Plasma levels of plant sterols and the risk of coronary artery disease: the prospective EPIC-Norfolk Population Study

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Abstract Some studies have suggested that a modest increase of plant sterol levels is a risk factor for coronary artery disease (CAD). We studied the relationship between plant sterol levels and CAD risk in a prospective nested case-control study consisting of 373 cases and 758 controls. Sitosterol and campesterol concentrations did not differ between cases and controls [sitosterol, 0.21 vs. 0.21 mg/dl (P = 0.1); campesterol, 0.31 vs. 0.32 mg/dl (P = 0.5)]. The sitosterol-to-cholesterol ratio was significantly lower in cases than in controls (1.19 vs. 1.29 µg/mg; P = 0.008), whereas the campesterol-to-cholesterol ratio did not differ significantly (1.78 vs. 1.88 µg/mg; P = 0.1). Plant sterol concentrations correlated positively with cholesterol levels and inversely with body mass index and triglyceride and lathosterol concentrations. Among individuals in the highest tertile of the sitosterol concentration, the unadjusted odds ratio (OR) for future CAD was 0.75 [95% confidence interval (CI) = 0.56–1.01]. After adjustment for traditional risk factors, the OR was 0.79 (95% CI = 0.56–1.13). For the campesterol concentration, the unadjusted OR was 0.95 (95% CI = 0.71–1.29) and the adjusted OR was 0.97 (95% CI = 0.68–1.39). In this large prospective study, higher levels of plant sterols, at least in the physiological range, do not appear to be adversely related to CAD in apparently healthy individuals.—Pinedo, S., M. N. Vissers, K. von Bergmann, K. Elharchaoui, D. Lütjohann, R. Luben, N. J. Wareham, J. J. P. Kastelein, K. T. Khaw, and S. M. Boekholdt. Plasma levels of plant sterols and the risk of coronary artery disease: the prospective EPIC-Norfolk Population Study. *J. Lipid Res.* 2007. **48:** 139–144.

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Dietary sterols consist mainly of animal-derived cholesterol and plant-derived phytosterols or plant sterols. The most abundant plant sterols are sitosterol and campesterol, with a chemical structure very similar to that of cholesterol, except for a single ethyl or methyl group in the side chain (1).

The usual daily intake of plant sterols in Western diets varies between 150 and 350 mg/day (2). In contrast to cholesterol, plant sterols cannot be synthesized in animals; they are derived exclusively from vegetables and vegetable oils and are thought to have cellular functions in these organisms that are similar to those of cholesterol in animals (3). In Western society, ~1,200–1,700 mg of cholesterol, of which approximately one-fourth is of dietary origin, enters the lumen of the small intestine every day (3). Approximately 50% of this cholesterol load is absorbed (4, 5), whereas for plant sterols this percentage ranges from merely 5% to sometimes 18%, depending on the type of sterol (6, 7).

The lower net absorption of plant sterols compared with cholesterol is attributable to the active resecretion of plant sterols back into the enteric lumen. This process is mediated by the ATP binding cassette (ABC) half-transporters ABCG5 and ABCG8 (8, 9). In individuals with dysfunctional ABCG5 and/or ABCG8, plant sterol net absorption is increased, resulting in 50-fold increased plasma levels of plant sterols. In patients with this rare inherited autosomal recessive disorder sitosterolemia, sitosterol levels range between 10 and 30 mg/dl (10), whereas in normal subjects, these levels seldom exceed 1 mg/dl (3). Phenotypical signs found in such patients with sitosterolemia are much like those of inherited hypercholesterolemia, i.e., devel-

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opment of xanthomas and premature coronary disease (8, 9, 11). These latter observations have led to the hypothesis that high levels of plant sterols are atherogenic.

In contrast, dietary plant sterols are currently being added to several food products for their reducing effect on plasma cholesterol levels. They compete with cholesterol for incorporation into micelles and thereby reduce cholesterol absorption in the intestine. Plasma levels of low density lipoprotein cholesterol (LDL-C) are reduced by ~10% as a consequence of foods enriched in plant sterols (12). However, consumption of such foods will also increase plasma plant sterol levels (13, 14), notwithstanding the fact such levels remain low compared with those in sitosterolemia patients. It is unclear whether such slightly increased plasma plant sterol concentrations over protracted periods of time, either physiologically or caused by plant sterol-enriched foods, constitute an additional risk factor for atherosclerosis.

