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Long-term consumption of plant stanol and sterol esters, vascular function and genetic regulation

Helena Gylling[1,2]*, Maarit Hallikainen[1], Olli T. Raitakari[3], Markku Laakso[2], Erkki Vartiainen[4], Pia Salo[5], Vesa Korpelainen[6], Jouko Sundvall[4] and Tatu A. Miettinen[7]

[1]*Department of Clinical Nutrition, University of Kuopio, Kuopio, Finland*
[2]*Department of Medicine, University Hospital of Kuopio, Kuopio, Finland*
[3]*Department of Clinical Physiology, University of Turku, Turku, Finland*
[4]*National Health Institute, Helsinki, Finland*
[5]*The Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland*
[6]*North-Karelia Centre for Public Health, Joensuu, Finland*
[7]*Division of Internal Medicine, Department of Medicine, University of Helsinki, Helsinki, Finland*

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Polymorphisms of the *ABCG5* and *ABCG8* genes interfere with cholesterol absorption and synthesis. We determined whether common polymorphisms of these genes regulate the responses of serum cholesterol and vascular function during long-term inhibition of cholesterol absorption. Mildly to moderately hypercholesterolaemic subjects (*n* 282) completed a 1-year study consuming plant stanol or sterol ester (2 g stanol or sterol) or control spread. Serum cholesterol and non-cholesterol sterols, markers of cholesterol absorption and synthesis, and variables of vascular function and structure were analysed in relation to common polymorphisms of *ABCG5* and *ABCG8*. At baseline, subjects with the 54K allele of *ABCG8* had higher brachial endothelial-dependent flow-mediated dilatation than those without it (5·79 (SE 0·31) *v.* 4·46 (SE 0·44) %; $P=0·049$), and subjects with the 632V allele of *ABCG8* had larger brachial artery diameter than those without it. Polymorphisms of *ABCG5* and *ABCG8* were neither associated with serum cholesterol reduction nor changes in cholesterol metabolism or in vascular function. However, in subjects with the 400K allele of *ABCG8*, intima media thickness (IMT) was increased in all groups more than in those without it ($P<0·05$). In conclusion, serum cholesterol lowering with absorption inhibition was not associated with polymorphic sites of *ABCG5* and *ABCG8*. However, regulation of baseline cholesterol metabolism and vascular function and structure, and IMT progression during 1 year seemed to share some of the common polymorphic sites of these genes, suggesting a gene-regulated interaction between cholesterol metabolism and vascular function and structure.

Vascular function: Intima media thickness: Cholesterol absorption: Phytosterol esters

Serum total and LDL-cholesterol levels can be lowered up to 10–14 % using dietary means by consuming plant stanol or sterol esters[1,2]. Serum cholesterol level is regulated by the homeostasis of absorption and synthesis of cholesterol. Of the putative cholesterol transporters, ABCG5, ABCG8[3] and Niemann-Pick C1-like protein[4] have been unravelled recently. It is not known, however, whether different polymorphisms of these genes affect the cholesterol-lowering response during inhibition of cholesterol absorption with plant stanols and sterols.

Up till now there has been no information on whether plant stanol or sterol ester consumption reduces cardiovascular morbidity or mortality. Plant stanol or sterol ester consumption seemed to have no effect on endothelial-dependent flow-mediated dilatation (FMD)[5–8], the surrogate marker of atherosclerosis. However, in more recent studies the consumption of plant stanol or sterol ester spreads was associated with beneficial changes in arterial elasticity and endothelial function in subjects with low baseline values[9,10].

The aims of the present study were to investigate whether any of the common polymorphic sites of the *ABCG5* and *ABCG8* genes regulate vascular function and structure and cholesterol metabolism in hypercholesterolaemic subjects at baseline and during 1-year inhibition of cholesterol absorption with plant stanol or sterol esters. In addition, we evaluated whether the polymorphic sites regulate the serum cholesterol response during the intervention.

Subjects and methods

Study population

Two hundred and ninety-seven subjects were recruited from the Joensuu area in North Karelia, Finland, by adverts in

Abbreviations: CAC, carotid artery compliance; FMD, flow-mediated dilatation; IMT, intima media thickness.

