the time their matched case-pair had an event) were matched to each case by center, sex, age (within 5 years), and time of enrolment (within 3 months). If needed, the same subjects could serve as a control more than once. Because we analyzed incident (new) cases, any individual who suffered a CHD event during the 10 year follow-up period was no longer eligible to be a control.

Dietary information

Information on usual food intake over the year before enrolment was collected by a validated computerized diet history questionnaire (21, 22). Energy and nutrient intakes were estimated using a conversion table in a computerized database especially compiled for the EPIC study in Spain. Intake of total phytosterols and their main components was estimated from the database of Spanish foods developed by Jiménez-Escrig, Santos-Hidalgo, and Saura-Calixto (23)

Laboratory measurements

Coded samples of plasma and blood cells (buffy coat) were shipped to a central laboratory and stored at −80°C until assay. Plasma glucose was measured by the glucose-oxidase method in fasting samples. Cholesterol and triglycerides were determined by enzymatic procedures; triglycerides were measured only in fasting samples. HDL-cholesterol was quantified after precipitation with phosphotungstic acid and magnesium chloride. The concentration of LDL-cholesterol was calculated as total cholesterol minus HDL-cholesterol minus triglycerides/5 when triglyceride levels were ⩽3.36 mmol/l in fasting samples, and by the homogeneous method of Daiichi Pure Chemicals (N-geneous® LDL; Genzyme Diagnostics, Cambridge, MA) when triglyceride levels were >3.36 mmol/l and in nonfasting specimens. The determinations were made in an ADVIA 1800 chemical analyzer (Siemens Healthcare Diagnostics, Madrid, Spain). Genomic DNA was obtained to determine the apolipoprotein E (apoE) genotype (24).

Serum noncholesterol sterol levels were determined by gas chromatography using a modification of the method of Heinemann, Axtmann, and von Bergmann (25). Epicoprostanol (2 μg)

was added to serum (0.1 ml) as internal standard. After alkaline hydrolysis, extraction, and derivatization to trimethylsilyl ethers, sterols were quantified on a 30-m nonpolar capillary column (TRB-Esterol, Teknokroma, Barcelona) equipped with flame ionization detection in a Perkin-Elmer GC Autosystem™ (Norwalk, CT) apparatus. Each run quantified lathosterol, campesterol, and sitosterol. Noncholesterol sterols are expressed as ratios to cholesterol (μg/mg cholesterol) because, like cholesterol, these molecules are transported exclusively in lipoprotein particles and their concentrations are altered by changes in carrier lipoprotein concentrations (2). Inter- and intra-assay coefficient variations were 5.0% and 3.2% for lathosterol, 1.9% and 1.6% for campesterol, and 2.0% and 1.8% for sitosterol, respectively.

Statistical analyses

Qualitative variables are expressed as numbers (percentage). Data for continuous variables are presented as mean ± SD. Phytosterol intakes and plasma levels of triglycerides and noncholesterol sterols had a skewed distribution and are presented as medians and interquartile ranges. Participants with more than 3 SDs from the mean of daily total energy intake were considered to have implausible dietary data and excluded from further analysis. We categorized control subjects by tertiles of both dietary phytosterol intake and plasma phytosterol-to-cholesterol ratios and used ANOVA, χ^2, and Kruskall-Wallis statistics, as appropriate, to calculate tests for trend for cardiovascular risk factors. Pearson correlation coefficients were constructed to test for relationships between intake of phytosterols and other dietary variables. Because phytosterol intake was strongly related to total energy consumption and showed age and gender differences, age-, gender-, and energy-adjusted phytosterol values were used when examining associations with other dietary variables or plasma lipid values.

Unpaired *t*-tests, χ^2 tests, or the Mann-Whitney test, as appropriate, were used for comparisons of variables between cases and controls. These statistical tests were two-tailed, and significance was set at $P < 0.05$. Analyses were performed using SPSS, version 15.0 (Chicago, IL).