Some studies have suggested that increased plasma plant sterol levels are associated with coronary artery disease (CAD) (15–18), but another study did not reveal any association with surrogate markers of atherosclerosis (19). Because the data are not consistent and data from prospective studies are limited, we examined the association between plasma levels of plant sterols and the risk of future CAD in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk cohort, comprising apparently healthy men and women who had never suffered a myocardial infarction or stroke.

METHODS

Study design

We performed a nested case-control study among participants of the EPIC-Norfolk Study, a prospective population study of 25,663 men and women aged between 45 and 79 years, resident in Norfolk, United Kingdom. All subjects completed a baseline questionnaire survey and attended a clinic visit (20). Participants were recruited from age-sex registers of general practices in Norfolk as part of a 10 country collaborative study (EPIC) designed to investigate dietary and other determinants of cancer. Additional data were obtained to enable the assessment of determinants of other diseases.

The design and methods of the study have been described previously in detail (20). In short, eligible participants were recruited by mail. At the baseline survey between 1993 and 1997, participants completed a detailed health and lifestyle questionnaire. Blood was taken by venipuncture into plain and citrate bottles. Blood samples were processed for assay at the Department of Clinical Biochemistry, University of Cambridge, or stored at −80°C. All individuals have been flagged for death certification at the UK Office of National Statistics, with vital status ascertained for the entire cohort. In addition, participants admitted to the hospital were identified using their unique National Health Service number by data linkage with ENCORE, the East Norfolk Health Authority database, which identifies all hospital contacts throughout England and Wales for Norfolk residents. Participants were identified as having CAD during follow-up if they had a hospital admission and/or died with CAD as the underlying cause. CAD was defined as codes 410 to 414 according to the International Classification of Diseases, 9th revision. We report results with follow-up to January 2003, an average of ~6 years. The study was approved by the Norwich District Health Authority Ethics Committee, and all participants gave signed informed consent.

Participants

For this analysis, we only considered individuals who did not report a history of heart attack or stroke at the baseline clinic visit. Of those individuals, 373 cases, in whom fatal or nonfatal CAD developed during follow-up, were randomly selected. Controls (758) were study participants who remained free of CAD during follow-up. Two controls were matched to each case by sex, age (within 5 years), and time of enrollment (within 3 months).

Biochemical analyses

Serum levels of total cholesterol, high density lipoprotein cholesterol (HDL-C), and triglycerides were measured on fresh samples with the RA 1000 (Bayer Diagnostics, Basingstoke, UK), and LDL-C levels were calculated with the Friedewald formula (21). In 2005, plasma samples for cases and controls were retrieved from frozen storage. The plasma noncholesterol sterols sitosterol, campesterol, and lathosterol were hydrolyzed, extracted, and analyzed as trimethylsilyl ethers by gas-liquid chromatography (Hewlett Packard 5890) using an automatic injection system (Hewlett Packard Automatic Sampler 7673A) with 5α-cholestane as the internal standard (22). Because noncholesterol sterols are transported in serum by lipoproteins, changes in lipoprotein concentrations also affect concentrations of noncholesterol sterols (23). Therefore, noncholesterol sterols are expressed in concentrations (mg/dl) as well as in ratios to cholesterol (μg/mg). To calculate the noncholesterol sterol-to-cholesterol ratios adequately, cholesterol levels were also analyzed by gas-liquid chromatography in the same run as the noncholesterol sterols.

Samples were analyzed in random order to avoid systemic bias. Researchers and laboratory personnel had no access to identifiable information and could identify samples by number only.

Statistical analysis

Baseline characteristics were compared between cases and controls using the GENMOD procedure of SAS (SAS Institute, Inc., Cary, NC), taking into account the matching between them. Data with a skewed distribution were first log-transformed, but in the tables we show untransformed medians and corresponding interquartile ranges (IQRs) or the distribution across tertiles for plant sterols.