* **Corresponding author:** Professor Helena Gylling, fax +358 17 162 792, email helena.gylling@uku.fi

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local newspapers, or the subjects had participated in earlier studies at the North Karelia Centre for Public Health. There were no differences between the groups regarding age, sex, BMI or lipid variables. Inclusion criteria were: age 25–70 years, serum cholesterol value 5·2–7·5 mmol/l and serum TAG ≤ 2·5 mmol/l. Exclusion criteria included the use of cholesterol-lowering medication or plant stanol or sterol products, BMI > 35 kg/m^2, insulin-treated diabetes, instable coronary artery disease, pregnancy or lactation, alcohol or drug abuse, or participation in some other study. Altogether 282 subjects, 129 men and 153 women with a mean age of 54 (SE 1) years (Table 1) completed the study, and were included in the final analyses. The most frequent reasons for drop-out were unwillingness to use the test spread in the indicated amount or personal reasons not related to the study.

All subjects gave their written informed consent. The investigation was carried out in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the North Karelia Central Hospital.

Study design

The study was a randomised, double-blind, parallel investigation with two intervention groups (stanol ester and sterol ester) and a control group. The randomisation was performed according to the frequency-matching principle with gender as the criterion. After randomisation, the subjects started the spreads for 12 months. To maintain the double-blindness, the packages were coded with different colours.

After randomisation, the subjects visited the North Karelia Centre for Public Health five times, at baseline and after 3, 6, 9 and 12 months; however, only baseline and 12-month values are given in the following. Blood samples after a 12 h fast were drawn, the subjects were weighed, and blood pressure was measured on every visit. Before the baseline and 12-month visits the subjects were asked neither to drink alcohol during 2 d nor smoke during 12 h before the measurements. Laboratory measurements including serum lipids, non-cholesterol sterols, blood glucose and high sensitive C-reactive protein, and sonographic measurements of carotid and brachial arteries were obtained at baseline and after 12 months. Common polymorphisms of the genes were analysed from the baseline blood sample. Compliance was checked on every visit by the number and fullness of returned containers.

Diet

The subjects continued their habitual home diet otherwise unchanged, but they replaced 25 g/d of their regular fat intake with the test spreads. The daily intake of plant stanols and sterols in the spreads were 2·13 and 2·15 g/d, respectively. The control spread contained no added plant stanols or sterols, but it contained a small amount of natural plant sterols (0·06 g per 25 g test spread). The amount of absorbable fat (15·1 g per

Table 1. Baseline characteristics of the study population before and after randomisation
(Mean values with their standard errors)

	Total (*n* 282)		STAEST group (*n* 93)		STEEST group (*n* 93)		Controls (*n* 96)		
Variables	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*P**
Sex (*n*)									
Males	129		45		42		42		
Females	153		48		51		54		
Age (years)	54·1	0·5	54·6	1·0	54·8	0·8	52·8	0·8	0·156
Weight (kg)	73·4	0·7	74·6	1·3	73·0	1·3	72·7	1·2	0·624
BMI (kg/m^2)	25·8	0·2	26·2	0·4	25·8	0·3	25·4	0·3	0·256
Waist circumference (cm)	87·9	0·7	90·9	1·9	87·6	1·1	86·6	1·0	0·264
Systolic BP (mmHg)	130	1	133	2	128	2	128	2	0·097
Diastolic BP (mmHg)	80	1	82	1	79	1	78	1	0·042
Serum cholesterol (mmol/l)	5·87	0·05	5·81	0·08	5·92	0·08	5·84	0·08	0·622
Plasma glucose (mmol/l)	5·30	0·03	5·38	0·06	5·33	0·06	5·20	0·05	0·074
hs-CRP (mg/l)	1·64	0·26	1·08	0·17	1·26	0·18	0·88	0·13	0·589
Serum squalene and non-cholesterol sterols ($10^2 \times \mu$mol/mmol cholesterol)									
Squalene	16	0	17	1	16	1	15	1	0·257
Desmosterol	79	1	81	2	78	2	79	2	0·588
Lathosterol	124	3	133	6	121	5	120	5	0·108
Campesterol	297	7	283	13	294	12	314	13	0·209
Sitosterol	141	3	137	6	140	6	148	6	0·352
Avenasterol	45	1	46	1	44	1	46	1	0·548
Cholestanol	149	2	151	3	146	3	150	3	0·441
Sitostanol	10	0	10	0	10	0	10	0	0·900
Sonographic measurements of carotid and brachial arteries									
IMT (mm)	0·64	0·01	0·66	0·01	0·64	0·01	0·63	0·01	0·419
Brachial artery diameter (mm)	3·66	0·04	3·69	0·06	3·70	0·06	3·59	0·07	0·287
FMD (%)	5·44	0·26	4·93	0·45	5·13	0·39	6·24	0·47	0·082
NMD (%)	21·5	0·5	20·2	0·9	21·2	0·8	22·9	0·9	0·080
CAC (%/10 mmHg)	1·36	0·04	1·32	0·07	1·37	0·06	1·38	0·06	0·769

