Accumulation of cholesterol precursors and plant sterols in human stenotic aortic valves

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Abstract   The pathogenesis of aortic valve stenosis (AS) is characterized by the accumulation of LDL-derived cholesterol in the diseased valves. Since LDL particles also contain plant sterols, we investigated whether plant sterols accumulate in aortic valve lesions. Serum samples were collected from 82 patients with severe AS and from 12 control subjects. Aortic valves were obtained from a subpopulation of 21 AS patients undergoing valve surgery and from 10 controls. Serum and valvular total cholesterol and noncholesterol sterols were measured by gas-liquid chromatography. Noncholesterol sterols, including both cholesterol precursors and sterols reflecting cholesterol absorption, were detected in serum samples and aortic valves. The higher the ratios to cholesterol of the cholesterol precursors and absorption markers in serum, the higher their ratios in the stenotic aortic valves ($r = 0.74$, $P < 0.001$ for lathosterol and $r = 0.88$, $P < 0.001$ for campesterol). The valvular ratio to cholesterol of lathosterol correlated negatively with the aortic valve area ($r = -0.47$, $P = 0.045$), suggesting attenuation of cholesterol synthesis with increasing severity of AS. The higher the absorption of cholesterol, the higher the plant sterol contents in stenotic aortic valves.▐▌ **These findings suggest that local accumulation of plant sterols and cholesterol precursors may participate in the pathobiology of aortic valve disease.**—Helske, S., T. Miettinen, H. Gylling, M. Mäyränpää, J. Lommi, H. Turto, K. Werkkala, M. Kupari, and P. T. Kovanen. **Accumulation of cholesterol precursors and plant sterols in human stenotic aortic valves.** *J. Lipid Res.* **2008.** 49: 1511–1518.

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The prevalence of nonrheumatic aortic valve stenosis (AS) is rapidly increasing due to general aging of the population, with clinically significant AS being present in 2% and even in 5.5% of individuals over 65 and 85 years of age, respectively (1, 2). Epidemiological risk factors of AS resemble those of atherosclerosis, including elevated serum LDL cholesterol, hypertension, smoking, diabetes, and male sex (1, 3, 4). Furthermore, LDL cholesterol accumulates in stenotic aortic valves (5–7), and experimental AS can be induced by dietary hypercholesterolemia in animal models (8–11). Finally, LDL cholesterol and its oxidation products may stimulate local inflammation in the valves and may also accelerate cell proliferation, bone matrix production, and subsequent calcification of the valves (6, 9, 12). In this respect, lowering the amount of circulating LDL, and thus also the amount of the LDL potentially entering the valve leaflets, could be beneficial to AS patients. Indeed, several retrospective studies have suggested that lowering serum LDL cholesterol by statins is associated with slower progression of AS (13–18). However, the only published prospective randomized trial failed to show a benefit for statin treatment in AS patients (19).

In addition to cholesterol, LDL particles contain noncholesterol sterols, including cholesterol precursors reflecting hepatic cholesterol synthesis (i.e., cholestenol, desmosterol, lathosterol, and squalene) and sterols reflecting intestinal cholesterol absorption (i.e., cholestanol and the plant sterols campesterol, sitosterol, and avenasterol) (20). While statin treatment attenuates cholesterol synthesis, it enhances the absorption of plant sterols from the intestine, thus elevating their concentrations in serum (20–23). Plant sterols or phytosterols compete with cholesterol for intestinal absorption, and food products containing plant sterols or their saturated derivatives, stanols, have been used alone or with statins to reduce serum cholesterol concentrations (24). Despite these generally

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accepted beneficial effects of plant sterols, issues of controversy regarding their protective effects on cardiovascular diseases exist (24, 25). Most convincingly, individuals with the rare genetic disorder phytosterolemia, who overabsorb plant sterols, also manifest tendon xanthomas and premature atherosclerotic cardiovascular disease (26, 27). Indeed, the development of extremely early atherosclerotic disease, despite the presence of normal or only slightly elevated serum cholesterol levels but markedly increased phytosterol levels in these patients, suggests that plant sterols could be injurious to cardiovascular tissue (24). Interestingly, early presentation of supravalvular aortic stenosis also has been described in a patient with phytosterolemia (28), suggesting that plant sterols could enter the supravalvular aortic tissue and perhaps even the aortic valve leaflets and participate in the pathogenesis of aortic valve disease in this rare metabolic condition.