Odds ratios (ORs) and 95% confidence intervals for CHD risk by plasma phytosterol ratios were calculated by conditional logistic regression using the PHREG procedure (SAS statistical software, v. 9; SAS Institute, Cary, NC), stratified by the case-control set. Risk estimates were computed as "crude" (adjustment for matching variables only), after adjustment by cardiovascular risk factors and apoE genotype, and after additional adjustment by intake of energy and nutrients, including plant sterols. We did not adjust for statin use because the few subjects under statin treatment at recruitment were excluded from calculations.

RESULTS

From the total number of 315 CHD cases, 16 were excluded, seven because they were under statin treatment, one due to implausible energy intake, and eight because plasma sterol determination failed due to insufficient or spoilt plasma samples; corresponding exclusions among the 630 control subjects were 8, 3, and 35.

Associations of dietary phytosterols in control subjects

The median total phytosterol intake of the whole cohort (n = 883) was 315 mg/day and was higher in men (337 mg/day, range 82–851) than in women (237 mg/day, range 26–501) ($P < 0.001$). After adjustment for energy intake, between-gender differences were blunted but still significant (321 mg/day in men and 293 mg/day in women, $P = 0.001$). **Table 1** shows that phytosterol intake in control subjects was associated directly with both the male sex and total energy intake and inversely with age, but was unre-

TABLE 1. Distribution of plasma lipids and noncholesterol sterol ratios by tertiles of total plant sterol intake in control subjects

Characteristics	Tertile 1	Tertile 2	Tertile 3	P^a
Plant sterol intake, mg/day	≤260.1	260.2–360.0	≥360.1	
n	194	195	195	
Sex, male (%)	109 (56)	166 (85)	180 (92)	<0.001
Age, years	55.4 ± 7.6	53.7 ± 6.8	53.3 ± 7.1	0.008
Total energy intake, kJ/day	8,348 ± 2,522	10,191 ± 2,553	12,576 ± 2,918	<0.001
Plasma lipids, mmol/l				
Total cholesterol				
Unadjusted	5.68 ± 0.86	5.66 ± 0.91	5.67 ± 0.91	0.982
Adjusted[b]	5.68	5.66	5.67	0.798
LDL-cholesterol				
Unadjusted	3.62 ± 0.80	3.65 ± 0.84	3.73 ± 0.83	0.391
Adjusted[b]	3.62	3.65	3.73	0.260
HDL-cholesterol				
Unadjusted	1.46 ± 0.36	1.37 ± 0.34	1.32 ± 0.33	<0.001
Adjusted[b]	1.46	1.37	1.32	0.077
Cholesterol/HDL ratio				
Unadjusted	4.11 ± 1.13	4.38 ± 1.24	4.57 ± 1.43	0.002
Adjusted[b]	4.11	4.38	4.57	0.058
Triglycerides[c]	1.09 (0.82–1.49)	1.23 (0.84–1.62)	1.14 (0.87–1.66)	0.365
Plasma noncholesterol sterol-to-cholesterol ratios, μmol/mmol				
Lathosterol	1.59 (1.10–2.09)	1.63 (1.33–2.18)	1.68 (1.31–2.17)	0.378
Campesterol	1.43 (1.16–2.03)	1.57 (1.23–1.86)	1.73 (1.25–2.11)	0.125
Sitosterol	1.27 (0.96–1.75)	1.34 (1.09–1.85)	1.53 (1.18–1.87)	0.029

Values are mean ± SD or number (percentage). Triglycerides and noncholesterol sterol ratios are medians (interquartile ranges).

[a] *P* for linear trend calculated by ANOVA, χ^2, or Kruskall-Wallis statistics, as appropriate.

[b] Adjusted by sex, age, and energy intake.

[c] Measured in 356 fasting samples.

lated to plasma lipid levels after adjusting by sex, age, and energy intake. Plasma campesterol- and sitosterol-to-cholesterol ratios increased across tertiles of phytosterol intake, but only the sitosterol increase was significant ($P = 0.029$). Sex-, age-, and energy-adjusted phytosterol intake was directly correlated ($P < 0.001$) with intake of vegetables ($r = 0.316$), fruits ($r = 0.475$), legumes ($r = 0.293$), cereals ($r = 0.238$), fiber ($r = 0.605$), vegetable protein ($r = 0.397$), and polyunsaturated fatty acids ($r = 0.269$) and inversely correlated ($P < 0.001$) with intake of animal protein ($r = -0.185$), saturated fatty acids ($r = -0.282$), and cholesterol ($r = -0.245$). Intake of measurable phytosterol subclasses showed similar associations (data not shown).