STAEST, stanol ester margarine group; STEEST, sterol ester margarine group; BP, blood pressure; hs-CRP, high sensitive C-reactive protein; IMT, intima media thickness; FMD, flow-mediated vasodilatation; NMD, nitrate-mediated vasodilatation; CAC, carotid artery compliance.
* Differences between groups. Sex was included as a covariate.

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25 g test spread) and fatty acid composition were similar in the test spreads. The fatty acid composition per 25 g test spread was as follows: SFA, 5·2 g; MUFA, 4·0 g; PUFA, 5·9 g (with *n*-6, 5·7 g and *n*-3, 0·2 g, respectively). The subjects kept a 3 d food record at baseline and at the end of the study, and the nutrients were calculated using Micro-Nutrica® dietary analysis software (The Social Insurance Institution, Turku, Finland) on the basis of Finnish Food Analyses and International Food Composition Tables[11].

Measurements

Blood pressure was measured with a mercury sphygmomanometer after 5 min rest. Plasma glucose was analysed with enzymic hexokinase and serum high sensitive C-reactive protein with latex immunoassay methods. Serum cholesterol, squalene and non-cholesterol sterols, i.e. cholestenol, desmosterol, lathosterol (markers of cholesterol synthesis)[12–14] and cholestanol, campesterol, sitosterol and avenasterol (markers of cholesterol absorption)[12,15] were measured with a single GLC run from non-saponifiable serum lipids. GLC was performed with a 50 m long SE-30 capillary column (Ultra 2; Agilent Technologies, Wilmington, DE, USA) principally as shown previously[16]. The values are given as ratios to cholesterol ($10^2 \times$ μmol/mmol of cholesterol).

Ultrasound studies

We used a Toshiba Xario mainframe (Toshiba, Tustin, CA, USA) with a 7–14 MHz linear array transducer (PLT1204AT). The digitally stored scans were analysed by one reader blinded to subjects' details.

Brachial artery test. Brachial artery diameter was measured as previously described[17]. In brief, a resting scan above the elbow was performed. Thereafter hyperaemia was induced by inflation of a cuff placed around the forearm followed by release. Subsequent scans were taken at 60 s after the cuff release. The tests were performed according to current guidelines[18]. FMD was calculated as the maximal percentage increase in arterial diameter during hyperaemia compared with the resting value. This dilation response reflects endothelium-dependent vasorelaxation capacity, because it can be blunted by simultaneous infusion of NO synthase inhibitor[19]. The vessel was allowed to recover for 10–15 min, after which a resting scan was taken. Then oral nitrate (isosorbide dinitrate spray, 1·25 mg) was given and after 4 min the vessel diameter was measured in order to determine the endothelium-independent nitrate-mediated vasodilatation. Between-visit CV were 3·2 % for brachial artery diameter measurements and 26·0 % for FMD[17].