Recent studies have portrayed the possibility of plant sterols being able to participate in the atherosclerotic process in the general population. Phytosterols have been identified in atherosclerotic plaques obtained from individuals with apparently normal absorption of plant sterols (29). Moreover, in some clinical studies, elevated circulating levels of plant sterols have been associated with the occurrence and severity of coronary artery disease (CAD) as well as with a positive family history of CAD (30–33). However, negative results regarding the relation of plant sterols and vascular disease also exist (34, 35), rendering additional large-scale investigations in this area mandatory. In the present study, we investigated whether plant sterols accumulate in aortic valve leaflets and whether the degree of such accumulation would be related to circulating concentrations of the respective plant sterols.

METHODS

Samples and study population

In the present study, we included 82 patients with clinically significant, symptomatic AS referred to the Helsinki University Central Hospital for valve replacement surgery. Only patients with isolated AS were included (i.e., those with more than mild aortic or mitral regurgitation or mitral stenosis were excluded). Other exclusion criteria included history of myocardial infarction or angiographically determined CAD (any proximal coronary artery stenosis > 50% of the luminal diameter), previous cardiac surgery, complicated diabetes, renal insufficiency (serum creatinine > 170 μmol/l), endocarditis, or malignancy. All patients underwent echocardiography and cardiac catheterization with coronary angiography. A more detailed description of the study population has been published elsewhere (36). The study protocol was approved by the Institutional Ethics Committee, and all participants signed an informed consent document. The investigation conformed with the principles outlined in the Declaration of Helsinki.

The mean age of the patients was 67 ± 10 years, and their mean body mass index (BMI) was 27 ± 4.5 kg/m². Mean (±SD) serum total cholesterol level was 5.15 ± 1.05 mmol/l, serum LDL was 3.14 ± 0.87 mmol/l, HDL was 1.44 ± 0.44 mmol/l, and triglycerides were 1.24 ± 0.44 mmol/l. Of the 82 patients, 20% received statin therapy (various agents), and 13 had consumed

margarine or yogurt supplemented with plant stanol (Benecol®; n = 9) or plant sterol esters (Becel Pro-active®; n = 3) regularly on a daily basis prior to the valve replacement. Plant stanol or sterol supplements were not used by 58 patients, and reliable information was not available from 11 individuals. Control blood samples were obtained from 12 subjects undergoing electrophysiological studies for tachyarrythmias or unexplained syncope. The control subjects were free of structural heart disease and had normal echocardiographic findings. Characteristics of the patients and controls, from whom the blood samples were obtained, are shown in **Table 1**. Blood samples were obtained from the femoral vein of all AS patients and control subjects. Serum was separated from the blood by mild centrifugation and stored at −70°C until analysis.

Stenotic aortic valves removed at valve replacement surgery were collected from a random subpopulation of the AS patients (n = 21). Of these, four patients had used margarine or yogurt supplemented with plant stanol (Benecol®; n = 2) or plant sterol esters (Becel pro-active®; n = 2) regularly prior to the surgery. In this subgroup of 21 patients, statin therapy was used by 8 individuals. As control tissue samples, a separate subset of nonstenotic aortic valves (n = 10) was obtained from medicolegal autopsies. The stenotic and nonstenotic valves were snap-frozen in liquid nitrogen and stored at −70°C until analysis.

Analysis of noncholesterol sterols and squalene in serum samples and aortic valves

Serum total and HDL cholesterol and triglycerides were quantified by routine methods used in our hospital. Serum noncholesterol sterols (cholestenol, desmosterol, lathosterol, campesterol, sitosterol, sitostanol, avenasterol, and cholestanol) and squalene were quantified by gas-liquid chromatography (GLC) using a 50 m long ULTRA-1 SE-30 column (Hewlett-Packard, Wilmington, DE) principally as shown elsewhere (37). For this purpose, 0.2 ml of serum was saponified after addition of 5α-cholestane as an

TABLE 1. Characteristics of the patients with AS and control subjects (mean ± SD or number of patients)