Plasma phytosterols and risk factors in control subjects

Table 2 shows the distribution of cardiovascular risk factors, plasma noncholesterol sterol ratios, and dietary phytosterol intake across tertiles of plasma sitosterol-to-cholesterol ratios in the control group. Plasma sitosterol tertiles were associated directly with HDL-cholesterol levels, plasma campesterol-to-cholesterol ratios, and phytosterol intake and inversely with BMI, waist circumference, plasma glucose and triglyceride levels, cholesterol/HDL ratios, and lathosterol-to-cholesterol ratios. The total cholesterol level increased nonsignificantly with increasing plasma sitosterol. Tertiles of plasma campesterol-to-cholesterol ratios showed similar associations (data not shown).

Characteristics of CHD cases and matched controls

The baseline characteristics of assessable CHD cases (n = 299) and controls (n = 584) are shown in **Table 3**. Matching secured that sex and age were comparable between cases and controls. Predictably, participants with incident CHD during follow-up had higher waist circumference and BMI and were more likely to smoke and have obesity, diabetes, hypertension, and hyperlipidemia than controls. Also, total cholesterol, LDL-cholesterol, and triglyceride levels were higher, and HDL-cholesterol was lower in cases than controls. apoE genotype frequency distribution and plasma noncholesterol sterol ratios were similar between the two groups. Intakes of total phytosterols and sitosterol, the main dietary noncholesterol sterol, were nonsignificantly lower in cases compared with controls. There were no case-control differences in energy or nutrient intake (data not shown).

Plasma phytosterols and CHD risk

Table 4 shows the unadjusted and adjusted ORs for future CHD by tertiles of plasma sitosterol- and campesterol-to-cholesterol ratios explored via conditional regression. Because this analysis requires strict case-control matching, 293 cases and 586 controls (33 used twice) were used by the model. The multivariable-adjusted risk for CHD was 41% lower in the highest plasma sitosterol-to-cholesterol tertile compared with the lowest tertile. The plasma campesterol ratios were not associated with the risk of CHD.

DISCUSSION

The results of this study in the prospective Spanish EPIC cohort suggest that elevated levels of plasma phytosterols are not associated with an increased risk of incident CHD.

TABLE 2. Distribution of cardiovascular risk factors and noncholesterol sterols by tertiles of plasma sitosterol-to-cholesterol ratios in control subjects

Characteristics	Tertile 1	Tertile 2	Tertile 3	P^a
Sitosterol-to-cholesterol ratio, μg/mg	≤1.16	1.17–1.62	≥1.63	
n	195	195	194	
Age, years	60.8 ± 7.1	60.6 ± 7.8	59.7 ± 7.7	0.321
Male sex, n (%)	148 (75.9)	158 (81.0)	149 (76.8)	0.428
BMI, kg/m^2	30.2 ± 4.3	28.4 ± 3.0	27.5 ± 3.2	<0.001
Waist circumference, cm	102.3 ± 10.2	97.7 ± 9.2	95.1 ± 9.9	<0.001
Hypertension, n (%)	46 (23.7)	54 (27.7)	43 (22.2)	0.425
Fasting glucose, mmol/l[b]	5.09 ± 1.93	4.57 ± 0.97	4.51 ± 0.88	0.002
Plasma lipids, mmol/l				
Total cholesterol	5.67 ± 0.91	5.59 ± 0.84	5.74 ± 0.92	0.255
LDL-cholesterol	3.66 ± 0.82	3.61 ± 0.74	3.72 ± 0.91	0.417
HDL-cholesterol	1.30 ± 0.31	1.36 ± 0.32	1.48 ± 0.39	<0.001
Triglycerides[b]	1.31 (0.93–2.09)	1.12 (0.84–1.52)	1.01 (0.78–1.36)	<0.001
Cholesterol/HDL ratio	4.57 ± 1.23	4.31 ± 1.16	4.18 ± 1.41	0.009
apoE genotype, n (%)[c]				0.534
apoE2	15 (8.8)	13 (7.7)	15 (8.4)	
apoE3	130 (76.0)	121 (72.0)	139 (78.1)	
apoE4	26 (15.2)	34 (20.2)	24 (13.5)	
Plasma noncholesterol sterol-to-cholesterol ratios, μmol/mmol				
Lathosterol	1.90 (1.35–2.36)	1.54 (1.21–1.96)	1.36 (0.99–1.78)	<0.001
Campesterol	1.07 (0.92–1.26)	1.53 (1.34–1.74)	2.17 (1.83–2.62)	<0.001
Total plant sterol intake, mg/day	286 (224–375)	315 (248–393)	317 (238–397)	0.041
Sitosterol	179 (141–227)	202 (156–245)	197 (149–253)	0.028
Campesterol	29 (22–36)	34 (24–42)	31 (25–40)	0.006