Carotid artery intima media thickness. The image was focused on the posterior (far) wall of the right carotid artery. The magnified image was recorded from the angle showing the greatest distance between the lumen–intima interface and the media–adventitia interface. At least four measurements of the common carotid far wall were taken 10 mm proximal to the bifurcation to derive mean carotid intima media thickness (IMT). The between-visit (two visits 3 months apart) CV for IMT measurements was 6·4 %[17].

Carotid artery elasticity. The best-quality cardiac cycle was selected from the 5 s clip image. The common carotid diameter 10 mm from the carotid bifurcation was measured from the B-mode images using ultrasonic calipers in end-diastole and end-systole, respectively. Brachial blood pressure was measured during the ultrasound study with an automated sphygmomanometer (Omron M4; Omron Matsusaka Co., Ltd., Matsusaka, Japan). The ultrasound and concomitant brachial blood pressure measurements were used to calculate carotid artery compliance (CAC):

$$\mathrm{CAC} = ((\mathrm{Ds} - \mathrm{Dd})/\mathrm{Dd})/(\mathrm{Ps} - \mathrm{Pd}),$$

where Dd is the diastolic diameter, Ds is the systolic diameter, Ps is the systolic blood pressure and Pd is the diastolic blood pressure[20]. Thus, compliance is a marker of arterial elasticity that measures the ability of the carotid arteries to expand as a response to pulse pressure caused by cardiac contraction and relaxation. Between-visit CV were 2·7 % for carotid artery diastolic diameter measurements and 19·5 % for CAC[20].

Polymorphism of ABCG5 *and* ABCG8

Previously identified five common polymorphisms of *ABCG5* and *ABCG8*[3,21] were assayed by PCR amplification and restriction fragment length polymorphism analysis. The restriction enzymes used were Tru1I (Thr400 → Lys, exon 8, *ABCG8*), NcoI (Ala632 → Val, exon 13, *ABCG8*) and PdmI (Gln604 → Glu, exon 13, *ABCG5*). The polymorphic sites Asp19 → His in exon 1 and Tyr54 → Cys in exon 2 of *ABCG8* were analysed with the ABI prism 3100 genetic analyser (Applied Biosystems, Foster City, CA, USA) BigDye® Terminator *v.* 1.1 Cycle sequencing kit.

Statistical analyses

The analyses were performed with SPSS for Windows 14.0 statistics program (SPSS, Chicago, IL, USA). Power analyses were performed as *a priori* analyses for both sexes separately, and the intervention was approximated to lower serum cholesterol by 10 % based on the previous 1-year plant stanol ester study[1]. The calculation confirmed the adequacy of the size of the study population. Univariate ANOVA was used to compare the baseline values and the changes between groups. ANOVA for repeated measurements was used to analyse the interaction of time and group, changes over time, and the effect of sex in between-group comparisons followed by *post hoc* comparisons. For sterols and variables of vascular function and structure, Pearson coefficients were calculated. To control the overall α concentration, Bonferroni adjustment was used. Most of the variables were normally distributed, but logarithmic transformations were used in the case of skewed distributions. Non-continuous variables were tested with the χ^2 test. A P value <0.05 was considered statistically significant.

Results

Baseline

None of the subjects had coronary artery disease or type 2 diabetes. Baseline characteristics are shown in Table 1. Diastolic blood pressure was the only variable differing between the groups. FMD was inversely associated with brachial

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Table 2. Differences in baseline variables between wild type and heterozygotes + homozygotes of common polymorphic sites of the *ABCG8* and *ABCG5* genes
(Mean values with their standard errors)