Characteristic	Patients with AS (n = 82)	Control Subjects (n = 12)
Age, years	67 ± 10	57 ± 4
Sex, male/female	39/43	7/5
New York Heart Association class, 1/2/3/4	2/50/28/2	All class 1
Aortic valve area index, cm²/m²	0.36 ± 0.10	
Mean pressure gradient, mmHg	49 ± 16	
Body mass index, kg/m²	26.8 ± 4.5	27.3 ± 3.1
Prevalence of		
Left ventricular hypertrophy[a]	63 (77%)	0
Hypertension	27 (33%)	2 (17%)
Diabetes	3 (4%)	0
Bicuspid/tricuspid valve/four cusps	12/69/1	0/12/0
Smoking, no/yes	68/14	
Plant stanol/plant sterol ester supplements (data were not available from 11 subjects)	9 (11%)/3 (4%)	Unknown
Medication		
Angiotensin-converting enzyme inhibitor/AT1 blocker	17 (21%)	2 (17%)
β-Blockers	53 (65%)	4 (33%)
Diuretics	28 (34%)	0
Statins	16 (20%)	2 (17%)
Digitalis	5 (6%)	1 (1.2%)

AS, aortic valve stenosis.
[a] Echocardiographic left ventricular mass index exceeding 110 g/m² in women and 134 g/m² in men.

internal standard. The sterols were extracted and converted after evaporation of the solvent to TMS derivatives for the GLC running. The values for the noncholesterol sterols and squalene are expressed as μg/100 ml serum or as their ratios to the respective serum cholesterol values ($10^2 \times$ μg/mg cholesterol).

To analyze the amount of noncholesterol sterols and squalene in valves, aortic valve tissue (100–360 mg) was weighted and the lipids were extracted by homogenizing the tissue with chloroform-methanol. Before extraction, 5α-cholestane and epicoprostanol were added as internal standards. Each homogenate was extracted three times, and the extract was evaporated and transferred in a small volume of ethyl ether onto a TLC plate coated with silica gel, and free and esterified sterol fractions were separated with hexane-ethyl ether (50:50, v/v). The fractions were extracted from the plate, and the ester fraction (including also 5α-cholestane and squalene) was saponified, after which the nonsaponifiable lipids were extracted with ethyl ether and the solvent was evaporated. The sterol fractions were silylated, and the noncholesterol sterols and squalene were quantified by GLC. The amounts of noncholesterol sterols and squalene in aortic valves are expressed as μg/100 g valvular tissue or as ratios of the sterols to the respective cholesterol values in the valves ($10^2 \times$ μg/mg cholesterol).

Statistics

For statistical calculations, SPSS software (version 11.0) was used. Differences between the groups were analyzed using Student's *t*-test or the Mann-Whitney *U*-test depending on data distribution. Normality of distribution was tested with the Kolmogorov-Smirnov method. The results are given as mean values and SD, or as medians and ranges. Differences were considered statistically significant at $P < 0.05$. Correlation coefficients were calculated with the Spearman rank correlation.

RESULTS

Serum concentrations of cholesterol, noncholesterol sterols, and squalene

The concentrations of cholesterol, noncholesterol sterols (cholestanol, lathosterol, desmosterol, campesterol, sitosterol, and avenasterol), sitostanol, and squalene in serum samples of AS patients and controls were in the same range (**Table 2**), the only statistically significant difference between patients and controls being the concentration and the ratio to cholesterol of cholestenol, which were higher in AS patients than in control subjects (Table 2; $P < 0.001$ for both). Serum concentrations of cholestenol, desmosterol, lathosterol, cholestanol, avenasterol, and squalene correlated positively with serum total cholesterol levels ($r = 0.42–0.83$, $P = 0.02$ to $P < 0.001$).

Comparison of the ratios to cholesterol of the noncholesterol sterols revealed that the ratios, which reflect cholesterol synthesis correlated positively with each other (e.g., $r = 0.62$, $P < 0.001$ for desmosterol and lathosterol). Similarly, the serum ratios to cholesterol of the plant sterols (avenasterol, sitosterol, and campesterol) and cholestanol correlated positively with each other (e.g., $r = 0.84$, $P < 0.001$ for campesterol and sitosterol). Furthermore, the serum ratios to cholesterol of lathosterol correlated negatively with those of campesterol ($r = -0.56$, $P = 0.001$) and sitosterol ($r = -0.58$, $P < 0.001$). The patients who

were treated with statins (n = 16) had lower serum total cholesterol levels ($P < 0.003$) than the subjects not receiving statins (n = 66). Similarly, the ratios to cholesterol of the cholesterol precursors desmosterol and lathosterol were lower in subjects receiving statin therapy ($P = 0.01$ and $P < 0.001$, respectively). In contrast, the serum ratios to cholesterol of the plant sterols campesterol and sitosterol were higher in patients treated with statins compared with individuals without statin therapy ($P = 0.03$ and $P = 0.006$, respectively). Serum concentrations of cholesterol and noncholesterol sterols did not differ significantly between patients with and without having consumed margarine or yogurt supplemented with plant stanol (Benecol®; n = 9) or plant sterol esters (Becel pro-active®; n = 3). However, data concerning the use of plant stanol or plant sterol ester supplements were not available from 11 patients.