[a] P value for linear trend calculated by ANOVA, χ^2, or Kruskall-Wallis statistics, as appropriate.
[b] Measured in 356 fasting samples.
[c] Determined in 524 participants and classified as apoE2 (E2/2+E2/3), apoE3 (E3/3), or apoE4 (E3/4+E4/4), with exclusion of seven subjects with the E2/4 genotype.

TABLE 3. Baseline characteristics of CHD cases and matched control subjects

Variables	Cases (n = 299)	Controls (n = 584)	P^a
Age, years	54.1 ± 7.2	54.3 ± 7.3	0.768
Men, n (%)	236 (78.9)	455 (77.9)	0.728
Smoking, n (%)			
Never	90 (30.1)	247 (42.3)	
Past	65 (21.7)	140 (24.0)	
Current	144 (48.2)	197 (33.7)	<0.001
BMI, kg/m^2	29.6 ± 3.7	28.7 ± 3.7	0.001
Waist circumference, cm	100.5 ± 9.8	98.3 ± 10.2	0.003
Obese, n (%)	122 (40.8)	176 (30.1)	0.002
Hypertension, n (%)	106 (35.5)	143 (24.5)	0.001
Type 2 diabetes, n (%)	41 (13.7)	39 (6.7)	0.001
Hyperlipidemia, n (%)	120 (40.3)	142 (24.3)	<0.001
Fasting glucose, mmol/l[b]	5.18 ± 1.60	4.72 ± 1.37	0.001
Total cholesterol, mmol/l	6.11 ± 0.95	5.70 ± 0.89	<0.001
LDL-cholesterol, mmol/l	4.07 ± 0.90	3.66 ± 0.82	<0.001
HDL-cholesterol, mmol/l	1.27 ± 0.38	1.38 ± 0.35	<0.001
Triglycerides, mmol/l[b]	1.39 (1.06–2.02)	1.14 (0.84–1.59)	<0.001
Cholesterol/HDL ratio	5.16 ± 1.48	4.36 ± 1.28	<0.001
apoE genotype, n (%)[c]			
apoE2	17 (6.4)	43 (8.3)	0.513
apoE3	201 (75.3)	390 (75.4)	
apoE4	49 (18.4)	84 (16.2)	
Total dietary plant sterols, mg/day	287 (227–372)	309 (234–386)	0.144
Campesterol, mg/day	30 (23–39)	31 (23–40)	0.517
Sitosterol, mg/day	178 (144–231)	192 (148–241)	0.100
Plasma noncholesterol sterol-to-cholesterol ratios, μmol/mmol			
Lathosterol	1.54 (1.24–2.06)	1.57 (1.15–2.06)	0.657
Campesterol	1.47 (1.18–1.94)	1.53 (1.19–1.97)	0.360
Sitosterol	1.29 (0.98–1.68)	1.36 (1.03–1.77)	0.087

Values are mean ± SD or number (percentage). Triglycerides and noncholesterol sterol ratios are medians (interquartile ranges).

[a] Unpaired *t*-test, χ^2, or Mann-Whitney test, as appropriate.