		Wild type			Heterozygotes + homozygotes			
Polymorphic sites	Variables	Mean	SE	*n*	Mean	SE	*n*	*P**
ABCG8:D19H	Lathosterol (10^2 μmol/mmol cholesterol)	121	3	216	139	9	41	0·042
	Campesterol (10^2 μmol/mmol cholesterol)	306	8	216	253	15	41	0·005
	Sitosterol (10^2 μmol/mmol cholesterol)	146	4	216	119	7	41	0·003
	Avenasterol (10^2 μmol/mmol cholesterol)	46	1	216	40	1	41	0·002
	Cholestanol (10^2 μmol/mmol cholesterol)	151	2	216	137	5	41	0·004
ABCG8:Y54K	Flow-mediated dilatation (%)	4·46	0·44	73	5·79	0·31	207	0·049
ABCG8:T400K	Campesterol (10^2 μmol/mmol cholesterol)	307	9	165	281	12	92	0·028
	Sitosterol (10^2 μmol/mmol cholesterol)	148	4	165	131	6	92	0·004
ABCG8:A632V	Brachial artery diameter (mm)	3·59	0·05	198	3·81	0·07	84	0·01
	Flow-mediated dilatation (%)	5·78	0·31	198	4·65	0·45	84	0·048
ABCG5:Q604E	Plasma glucose (mmol/l)	5·26	0·03	198	5·46	0·08	84	0·032
	Cholestenol (10^2 μmol/mmol cholesterol)	21	0	200	23	1	57	0·044
	Sitosterol (10^2 μmol/mmol cholesterol)	145	4	200	129	6	57	0·044

* Differences between wild and heterozygotes + homozygotes. Sex and age were included as covariates in the analysis.

artery diameter (r −0·300; $P<0·001$) and IMT (r −0·220; $P<0·001$). Brachial artery diameter was associated with serum squalene (r 0·261; $P<0·001$) and desmosterol (r 0·393; $P<0·001$).

The number of subjects analysed for the polymorphic sites of *ABCG5* and *ABCG8* varied from 257 to 282. The polymorphic sites were in Hardy–Weinberg equation, and were evenly distributed between the groups. The presence of the 19H allele of *ABCG8* was associated with higher serum lathosterol and with lower absorption marker sterols than without it (Table 2). Similar results regarding serum campesterol and sitosterol were observed with the 400K allele of *ABCG8*. In subjects with the 604E allele of *ABCG5*, plasma glucose and serum cholestenol were higher and sitosterol lower than in subjects without the allele. Subjects with the 54K allele of *ABCG8* had higher FMD than those without it; subjects with the 632V allele of *ABCG8* had larger brachial artery diameter and smaller FMD than those without it.

Intervention

Body weight, waist circumference, blood pressure and high sensitive C-reactive protein did not differ between the

Table 3. Dietary data
(Mean values with their standard errors)

		STAEST (*n* 93)		STEEST (*n* 93)		Controls (*n* 96)			
	Time (months)	Mean	SE	Mean	SE	Mean	SE	*P**	*P*†
Energy (MJ/d)	0	7·7	0·2	8·1	0·2	8·0	0·3		
	12	8·0	0·2	7·9	0·2	8·2	0·2	0·118	0·134
Fat (% energy)	0	31·5	0·7	32·7	0·6	31·4	0·7		
	12	32·6	0·6	34·4	0·6	34·0	0·5	0·287	<0·001
SFA (% energy)	0	11·8	0·3	12·6	0·3	12·0	0·3		
	12	11·8	0·3	12·7	0·3	12·4	0·3	0·566	0·338
MUFA (% energy)	0	10·5	0·3	10·9	0·3	10·6	0·3		
	12	10·1	0·2	10·9	0·3	10·7	0·2	0·438	0·519
PUFA (% energy)	0	5·4	0·1	5·3	0·2	5·3	0·2		
	12	7·2	0·2	7·3	0·2	7·4	0·2	0·336	<0·001
Proteins (% energy)	0	17·0	0·4	16·8	0·3	16·7	0·3		
	12	16·2	0·3	16·3	0·3	16·3	0·3	0·874	0·001
Carbohydrates (% energy)	0	43·6	0·8	43·2	0·7	43·8	0·7		
	12	43·7	0·7	42·1	0·7	42·8	0·6	0·383	0·078
Alcohol (% energy)	0	3·2	0·6	2·5	0·5	3·3	0·6		
	12	2·8	0·5	2·5	0·4	2·3	0·3	0·244	0·104
Cholesterol (mg/MJ)	0	30·8	1·1	32·7	1·2	30·5	1·1		
	12	26·4	0·9	29·7	1·1	27·1	0·8	0·652	<0·001
Fibre (g/MJ)	0	3·1	0·1	2·9	0·1	2·9	0·1		
	12	3·2	0·1	3·0	0·1	3·1	0·1	0·827	0·002

STAEST, stanol ester margarine group; STEEST, sterol ester margarine group.
* Group × time interaction.
† Overall time effect during the study.