Concentrations of cholesterol, noncholesterol sterols, and squalene in aortic valves

Mean concentrations and the variations of cholesterol, noncholesterol sterols, and squalene (μg/100 g tissue ± SD) in aortic valves are shown in Table 2.

Analysis of aortic valves with GLC revealed that, besides cholesterol, the cholesterol precursors cholestenol, des-

TABLE 2. Concentrations of cholesterol, noncholesterol sterols, and squalene in serum (S) and in aortic valves (AV) of patients with AS and control subjects (mean ± SD)

Variable	Patients with AS (n = 82)	Controls (n = 12)[a]
Total cholesterol[b]		
S (mg/100 ml) (n = 82)	183 ± 37	190 ± 38
AV (mg/100 g tissue) (n = 21)	813 ± 385	787 ± 948
Squalene		
S (μg/100 ml)	28 ± 13	36 ± 19
AV (μg/100 g tissue)	693 ± 1,099	1,247 ± 752
Cholestenol		
S (μg/100 ml)	94 ± 49	36 ± 16[c]
AV (μg/100 g tissue)	146 ± 64	129 ± 90
Lathosterol		
S (μg/100 ml)	206 ± 98	221 ± 121
AV (μg/100 g tissue)	590 ± 354	508 ± 397
Desmosterol		
S (μg/100 ml)	187 ± 70	172 ± 60
AV (μg/100 g tissue)	918 ± 460	831 ± 784
Campesterol		
S (μg/100 ml)	483 ± 271	507 ± 169
AV (μg/100 g tissue)	2,407 ± 1,480	1,887 ± 2108
Sitosterol		
S (μg/100 ml)	227 ± 116	241 ± 79
AV (μg/100 g tissue)	1,193 ± 662	926 ± 1017
Sitostanol		
S (μg/100 ml)	14 ± 7	13 ± 5
AV (μg/100 g tissue)	122 ± 70	132 ± 60
Avenasterol		
S (μg/100 ml)	71 ± 23	74 ± 15
AV (μg/100 g tissue)	365 ± 149	420 ± 237
Cholestanol		
S (μg/100 ml)	284 ± 76	302 ± 76
AV (μg/100 g tissue)	1,654 ± 872	2,263 ± 3151

[a] The control group consisted of two subgroups. Serum samples were collected from the first subgroup (undergoing electrophysiological studies), and aortic valves were obtained from the second subgroup (from medicolegal autopsies).
[b] Analyzed with gas-liquid chromatography.
[c] $P < 0.001$.

mosterol, lathosterol, and squalene were present in aortic valvular tissue. Furthermore, cholestanol, plant sterols including campesterol, sitosterol, and avenasterol, and the plant stanol sitostanol were detected in the valves. The concentrations of all cholesterol precursors (except squalene), cholestanol, and plant sterols in the valves correlated with those of cholesterol in the valves (r values ranged from 0.53 for sitostanol to 0.95 for desmosterol, $P < 0.001$ for all). The correlation between valvular cholesterol and sitosterol is shown in **Fig. 1A**. Furthermore, the concentrations of noncholesterol sterols (including cholesterol precursors and plant sterols) in aortic valves correlated positively with each other (r values ranged from 0.38 for cholestenol-sitostanol, $P = 0.037$, to 0.98 for sitosterol-campesterol, $P < 0.001$). Squalene, in contrast, correlated positively only with valvular sitostanol concentrations ($r = 0.48$, $P = 0.007$). Patients using statin therapy (n = 8) had a trend toward higher levels of plant sterols (campesterol, sitosterol, and avenasterol) and cholestanol in their aortic valves, although these differences were not statistically significant. The concentration of sitostanol, instead, was

significantly higher in subjects receiving statins compared with that of subjects not receiving statins ($P = 0.048$).