To determine relationships between plant sterols and traditional cardiovascular risk factors, mean risk factor levels were calculated per sitosterol or campesterol tertile. Tertiles were based on the distribution in controls. The linearity between risk factor levels and plant sterol tertiles was calculated with the General Linear Models procedure in SAS. The GENMOD procedure was used for logistic regression analysis to calculate odds ratios (ORs) and corresponding 95% confidence intervals (CIs) as estimates of the relative risk of incident CAD, adjusted for matching variables. The lowest tertile was used as the reference category. ORs were adjusted for the following cardiovascular risk factors: age, sex, systolic blood pressure, total cholesterol, HDL-C, body mass index (BMI), smoking (never, past, current), and diabetes. ORs were also estimated after additional adjustment for the lathosterol concentration, a marker for cholesterol synthesis. ORs based on the plant sterol-to-cholesterol ratios were additionally adjusted for the lathosterol-to-cholesterol ratio instead of the lathosterol concentration. $P < 0.05$ was considered significant. Statistical analyses were computed with SAS software, version 9.1.

RESULTS

From the total number of 373 cases, 89 patients (24%) died from CAD and 284 patients (76%) had nonfatal CAD events. Baseline characteristics of cases and controls are presented in **Table 1**. Matching ensured that age and sex were comparable between cases and controls. As expected, individuals in whom CAD developed during follow-up were more likely to smoke and have diabetes than controls. Also, total cholesterol, LDL-C and triglycerides levels, systolic and diastolic blood pressure, and BMI were significantly higher in cases than in controls, whereas HDL-C levels were significantly lower.

As shown in Table 1, the baseline plasma sitosterol and campesterol concentrations did not differ between cases and controls. The median sitosterol concentration of 0.21 mg/dl (IQR, 0.15–0.28 mg/dl) in cases was equal to that in controls (0.21 mg/dl; IQR, 0.17–0.29 mg/dl; $P = 0.1$). The median campesterol concentration was 0.31 mg/dl in cases (IQR, 0.21–0.44 mg/dl) and 0.32 mg/dl in controls (IQR, 0.23–0.44 mg/dl; $P = 0.5$). However, the baseline sitosterol-to-cholesterol ratio was significantly lower in cases (1.19 µg/mg; IQR, 0.92–1.55 µg/mg) than in controls (1.29 µg/mg; IQR, 1.01–1.63 µg/mg; $P = 0.008$), whereas the campesterol-to-cholesterol ratio did not differ significantly between cases (1.78 µg/mg; IQR, 1.37–2.43 µg/mg) and controls (1.88 µg/mg; IQR, 1.43–2.51 µg/mg; $P = 0.1$). **Table 2** shows the distribution of cardiovascular risk factors by the tertiles of sitosterol and campesterol concentrations. The tertiles of the plant sterols were positively associated with plasma levels of total cholesterol, LDL-C, and HDL-C and inversely associated with BMI, plasma triglyceride levels, and lathosterol concentrations. When relationships were calculated using tertiles based on the plant sterol-to-cholesterol ratios, the significant associations with total cholesterol, LDL-C, and HDL-C were no longer apparent. The other associations were similar to those with plant sterol concentrations, except for an additional positive association between both plant sterol ratios and male gender and an inverse association between campesterol ratio and age (data not shown).