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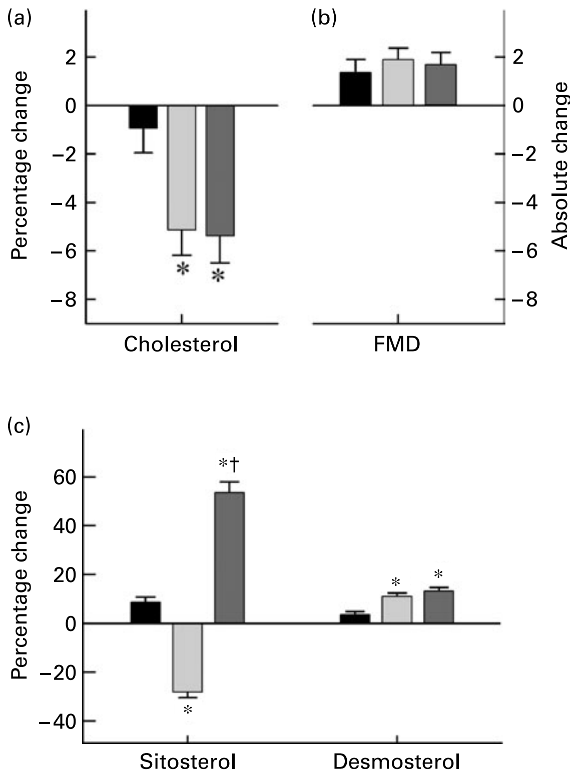

Fig. 1. Changes in serum cholesterol (a), flow-mediated dilatation (FMD) (b) and serum sitosterol and desmosterol ratios to cholesterol (c) in hypercholesterolaemic subjects consuming plant stanol (*n* 93; ◻) or sterol (*n* 93; ▩) ester spread or control spread (*n* 96; ■) for 1 year. Values are means, with standard errors represented by vertical bars. * Mean value was significantly different from that of the control group ($P<0·05$). † Mean value was significantly different from that of the stanol ester margarine group ($P<0·05$).

groups and were unchanged (data not shown). Mean plasma glucose was significantly increased by 1–3 % in all three groups from baseline (NS between the groups); however, the change or the 1-year glucose values were not associated with lipids or the vascular variables.

No significant differences in nutrient intakes occurred between the groups. However, the intakes of fat, PUFA and fibre increased, and that of protein and dietary cholesterol decreased similarly in all groups ($P<0·05$ for all from baseline) (Table 3). Compliance was good and similar between the groups.

Serum cholesterol was decreased from baseline by −0·9 (SE 1·0) % (NS) in controls, −5·1 (SE 1·0) % ($P=0·001$) in the stanol ester group and −5·4 (SE 1·1) % ($P<0·001$) in the sterol ester group, respectively (Fig. 1). The control-related reductions in serum cholesterol were −4·2 (SE 1·5) % ($P<0·01$) with stanol esters and −4·4 (SE 1·5) % ($P<0·01$) with sterol esters. The changes were similar in men and women, in subjects with different BMI, and with low to high serum cholestanol indicating low to high cholesterol absorption (data not shown). The absolute 12-month serum cholesterol values did not differ between the sterol ester and control groups (5·56 (SE 0·07) *v.* 5·76 (SE 0·08) mmol/l; NS).

Synthesis marker sterols were increased with stanol and sterol esters compared with baseline and compared with controls, as shown for desmosterol in Fig. 1. Serum cholestanol was decreased with stanol (−3·2 (SE 1·0) %) and sterol esters (−4·9 (SE 1·0) %) compared with baseline and with sterol esters compared with controls (−6·7 (SE 1·3) %; $P<0·001$ for all). Serum plant sterols decreased with stanol and increased with sterol esters, as shown for sitosterol in Fig. 1.