When valvular noncholesterol sterols and squalene concentrations were proportioned to valvular cholesterol concentrations, a positive correlation appeared between the cholesterol precursors cholestenol, desmosterol, lathosterol, and squalene ($r = 0.37$–0.84, $P < 0.05$ to $P < 0.001$). Similarly, the ratios of plant sterols (campesterol, sitosterol, and avenasterol) to cholesterol correlated positively with each other ($r = 0.38$–0.84, $P < 0.05$ to $P < 0.001$). The aortic valvular total cholesterol levels correlated negatively with the ratios to cholesterol of cholestenol ($r = -0.61$, $P < 0.001$), lathosterol ($r = -0.48$, $P = 0.007$), sitostanol ($r = -0.60$, $P < 0.001$), avenasterol ($r = -0.72$, $P < 0.001$), and squalene ($r = -0.73$, $P < 0.001$). Interestingly, ratios of valve sitostanol to cholesterol correlated positively with those of the cholesterol precursors desmosterol ($r = 0.54$, $P = 0.002$) and lathosterol ($r = 0.36$, $P = 0.046$). In aortic valvular tissue, the ratio to cholesterol of the cholesterol precursor lathosterol correlated negatively with aortic valve area (AVA) ($r = -0.47$, $P = 0.045$). Of those 21 AS patients from whom aortic valves were obtained, only 4 individuals had consumed margarine or yogurt supplemented with plant stanol (Benecol®; n = 2) or plant sterol esters (Becel Pro-active®; n = 2). In these patients, aortic valve concentrations of cholesterol, noncholesterol sterols, and squalene, as well as their ratios to cholesterol, were similar to those of subjects not consuming these products. However, the ratio to cholesterol of lathosterol, a marker of hepatic cholesterol synthesis, was higher in aortic valves of patients who had consumed plant sterol ester supplements (136.5 ± 2.1 vs. 68.8 ± 30.7 $10^2 \times \mu g/mg$ cholesterol; $P = 0.007$).

Negative correlation between BMI and the amount of plant sterols in serum and in the stenotic aortic valves

BMI correlated negatively with the ratios to cholesterol of serum sitosterol ($r = -0.32$, $P = 0.003$) and avenasterol ($r = -0.23$, $P = 0.03$), whereas it correlated positively with the serum total cholesterol levels ($r = 0.24$, $P = 0.03$) and the ratios to cholesterol of the serum cholesterol precursors desmosterol ($r = 0.25$, $P = 0.02$) and lathosterol ($r = 0.36$, $P = 0.001$). Furthermore, a negative correlation appeared between BMI and the valvular ratios to cholesterol of the plant sterols campesterol ($r = -0.48$, $P = 0.028$), sitosterol ($r = -0.70$, $P < 0.001$; Fig. 1B), and avenasterol ($r = -0.48$, $P = 0.027$).