[b] Measured in 540 fasting samples (184 cases and 356 controls).

[c] Determined in 808 participants and classified as apoE2 (E2/2+E2/3), apoE3 (E3/3), or apoE4 (E3/4+E4/4), with exclusion of 10 subjects with the E2/4 genotype.

Instead, the upper tertile of cholesterol-adjusted plasma sitosterol, the main dietary phytosterol, was inversely related to CHD risk both unadjusted and after controlling for various confounders. Furthermore, phytosterol intake with natural foods, a measure of healthy dietary choices, and HDL-cholesterol increased, and adiposity measures, cholesterol/HDL ratios, and levels of fasting glucose, triglycerides, and lathosterol, a cholesterol precursor, decreased across plasma sitosterol tertiles. Our results suggest that plasma phytosterols are a marker of a healthy diet and a lower cardiometabolic risk and are thus associated with a reduced risk for CHD.

The controversy on whether raised plasma phytosterols are a CHD risk factor in nonsitosterolemic individuals has been fed by the inconsistent findings of epidemiological studies (9–15). Other contradictory evidence has been presented recently. An investigation from the Dallas Heart Study showed no relationship between plasma phytosterols and a surrogate marker for CHD, namely, coronary calcium scores measured by electron beam computed tomography (26). Miettinen et al. (27) reported an association between raised plasma phytosterol levels and increased phytosterol content of surgically removed carotid plaques, while Weingärtner et al. (28) showed that consumption of phytosterol-enriched margarine correlated with increased plasma concentrations and tissue deposition in aortic valves obtained at surgery in patients with aortic stenosis. Silbernagel et al. (29) showed a weak association of plasma phytosterol ratios with increased severity of angiographically assessed coronary artery disease. Finally, recent data from a case-control study within the Framingham offspring cohort (30) suggest that elevated plasma phytosterols and concurrently decreased synthesis markers are associated with cardiovascular disease or carotid stenosis. It is clear that the topic of plasma phytosterols and cardiovascular risk is controversial. Our results agree with those of some recent studies (13–15) in suggesting a lack of association and even a protective role of elevated plasma phytosterol concentrations on risk of future CHD.

The median phytosterol intake (315 mg/day) in the EPIC-Spain cohort was similar to that previously reported from another Spanish population (23) and from the EPIC-Norfolk study (16). However, it was slightly higher than that reported from a Swedish population study (17) and nearly 3 times higher than that described in the CORA study from Germany (14), which casts doubt on the reliability of the phytosterol database used in that study. As previously reported (16, 17), the phytosterol content in the usual diet of EPIC-Spain participants correlated directly with consumption of healthy foods and nutrients, such as fruits and seeds, vegetable protein, fiber, and poly-

TABLE 4. ORs (95% confidence intervals) for incident CHD across tertiles of plasma phytosterol to cholesterol ratios

				Adjusted OR	
	Cases No. (%)	Controls No. (%)	Unadjusted OR	Model[a]	Model[b]
Sitosterol-to-cholesterol ratio					
⩽1.14	109 (37.2)	168 (30.4)	1	1	1
1.15–1.59	100 (34.1)	185 (33.5)	0.834 (0.585–1.188)	0.904 (0.576–1.419)	0.921 (0.575–1.475)
⩾1.60	84 (28.7)	200 (36.2)	0.645 (0.450–0.927)	0.758 (0.395–1.003)	0.592 (0.363–0.965)
	P for trend		0.018	0.047	0.032
Campesterol-to-cholesterol ratio					
⩽1.29	103 (35.2)	174 (31.5)	1	1	1
1.30–1.78	102 (34.8)	181 (32.7)	0.933 (0.655–1.327)	1.084 (0.699–1.680)	1.046 (0.658–1.661)
⩾1.79	88 (30.3)	198 (35.8)	0.720 (0.500–1.036)	0.898 (0.568–1.420)	0.763 (0.464–1.254)
	P for trend		0.076	0.643	0.285

[a] Adjusted by smoking, BMI, diabetes, hypertension, total cholesterol, HDL-cholesterol, and apoE genotype.
[b] Additionally adjusted by intake of energy, protein, fiber, saturated, monounsaturated and polyunsaturated fatty acids, alcohol, cholesterol, and plant sterols.