Table 3 shows the unadjusted and adjusted ORs for future CAD by plant sterol tertiles. There was no significant interaction between sex and sitosterol or campesterol concentrations in the unadjusted and adjusted regression models [sitosterol, $P = 0.5$ (unadjusted) and $P = 0.8$ (adjusted); campesterol, $P = 0.4$ (unadjusted) and $P = 0.6$ (adjusted)]. Therefore, data for men and women were pooled. In the unadjusted analyses, the sitosterol concentration was inversely associated with the risk of CAD in the middle and highest tertile compared with the lowest tertile; the unadjusted ORs were 0.63 (95% CI = 0.46–0.86) and 0.75 (95% CI = 0.56–1.01), respectively, with P for linearity = 0.05. However, even though the OR in the middle tertile of the sitosterol concentration remained significant after adjustment for traditional risk factors, the OR in the highest tertile and the P for linearity were no longer significant after adjustment (OR = 0.79, 95% CI = 0.56–1.13; $P = 0.2$). Campesterol concentrations were not significantly associated with the risk of CAD. Among individuals in the highest tertile, the unadjusted OR for future CAD was 0.95 (95% CI = 0.71–1.29; $P = 0.8$) and the adjusted OR was 0.97 (95% CI = 0.68–1.39; $P = 0.9$). Additional adjustment for the lathosterol-to-cholesterol ratio did not fundamentally change the ORs (Table 3). When plant sterol-to-cholesterol ratios were used instead of concentrations for the statistical analyses, the ORs were not essentially altered (Table 3).

TABLE 1. Baseline characteristics

Characteristics	Controls	Cases	P
Subjects, n	758	373	
Age, years	64.8 ± 8.1	64.7 ± 8.2	Matched
Male sex, n	465 (61.4)	232 (62.2)	Matched
Smoking			<0.0001
Never	332 (43.8)	118 (31.6)	
Past	361 (47.6)	186 (49.9)	
Current	59 (7.8)	63 (16.9)	
BMI, kg/m²	26.2 ± 3.5	27.4 ± 3.9	<0.0001
Total cholesterol, mmol/l	6.29 ± 1.21	6.53 ± 1.25	0.001
LDL cholesterol, mmol/l	4.05 ± 0.99	4.31 ± 1.09	<0.0001
HDL cholesterol, mmol/l	1.41 ± 0.41	1.28 ± 0.36	<0.0001
Triglycerides, mmol/l	1.6 [1.1–2.3]	1.9 [1.4–2.7]	<0.0001
Systolic blood pressure, mmHg	137.9 ± 17.1	142.0 ± 18.2	<0.0001
Diastolic blood pressure, mmHg	83.2 ± 11.0	85.4 ± 11.6	0.002
Diabetes	14 (1.9)	19 (5.1)	0.003
Noncholesterol sterols			
Sitosterol, mg/dl	0.21 [0.17–0.29]	0.21 [0.15–0.28]	0.1
Campesterol, mg/dl	0.32 [0.23–0.44]	0.31 [0.21–0.44]	0.5
Lathosterol, mg/dl	0.19 [0.14–0.26]	0.20 [0.15–0.27]	0.07
Sitosterol-to-cholesterol ratio, µg/mg	1.29 [1.01–1.63]	1.19 [0.92–1.55]	0.008
Campesterol-to-cholesterol ratio, µg/mg	1.88 [1.43–2.51]	1.78 [1.37–2.43]	0.1
Lathosterol-to-cholesterol ratio, µg/mg	1.15 [0.87–1.51]	1.19 [0.90–1.53]	0.2

BMI, body mass index. Data are presented as means ± SD, median [interquartile ranges], or number (%). Analyses were performed by the GENMOD procedure in SAS 9.1 and were adjusted for matched pairs.

TABLE 2. Distribution of cardiovascular risk factors by tertiles of sitosterol or campesterol concentrations