Control, stanol and sterol ester spreads increased FMD (Fig. 1), IMT and CAC, and decreased brachial artery diameter from baseline, but did not affect the brachial maximum post-ischaemic diameter, suggesting that the change in FMD results from the decreased brachial artery diameter (Table 4). The changes in FMD, IMT and CAC were related to their respective baseline values in the groups (for example, CAC; change *v.* baseline r −0·444 to r −0·666; $P<0·001$). The changes in FMD, IMT and CAC were not related to BMI or baseline cholesterol or non-cholesterol sterol values. However, the change in serum cholesterol by stanol ester was positively related to the change in brachial artery diameter (r 0·322;

Table 4. Sonographic measurements of carotid and brachial arteries
(Mean values with their standard errors)

Variables	Time (months)	STAEST (*n* 93)		STEEST (*n* 93)		Controls (*n* 96)			
		Mean	SE	Mean	SE	Mean	SE	*P**	*P*†
IMT (mm)	0	0·66	0·01	0·64	0·01	0·63	0·01		
	12	0·68	0·01	0·68	0·01	0·66	0·01	0·312	<0·001
Brachial diameter baseline (mm)	0	3·69	0·06	3·70	0·06	3·59	0·07		
	12	3·63	0·07	3·64	0·06	3·54	0·07	0·983	0·006
Brachial diameter hyperaemia (mm)	0	3·86	0·61	3·88	0·63	3·80	0·65		
	12	3·87	0·64	3·88	0·62	3·80	0·64	0·968	0·97
FMD (%)	0	4·93	0·45	5·13	0·39	6·24	0·47		
	12	6·83	0·45	6·82	0·43	7·56	0·51	0·502	<0·001
NMD (%)	0	20·2	0·9	21·2	0·8	22·9	0·9		
	12	21·9	0·9	21·4	0·8	22·9	0·9	0·288	0·268
CAC (%/10 mmHg)	0	1·32	0·07	1·37	0·06	1·38	0·06		
	12	1·57	0·06	1·59	0·07	1·68	0·08	0·754	<0·001

STAEST, stanol ester group; STEEST, sterol ester group; IMT, intima media thickness; FMD, flow-mediated vasodilatation; NMD, nitrate-mediated vasodilatation; CAC, carotid artery compliance.
* Group × time interaction.
† Overall time effect.

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Table 5. Polymorphism of the *ABCG8* gene and change in intima media thickness (IMT) by the intervention

(Mean values with their standard errors)

	STAEST						STEEST						Controls						
	Wild type			Heterozygotes + homozygotes			Wild type			Heterozygotes + homozygotes			Wild type			Heterozygotes + homozygotes			
Changes in variables	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	*P**
ABCG8T400K IMT (%)	2·3	1·9	63	7·3†	2·2	30	5·5	1·7	61	10·1†	2·9	31	4·3	1·6	62	6·7†	2·8	33	0·830

STAEST, stanol ester group; STEEST, sterol ester group.
* Group × genotype interaction.
† $P < 0.05$ between genotypes.

$P=0.009$), whereas the respective association was negative with sterol ester (r −0·302; $P=0.015$). The change in FMD correlated with the change in brachial artery diameter (stanol ester r −0·475, $P<0.001$; sterol ester r −0·291, $P=0.015$; controls r −0·290, $P=0.012$).

In subjects with the 400K allele of *ABCG8*, the increase in IMT was larger in all groups than in subjects without the allele (Table 5). There were no other associations between the polymorphic sites of the genes and the changes in serum cholesterol, non-cholesterol sterols and in vascular function and structure.