Relation of noncholesterol sterol ratios in serum and in aortic valves

The higher the ratios to cholesterol of the various cholesterol precursors and absorption sterols in serum, the higher their ratios in the stenotic aortic valves (e.g., $r = 0.74$, $P < 0.001$ for lathosterol and $r = 0.88$, $P < 0.001$ for campesterol; **Fig. 2**). Importantly, a strong positive correlation appeared between the ratio of sitosterol (indicating sterol absorption) and lathosterol (indicating cholesterol synthesis) in serum and in stenotic aortic valves ($r = 0.84$, $P < 0.0001$; **Fig. 3**). Thus, the higher the ratio of the absorption sterols (e.g., sitosterol) and the cholesterol precursors
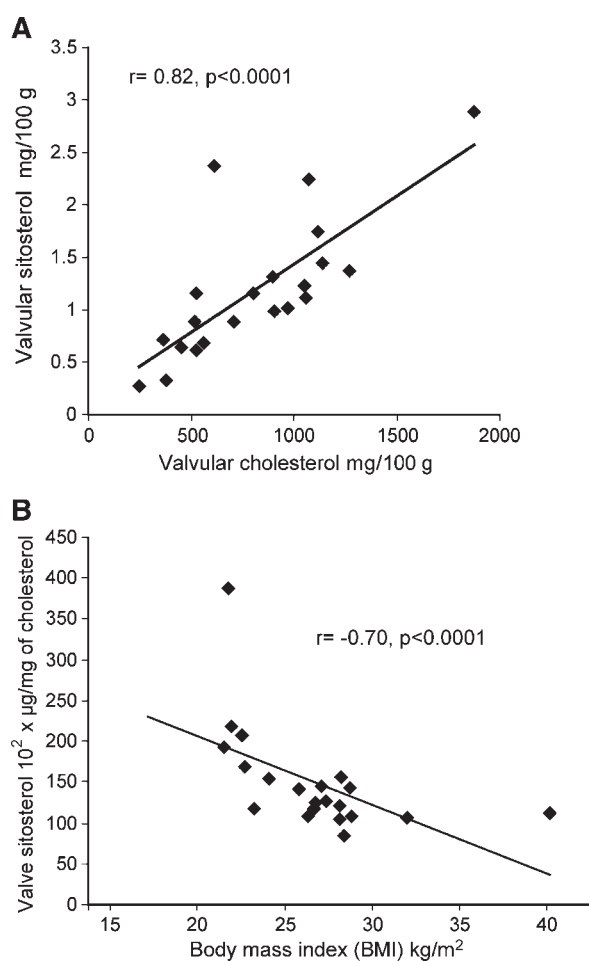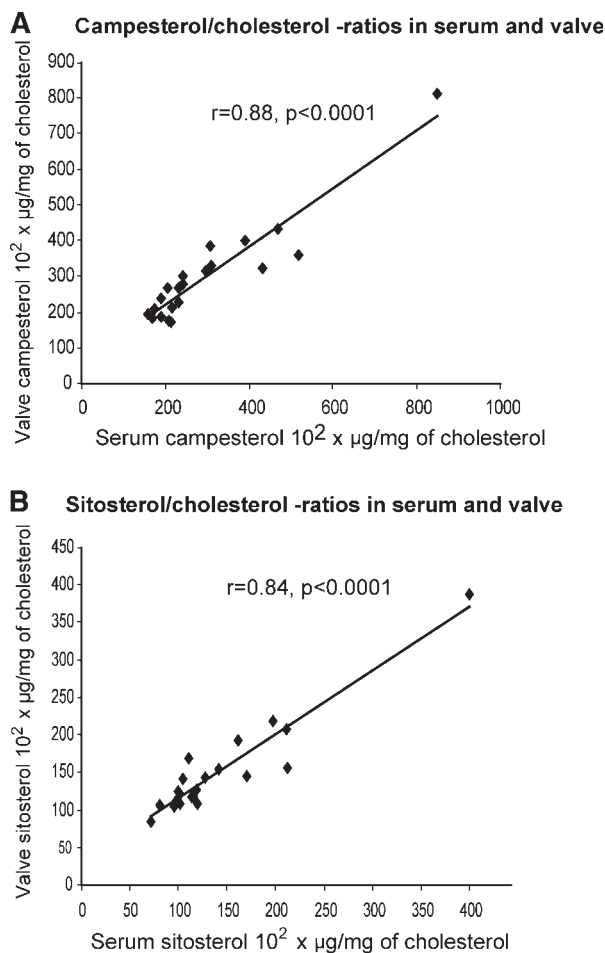

Fig. 1. A: Correlation between aortic valve cholesterol content (mg/100 g) and aortic valve sitosterol concentration (mg/100 g). B: Correlation of aortic valve sitosterol content (relative to cholesterol) with body mass index.

A

Campesterol/cholesterol -ratios in serum and valve

r=0.88, p<0.0001

B

Sitosterol/cholesterol -ratios in serum and valve

r=0.84, p<0.0001

Fig. 2. Correlation of serum ratios of campesterol (A) and sitosterol (B) to cholesterol with those of aortic valve campesterol and sitosterol.

(e.g., lathosterol) in the circulation, the higher their ratio in the diseased valves. This correlation remained highly significant also when the serum ratios to cholesterol of sitosterol/lathosterol were correlated with the valvular ratios to cholesterol of sitosterol/lathosterol ($r = 0.84$, $P < 0.0001$).
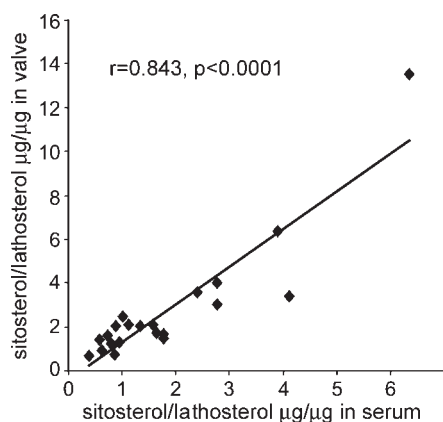

r=0.843, p<0.0001

Fig. 3. Correlation between the ratio of sitosterol (indicating absorption) and lathosterol (indicating cholesterol synthesis) in stenotic aortic valves and in serum samples.