Characteristics	Sitosterol				Campesterol			
	Tertile 1 (n = 408)	Tertile 2 (n = 352)	Tertile 3 (n = 371)	P	Tertile 1 (n = 381)	Tertile 2 (n = 373)	Tertile 3 (n = 377)	P
Age, years	65.1 ± 8.0	64.4 ± 8.0	64.8 ± 8.4	0.6	65.6 ± 7.4	64.5 ± 8.4	64.2 ± 8.5	0.02
Male sex, n (%)	258 (63.2)	224 (63.6)	215 (58.0)	0.1	234 (61.4)	231 (61.9)	232 (61.5)	1.0
Smoking				0.7				0.5
Never	167 (41.6)	130 (37.3)	153 (41.5)		163 (43.8)	134 (35.9)	153 (40.9)	
Past	182 (45.4)	186 (53.3)	179 (48.5)		170 (45.7)	195 (52.3)	182 (48.7)	
Current	52 (13.0)	33 (9.5)	37 (10.0)		39 (10.5)	44 (11.8)	39 (10.4)	
BMI, kg/m^2	27.7 ± 4.1	26.6 ± 3.4	25.5 ± 3.2	<0.0001	27.6 ± 3.8	26.6 ± 3.7	25.7 ± 3.3	<0.0001
Total cholesterol, mmol/l	5.98 ± 1.09	6.35 ± 1.12	6.81 ± 1.34	<0.0001	5.91 ± 1.08	6.37 ± 1.25	6.83 ± 1.19	<0.0001
LDL cholesterol, mmol/l	3.81 ± 0.94	4.09 ± 0.94	4.52 ± 1.08	<0.0001	3.73 ± 0.93	4.09 ± 0.92	4.58 ± 1.06	<0.0001
HDL cholesterol, mmol/l	1.29 ± 0.38	1.37 ± 0.41	1.44 ± 0.41	<0.0001	1.30 ± 0.40	1.37 ± 0.39	1.42 ± 0.41	<0.0001
Triglycerides, mmol/l	2.06 ± 1.19	2.03 ± 1.13	1.91 ± 1.66	0.005	2.03 ± 1.19	2.07 ± 1.69	1.90 ± 1.07	0.07
Systolic blood pressure, mmHg	138.7 ± 17.5	140.9 ± 17.2	138.2 ± 17.8	0.8	138.6 ± 17.6	140.9 ± 17.0	138.4 ± 17.9	0.9
Diastolic blood pressure, mmHg	83.8 ± 11.4	84.8 ± 11.0	83.2 ± 11.3	0.5	83.3 ± 11.4	84.7 ± 10.6	83.6 ± 11.7	0.6
Diabetes, n (%)	16 (3.9)	7 (2.0)	10 (2.7)	0.3	12 (3.2)	12 (3.2)	9 (2.4)	0.5
Sitosterol, mg/dl	0.14 ± 0.03	0.22 ± 0.02	0.35 ± 0.09	<0.0001	0.15 ± 0.04	0.22 ± 0.05	0.33 ± 0.10	<0.0001
Campesterol, mg/dl	0.21 ± 0.07	0.33 ± 0.08	0.52 ± 0.16	<0.0001	0.19 ± 0.05	0.32 ± 0.04	0.54 ± 0.14	<0.0001
Lathosterol, mg/dl	0.22 ± 0.10	0.21 ± 0.09	0.20 ± 0.12	0.08	0.22 ± 0.11	0.21 ± 0.11	0.20 ± 0.10	0.008
Sitosterol-to-cholesterol ratio, µg/mg	0.96 ± 0.24	1.29 ± 0.26	1.82 ± 0.49	<0.0001	1.00 ± 0.28	1.28 ± 0.32	1.76 ± 0.53	<0.0001
Campesterol-to-cholesterol ratio, µg/mg	1.42 ± 0.51	1.93 ± 0.54	2.76 ± 0.87	<0.0001	1.30 ± 0.38	1.89 ± 0.42	2.87 ± 0.80	<0.0001
Lathosterol-to-cholesterol ratio, µg/mg	1.43 ± 0.60	1.21 ± 0.42	1.05 ± 0.61	<0.0001	1.48 ± 0.60	1.22 ± 0.60	1.01 ± 0.42	<0.0001

Data are presented as means ± SD or number (%) per tertile. P indicates P for linearity between plant sterol tertiles and risk factor levels.

DISCUSSION

Increased plasma levels of plant sterols were not associated with the risk of CAD in apparently healthy individuals in this prospective study. On the contrary, the sitosterol-to-cholesterol ratio was inversely associated with CAD, but significance was lost after adjustment for traditional risk factors.