Discussion

The new observations in the present study were, first, that at baseline the polymorphisms of *ABCG8* were associated with variables of endothelial function. Second, the polymorphisms were not associated with changes in serum cholesterol or absorption sterols during the intervention. Third, the increase in PUFA in the test spreads together with other dietary changes improved the vascular function and arterial elasticity in all groups. However, the addition of stanol or sterol ester to spreads in spite of serum cholesterol reduction led to no additional improvement in vascular function. Finally, the magnitude of the 1-year increase in IMT was related to the polymorphic site of *ABCG8*, so that subjects with the 400K allele had larger increases in IMT than subjects without it.

Two polymorphic sites of *ABCG8* and *ABCG5* were associated with baseline cholesterol metabolism, similar to previous findings[3,22,23]. The new observation was the association of plasma glucose with the 604E allele of *ABCG5*. Previously this allele was associated with obesity, high serum insulin level and high synthesis and low absorption of cholesterol[22]. The profile of high synthesis–low absorption of cholesterol is considered typical for the metabolic syndrome[24,25]. Accordingly, the new finding in the present study strengthens the idea that the ABCG5:G604E polymorphic site separates subjects with and without the characteristics of the metabolic syndrome. Contrary to the baseline regulation of cholesterol metabolism, the polymorphic sites were neither associated with the changes of serum cholesterol nor of absorption and synthesis markers during the intervention. The present results differ from a previous study conducted in healthy subjects, in whom the serum plant sterol response to stanol esters was associated with the *ABCG8* T400K genotype[23]. This discrepancy might result from the differences in study populations.

Of the variables of arterial health, brachial artery diameter was associated with the markers of cholesterol synthesis (squalene and desmosterol), suggesting that the higher the cholesterol synthesis, the larger is the arterial diameter. Similar results were obtained in our previous study[8]. Furthermore, it was shown earlier that brachial artery diameter was associated with serum TAG and glucose levels, and inversely with HDL-cholesterol and markers of cholesterol absorption[8]. Accordingly, large arterial diameter is correlated with variables of the metabolic syndrome. The pathophysiological mechanisms between large brachial artery diameter and the unfavourable metabolic variables remain open to discussion. The most prominent difference between stanol and sterol ester consumption was the change in serum plant sterol levels (Fig. 1). In linear proportion to their serum

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concentrations, plant sterols accumulate in tissue cells (for example, erythrocytes)[26] and in arterial wall atheromata[27]. It remains to be shown whether these changes might explain the different behaviour of brachial artery diameter during cholesterol reduction with plant stanols or sterols.

Consumption of the spreads increased the intake of PUFA. In addition, the intakes of total fat, cholesterol, fibre and protein were changed. Probably all these changes (except perhaps the change in protein intake) were responsible for the favourable effects on FMD, CAC and brachial artery diameter, even though in analysis of covariance none of the dietary changes explained the variability in vascular function and structure. However, the dietary changes could not stop the unfavourable age-related increment of IMT. In concert with earlier studies[5–8] the addition of stanol or sterol ester had no additional effect on endothelial function. One possibility is that the serum cholesterol reduction obtained is not large enough to affect the vascular function and structure.

At baseline both brachial artery diameter and FMD were genetically regulated, and even two different polymorphic sites of *ABCG8* were associated with FMD. However, the association between ABCG8:A632V and FMD can reflect the association of the polymorphic site with brachial artery diameter; the larger the arterial diameter, the smaller is FMD. During the intervention the T400K polymorphic site of *ABCG8* was associated with the increase in IMT. Stanol or sterol esters had no role in this regulation. This is a new observation, suggesting that one of the regulators of the age-dependent progression of IMT is *ABCG8*. Since at baseline the same T400K polymorphic site of *ABCG8* separated subjects with a profile of cholesterol metabolism characteristic for the metabolic syndrome, we assume that low cholesterol absorption and high synthesis as part of the metabolic syndrome is unfavourable for the progression of IMT.

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All authors participated in the planning of the study and critical review of the manuscript. In addition, V. K. was responsible for the recruitment of the study population and the intervention; O. T. R. analysed the ultrasound scans; M. L., J. S. and T. A. M. were responsible for the different laboratory analyses; M. H. and E. V. performed the statistical analyses and H. G. prepared the manuscript.

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