unsaturated fatty acids and inversely with intake of unhealthy diet components, such as animal protein, saturated fatty acids, and cholesterol. Cholesterol-adjusted plasma sitosterol levels were weakly associated with phytosterol intake. In the absence of supplementation with phytosterol-enriched foods, not commercially available in Spain at the time the EPIC cohort was recruited in 1992–1996, this finding suggests that plasma phytosterol levels are related in part to phytosterol intake with the usual diet, which in turn reflects healthy food choices.

Besides diet, other factors may influence plasma phytosterol levels (see review in Ref. 7). The apoE genotype has been studied in this regard because circulating phytosterols are markers for cholesterol absorption, and apoE plays a major role in lipid transport, but findings have been inconsistent (7). In this study, apoE variants were unrelated to plasma phytosterols. We did not investigate variability at the ABCG5/8 gene loci, a potent heritable factor influencing phytosterol levels (31). An additional factor and an important one determining circulating levels of noncholesterol sterols is adiposity. Recent work by Miettinen and colleagues (32–35) has shown that insulin resistance states, such as obesity, the metabolic syndrome, and type 2 diabetes, are associated with increased cholesterol synthesis (i.e., high plasma lathosterol-to-cholesterol ratios) and reduced cholesterol absorption (i.e., low plasma sitosterol-to-cholesterol ratios). When obese diabetic subjects underwent weight reduction, their cholesterol synthesis decreased while absorption increased (35). Indeed, there is evidence that a synergy exists whereby changes in cholesterol synthesis result in an opposite response of cholesterol absorption to maintain cholesterol homeostasis (36). Concurring with these concepts, our results show that adiposity, fasting glucose, triglycerides, and the cholesterol synthesis marker lathosterol decreased across tertiles of plasma sitosterol ratios. As measures of the efficiency of sterol absorption, plasma sitosterol ratios were associated with nonsignificant increases of the total cholesterol level. However, because HDL-cholesterol increased proportionately to a greater extent, the cholesterol/HDL ratio was lower. Thus, increased plasma phytosterols signaled leaner individuals with a lower overall cardiometabolic risk.

An additional factor that influences plasma phytosterol-to-cholesterol ratios is statin treatment (7, 36), a reason why statin users were excluded from the analyses. Finally, the plasma sitosterol and campesterol ratios to cholesterol were in the same physiological range as reported in previous studies (9–15, 30), strengthening the reliability of our data.

This study has limitations. The observational design does not exclude the possibility that potential confounders could have influenced the associations observed. Cases and controls were matched for gender, age, and time of recruitment, but not for cardiovascular risk factors. Although we controlled for factors known to be associated with cholesterol homeostasis and CHD risk in the multivariate analyses, we cannot exclude the influence of unmeasured confounders. Second, only a single measure of dietary exposure and risk factor status at baseline was available; therefore, changes in diet and other lifestyle variables that might have influenced CHD risk throughout the ~10 year follow-up were not accounted for. Finally, it could be argued that the findings are specific for the Mediterranean cohort studied, but the similarity of plasma noncholesterol levels with those reported in epidemiologic studies from the US (9, 26, 30), Finland (10), Germany (11, 12, 14), the UK (13), and The Netherlands (15) suggests that they may be generalized to other populations.

In conclusion, circulating phytosterols are unrelated to the risk of incident CHD in the prospective cohort of the Spanish EPIC study. They are also indicative of an efficient intestinal cholesterol absorption, which only marginally raises total cholesterol levels but is strongly and inversely related to features of the metabolic syndrome. Plasma concentrations of the main phytosterols, sitosterol and campesterol, appear to be markers of a lower cardiometabolic risk. These results suggest that moderately elevated plasma sitosterol, but not campesterol, might signal individuals with a reduced risk for CHD. However, taking into consideration all the epidemiological studies dealing with this topic (9–15, 26–31), we still cannot answer the important question of whether circulating phytosterols are proatherogenic, antiatherogenic, or neutral.

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