The hypothesis that plasma plant sterol levels may be atherogenic has been supported by a few previous studies (15–18, 24). Glueck et al. (15) measured plant sterol concentrations in nonsitosterolemic individuals and concluded that plant sterols are associated with a personal or family history of CAD in subjects younger than 55 years. However, only individuals with hypercholesterolemia, who are already at higher risk, were included, and it is difficult to disentangle the atherogenic effect of cholesterol versus plant sterols in that study. A nested case-control study from the original Prospective Cardiovascular Munster Study among only men (n = 477) showed that subjects with increased sitosterol levels had a 1.8-fold risk of major coronary events compared with subjects with low sitosterol levels. Moreover, in men with a >20% coronary event risk over 10 years, high sitosterol levels were associated with an additional 3-fold increase in risk (17). However, only univariate analysis was performed, and it is unclear whether the positive association between plasma sitosterol levels and coronary events would have remained significant if multivariate analyses had been performed to adjust for potential confounders. Finally, recent findings in the Framingham Offspring Study also showed a significant association between plant sterol-to-cholesterol ratios and CAD (24). However, in that cohort, lipids and lipoproteins were not associated with CAD, which could cast doubts on the validity of the selection of cases and controls. In summary, even though some studies have suggested a positive association between plant sterol levels and CAD risk, these data should be interpreted with caution.

Our findings indicate that plant sterols are not associated with CAD risk, consistent with the results described by Wilund and coworkers (19). They investigated a possible relation between plant sterol-to-cholesterol ratios and coronary atherosclerosis by measuring coronary calcium with electron beam computed tomography scanning in the Dallas Heart Study. They found no difference in median plasma sitosterol and campesterol ratios between subjects with and without positive calcium scores, and there was no correlation between plasma levels of plant sterols and the amount of electron beam computed tomography-detected calcium. Thus, our findings, showing no positive association between plasma plant sterol ratios and clinical cardiovascular end points in a nested case-control analysis from a large prospective study, endorse the findings of Wilund and colleagues (19), which were based on a surrogate marker of CAD.

In our study, sitosterol levels unexpectedly tended to be inversely associated with CAD risk, in contrast to the positive association found by others (15–18, 24). Even though

TABLE 3. ORs for future CAD events by plant sterol tertiles, in absolute concentrations and in ratios to cholesterol, in men and women together

Variable	Tertile 1	Tertile 2	Tertile 3	P
Sitosterol, mg/dl	<0.1836	0.1836–0.2586	>0.2586	
n	408	352	371	
OR unadjusted	1.00	0.63 (0.46–0.86)	0.75 (0.56–1.01)	0.05
OR, adjusted (1)	1.00	0.68 (0.49–0.95)	0.79 (0.56–1.13)	0.2
OR, adjusted (2)	1.00	0.68 (0.49–0.95)	0.78 (0.55–1.11)	0.2
Campesterol, mg/dl	<0.2564	0.2564–0.3893	>0.3893	
n	381	373	377	
OR unadjusted	1.00	0.92 (0.68–1.25)	0.95 (0.71–1.29)	0.8
OR, adjusted (1)	1.00	0.92 (0.65–1.29)	0.97 (0.68–1.39)	0.9
OR, adjusted (2)	1.00	0.90 (0.64–1.28)	0.94 (0.65–1.35)	0.7
Sitosterol-to-cholesterol ratio, µg/mg	<1.102	1.102–1.502	>1.502	
n	411	361	359	
OR unadjusted	1.00	0.67 (0.50–0.91)	0.66 (0.49–0.89)	0.006
OR, adjusted (1)	1.00	0.74 (0.53–1.04)	0.90 (0.64–1.26)	0.5
OR, adjusted (2)	1.00	0.73 (0.52–1.01)	0.85 (0.60–1.21)	0.3
Campesterol-to-cholesterol ratio, µg/mg	<1.562	1.562–2.267	>2.267	
n	384	379	368	
OR unadjusted	1.00	0.94 (0.70–1.28)	0.86 (0.63–1.17)	0.3
OR, adjusted (1)	1.00	0.99 (0.71–1.39)	1.09 (0.77–1.54)	0.6
OR, adjusted (2)	1.00	0.98 (0.69–1.38)	1.05 (0.72–1.51)	0.8