Furthermore, the serum ratios to cholesterol of sterols reflecting cholesterol absorption (cholestanol, avenasterol, sitosterol, and campesterol) correlated negatively with the valvular ratios to cholesterol of sterols reflecting cholesterol synthesis (lathosterol and cholestenol) (e.g., $r = -0.72$, $P < 0.001$ between the ratios to cholesterol of serum avenasterol and valvular cholestenol).

DISCUSSION

The key observation of the present study was that serum noncholesterol sterols, including cholesterol precursors, plant sterols, and cholestanol, accumulate in stenotic aortic valve leaflets in a direct relation to their respective concentrations in serum. These findings support the hypothesis that infiltration of cholesterol in aortic valves contributes to the development of AS and suggests that both plasma-derived cholesterol and its precursors are trapped in aortic valve lesions. Furthermore, the present findings demonstrate that dietary plant sterols are capable of entering aortic valve leaflets and thus could exert local effects in the valves.

Dietary plant sterols or phytosterols are known for their serum LDL cholesterol-lowering effect, which results from their ability to compete with dietary and biliary cholesterol for intestinal absorption (38). Contrary to this well-known beneficial action of intestinal plant sterols on plasma LDL cholesterol level, recent studies suggest that circulating plant sterols may actually exert direct detrimental effects on the vasculature (24, 25). Indeed, in patients with the rare genetic abnormality phytosterolemia, plant sterols accumulate in tissues, resulting in tendon xanthomas and premature atherosclerosis, including CAD and sudden cardiovascular death (26–28, 39). Furthermore, plant sterols and cholesterol may accumulate in aortic tissue and even lead to supravalvular aortic stenosis, as has been found in a phytosterolemic patient (28). While normally, <5% of the phytosterols are absorbed from the intestinal lumen, in patients with phytosterolemia, the intestinal absorption of phytosterols is strongly increased and ranges from 16% to 63% of ingested plant sterols (24, 25). Interestingly, only a minor fraction of the sterols in the tendon xanthomata of phytosterolemic patients is composed of plant sterols (<18%), the remaining sterols being free and esterified cholesterol (26). Therefore, it is conceivable that phytosterols could facilitate the entry of cholesterol into tissues, including atherosclerotic arterial wall and even sclerotic or stenotic aortic valves. Indeed, in the present study, the concentrations of plant sterols in aortic valve leaflets correlated closely with the cholesterol concentrations of the valves.

Accumulation of LDL cholesterol in aortic valve leaflets is a central feature in the development of AS (5, 7). Lipid-loaded foam cells are present in the progressing lesions of AS but are absent from normal nonstenotic valves (5). Moreover, oxidized LDL is found in stenotic aortic valves and colocalizes with infiltrates of T-lymphocytes and calcium deposits, suggesting that oxidized lipids participate in the pathogenesis of AS (6). Further support for the role of cholesterol in AS progression originates from animal

models, in which hypercholesterolemia increased aortic valve cholesterol content and induced bone matrix production and calcification of the valves, which could be inhibited by statin treatment (9, 10). Besides increasing valvular lesion size and promoting the entry of cholesterol in the affected valves, locally accumulated plant sterols could accentuate inflammation in the valves, which is a key element in lesion development and contributes to valve calcification and progression of the disease (5, 40–46). Interestingly, plant sterols are more avidly oxidized than cholesterol in serum (47), suggesting that oxidized plant sterols, like oxidized cholesterol, could serve as triggers of inflammation in aortic valves (48). It is tempting to hypothesize that oxidized plant sterols could also induce local calcification in aortic valve leaflets, in analogy to products of cholesterol oxidation, such as 25-hydroxycholesterol, which has been shown to accelerate aortic valve calcification in vitro (12). Importantly, the present findings suggest that the relative quantity of circulating cholesterol and noncholesterol sterols in blood is the major determinant of their composition in aortic valves. Indeed, the higher the ratio of the cholesterol precursors (e.g., lathosterol) and the absorption sterols (e.g., sitosterol) in the circulation, the higher their ratio in stenotic aortic valves. Besides the amounts of circulating cholesterol and noncholesterol sterols entering the leaflets, additional local effectors in the valves, such as infiltration of inflammatory cells, expression of growth factors and cytokines, and probably genetic factors, could further determine whether valve sclerosis and stenosis will ensue. Interestingly, the ratio to cholesterol of the hepatic cholesterol precursor lathosterol in stenotic valves correlated negatively with AVA, raising the possibility that cholesterol synthesis was accentuated with decreasing AVA (i.e., with increasing severity of AS).