CAD, coronary artery disease; OR, odds ratio. ORs for the risk of CAD for sexes combined, adjusted for sex and age. (1) Adjustment for sex, age, systolic blood pressure, total cholesterol, high density lipoprotein cholesterol, BMI, smoking, and diabetes; (2) adjustment for the variables above and in addition lathosterol-to-cholesterol ratio. P indicates P for linearity between plant sterol tertiles and CAD risk.

this finding may be surprising, all other associations between plant sterol levels and traditional CAD risk factors, as well as those between traditional risk factors and CAD risk, were in the expected direction. For instance, levels of total cholesterol, LDL-C, triglycerides, systolic and diastolic blood pressure, and BMI were significantly higher, whereas HDL-C levels were significantly lower in cases than in controls. Also, as reported previously (18, 25), the sitosterol and campesterol concentrations and their ratios to cholesterol were inversely correlated with BMI, plasma triglycerides, and lathosterol concentrations, a marker of cholesterol synthesis (2). Furthermore, the plasma sitosterol and campesterol levels and their ratios to cholesterol were in the same range as shown in previous studies (15–19). For instance, median plasma sitosterol and campesterol levels were 0.21 and 0.32 mg/dl, respectively. Those concentrations correspond with those reported in other studies, in which mean plasma sitosterol and campesterol concentrations ranged from 0.17 to 0.40 mg/dl and from 0.21 to 0.50 mg/dl, respectively (15–18). These identical plant sterol concentrations and associations with traditional risk factors of CAD in this study compared with previous studies strengthen the reliability of our data, which indicate that plant sterol levels are not associated with CAD risk, at least not positively.

Consumption of food products enriched with plant sterols has been shown to reduce cholesterol absorption and thereby plasma LDL-C levels. Clinical trials have shown that an intake of 2–3 g/day plant sterols significantly reduces serum LDL-C concentrations from 9% to 14% (12, 26). They do so by reducing the absorption of cholesterol from the intestine by competing with cholesterol for incorporation into mixed micelles, the latter being essential for the transport of sterols to and their uptake by mucosal cells. Total concentrations of plant sterols in plasma in subjects consuming sterol ester products are within the range of 0.6–2.0 mg/dl (13, 14). Although this is 20 to 100 times lower than in patients homozygous for sitosterolemia, some concerns have been raised that the increase in plasma plant sterol levels attributable to the use of plant sterol-enriched food products as a cholesterol-lowering agent may in fact be atherogenic. Our study cannot address this issue, because the participants did not use food products enriched with plant sterols. However, based on our data, we speculate that it is unlikely that such small concentrations of plasma plant sterols are detrimental, especially when taking the cholesterol-lowering effect of such food products into account.

A number of issues have to be taken into account when interpreting the results of our study. First, CAD events were ascertained through death certification and hospital admission data, which are likely to lead to both under-ascertainment and misclassification of cases. However, previous validation studies in our cohort indicate high specificity of such case ascertainment (27), which was at least equivalent to that of other large prospective cohort studies. Second, we cannot exclude the possibility that sample storage at −80°C for 10 years may have affected the plant sterol concentrations of plasma in the tubes. However, data on 10 year stability of plant sterols in frozen plasma are lacking. Nonetheless, plant sterol concentrations in our cohort were comparable to those in previous studies (15, 17, 28); hence, a loss of plant sterols during storage seems unlikely. Finally, plasma levels of plant sterols were determined in a single sample that was obtained from nonfasting subjects and at a nonuniform time of the day. Diurnal variation and variation over time could have affected the plant sterol concentrations. However, intraindividual variation in the plasma concentrations of noncholesterol sterols is minimal (29). Furthermore, the half-lives of sitosterol and campesterol are 3 and 4 days, respectively, and excessive variation during the day is not

expected (30, 31). Therefore, it is unlikely that variation over time would have influenced our results.

We conclude that slightly increased plasma levels of plant sterols, at least in the physiological range, are not positively associated with the risk of CAD and do not appear to be adversely related to CAD in apparently healthy individuals. Within this range, these findings provide reassurance for the safe addition of plant sterols to food products.▮

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