Regarding the significance of circulating plant sterols in the pathogenesis of atherosclerosis in the general population, considerable controversy exists. Importantly, plant sterols have been detected in atherosclerotic carotid artery plaques of normal adults, suggesting that plant sterols could participate in the development of atherosclerotic lesions even in the absence of genetically abnormal phytosterol metabolism (29). In some epidemiological studies, serum levels of plant sterols have been shown to associate with the occurrence of CAD independent of serum cholesterol levels (30–33), while other investigators have failed to detect an association between the levels of serum plant sterols and a family history of CAD, or between serum plant sterol levels and coronary artery calcium score (34, 35). Moreover, recent animal studies have suggested that phytosterols could rather reduce than increase atherosclerosis (49–53). In conclusion, several studies suggest that plant sterols could inadvertently increase cardiovascular risk, but since conflicting results exist, additional studies are urgently needed.

In the present study, lower BMI was associated with increased levels of plant sterols in both sera and diseased valves. This is of considerable interest, since in the Helsinki Aging Study, low BMI was identified as a risk factor for aortic valve calcification (3). On the other hand, the metabolic syndrome has been identified as an independent risk factor of AS progression (54). Regarding the risk factors of AS, traditional risk factors of atherosclerosis, including hypercholesterolemia, also predispose to the development of calcific aortic valve disease (1), raising the idea that atherosclerotic risk factors could serve as possible therapeutic targets in AS. At present, several experimental and retrospective clinical studies have provided data suggesting that treatment with statins may retard AS progression (13–18). In contrast, the only published prospective randomized trial failed to find a benefit for statin therapy in AS patients (19). It is noteworthy that, while statins lower serum LDL cholesterol levels, they simultaneously increase the levels of serum plant sterols, apparently reflecting accentuated sterol absorption (22). Consistent with these earlier findings, patients receiving statins in this study also demonstrated elevated serum levels of plant sterols. If plant sterols were deleterious to the vasculature and the aortic valves, some benefit gained from statins might be blunted due to elevated levels of plant sterols circulating in the blood and entering the stenotic aortic valves. In the ongoing Simvastatin and Ezetimibe in Aortic Stenosis study (55), ezetimibe, which interferes with the absorption of both cholesterol and plant sterols (56, 57), is being used in combination with simvastatin in patients with AS. When finished, this trial will hopefully greatly enhance our knowledge about the potential adverse role of circulating plant sterols in this disease.

Some limitations of the present study exist. First, the control aortic valves were obtained from different subjects than the control blood samples. The control nonstenotic valves were collected at medicolegal autopsies, but freshly drawn serum samples were not available from these subjects; thus, control blood samples had to be collected from a separate study group. Therefore, we were unable to determine whether the observed relationship between serum and valve sterol concentrations in the AS group also existed in the control group. Second, there were no significant differences in the valvular sterol contents between the stenotic and control valves. This lack of difference may partly relate to the fact that the variation of the sterol values was particularly high in the control group. It is also conceivable that the distribution of sterols between the intracellular and extracellular pools was different in the AS and control samples. Another possible explanation for the lack of the expected difference in the sterol contents between the two groups is that the stenotic valves were freshly obtained from valve replacement procedures, whereas the control valves were collected at autopsy several days after death.

It is also unfortunate that we were not able to detect differences in plant sterol concentrations in serum or aortic valves between individuals with and without using plant sterol or stanol supplements in their regular diet. However, the data concerning the use of plant sterols or stanols were collected retrospectively, and the duration of the use and the daily doses were not characterized. Furthermore, information of plant sterol or stanol supplements was not available from 11 patients. Therefore, appropriate conclusions of the effects of dietary supplementation with plant sterols

on aortic valves cannot be drawn from this study, and this issue remains an interesting challenge for future studies.

In summary, the present study demonstrates that plant sterols accumulate in aortic valves even in individuals with normal serum phytosterol levels, raising the possibility that phytosterols are a novel risk factor of AS. Furthermore, increases in the levels of circulating plant sterols resulted in elevated levels of plant sterols in aortic valves, revealing that the mechanism of plant sterol deposition in stenotic valves is closely related to their respective levels in the circulation. Since plant sterols are carried in plasma LDL particles together with cholesterol, these findings provide a novel link between one of the traditional risk factors of AS, namely hypercholesterolemia, and the pathobiology of the disease. Additional studies are needed to uncover the mechanism(s) by which plant sterols act on the valvular cells.▮

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