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A Plant Stanol Yogurt Drink Alone or Combined with a Low-Dose Statin Lowers Serum Triacylglycerol and Non-HDL Cholesterol in Metabolic Syndrome Patients[1–3]

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Abstract

We evaluated the effects of 2 commonly available strategies (plant stanol ester drink and 10 mg simvastatin) on coronary heart disease (CHD) risk variables in participants with metabolic syndrome. Metabolic syndrome patients are at increased risk to develop CHD, partly due to high triacylglycerol (TAG) and low HDL cholesterol (HDL-C) concentrations and a low-grade inflammatory profile. Effects of plant stanol esters on TAG concentrations in these participants are unknown. After a 3-wk run-in period in which individuals consumed placebo yogurt drinks and placebo capsules, participants were randomly divided into 4 groups: placebo ($n = 9$), simvastatin + placebo drink ($n = 10$), placebo + stanol drink ($n = 9$), and simvastatin + stanol drink ($n = 8$). After 9 wk, we evaluated the effects on serum lipids, low-grade inflammation, and endothelial dysfunction markers. In metabolic syndrome patients, stanol esters (2.0 g/d), simvastatin, or the combination lowered non-HDL-C by 12.8% ($P = 0.011$), 30.7% ($P < 0.001$), and 35.4% ($P < 0.001$), respectively, compared with placebo. TAG were lowered by 27.5% ($P = 0.044$), 21.7% ($P = 0.034$), and 32.7% ($P < 0.01$), respectively. The total-:HDL-C ratio was significantly lowered in all 3 intervention groups. We found no treatment effects on the apolipoprotein CII:CIII ratio, cholesterol ester transfer protein mass, FFA concentrations, and markers for low-grade inflammation or endothelial dysfunction. This study shows that in metabolic syndrome patients, plant stanol esters lower not only non-HDL-C, but also TAG. Effects on TAG were also present in combination with statin treatment, illustrating an additional benefit of stanol esters in this CHD risk population. J. Nutr. 139: 1–7, 2009.

Introduction

Consumption of functional foods enriched with plant stanol esters and treatment with statins are recommended as LDL cholesterol (LDL-C)[6]–lowering strategies to reduce cardiovascular risk (1). Besides lowering LDL-C, statins also elevate HDL cholesterol (HDL-C) concentrations and lower those of triacylglycerol (TAG) (2) and may decrease the proinflammatory profile (3). For plant stanol esters, there is very little evidence for effects other than lowering LDL-C. However, results of our recent meta-analysis (4) suggested that plant stanol esters may lower TAG, especially in individuals with elevated baseline TAG concentrations. The main reason why these effects have not been observed in earlier studies may have been due to the lack of statistical power in relation to the low baseline TAG values of the participants. Thus, what is lacking is a study, e.g., in a population of participants with metabolic syndrome (which are, among others, characterized by elevated TAG concentrations) to evaluate the effects of plant stanol esters. Moreover, metabolic syndrome patients are characterized by an increased risk to develop coronary heart disease (CHD), which is not primarily associated with elevated LDL-C. Instead, other cardiovascular risk markers such as elevated TAG concentrations, low HDL-C concentrations, and a proinflammatory profile may contribute extensively. Therefore, the first aim of our study was to evaluate whether plant stanol esters improve the serum lipid profile in metabolic syndrome patients, with special attention to changes in serum TAG concentrations.

Whether plant stanol esters affect inflammatory profiles has scarcely been studied. In an earlier study, we did not show any effects of plant stanol esters on high sensitivity C-reactive protein

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[6] Abbreviations used: apo, apolipoprotein; CETP, cholesterol ester transfer protein; CHD, coronary heart disease; en%, energy percent; HDL-C, HDL cholesterol; hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; LDL-C, LDL cholesterol; LPL, lipoprotein lipase; LXR, liver X-receptor; OTC, over–the-counter; PBMC, peripheral mononuclear blood cell; SAA, serum myloid A; sE-selectin, soluble endothelial selectin; sICAM, soluble intercellular adhesion molecule; sVCAM, soluble vascular adhesion molecule; TAG, triacylglycerol.
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(hsCRP) and markers reflecting endothelial dysfunction such as soluble intercellular adhesion molecule (sICAM), soluble vascular adhesion molecule (sVCAM), and soluble endothelial selectin (sE-selectin) despite decreased LDL-C (*5*). However, in that study, plant stanol esters were consumed by patients who had already been receiving stable statin treatment for several years and therefore masked the plant stanol effect. Of note, others have shown effects of plant stanol esters on hsCRP in populations not receiving statin treatment (*6*). Therefore, the second aim of the study was to evaluate the effects of plant stanol ester on a wide range of markers reflecting low-grade inflammation and endothelial dysfunction in metabolic syndrome patients with and without statin treatment.

Methods

Participants

All volunteers were recruited via advertisements in local newspapers and posters in the university and hospital buildings. Individuals from Maastricht and surrounding municipalities willing to participate were invited for 2 screening visits. Thirty-six participants met all of our eligibility criteria. They were required to have at least 3 of the 5 characteristics of metabolic syndrome according to the National Cholesterol Education Program adult treatment panel guidelines (*1*). In addition, the volunteers had to fulfill the following additional criteria, which apply to the target population for the low-dose (10 mg), over-the-counter (OTC) statins on the UK market. Volunteers had to be 45–70 y (men) or 55–70 y (women) with at least 1 of the 2 following criteria: a family history of CHD in a firs-degree relative in which only CHD events in male relatives <55 y old and female relatives <65 y old were considered, and/or overweight as defined by a BMI >25 kg/m^2 or abdominal obesity (waist circumference >102 cm for men, >88 cm for women). This means that the metabolic syndrome participants in this study were a specific subpopulation characterized by either elevated BMI or a positive CHD family history in a first-degree relative. Whether this affected the results remains unknown but seems unlikely. Exclusion criteria were: smoking, >2 alcoholic beverages (e.g. 40 g alcohol)/d, active cardiovascular disease such as congestive heart failure or a recent (<6 mo) event (defined as a myocardial infarction or cerebrovascular attack), peripheral vascular disease, familial hypercholesterolemia, impaired renal (as evidenced by increased serum creatinine concentrations >133 μmol/L) or hepatic function (as manifested by concentrations of alanine-aminotransferase, asparagine-aminotransferase, γ-glutamyl transpeptidase, total billirubin or alkaline phosphatase > 2 times the upper limit of normal), or other severe medical conditions that might have interfered with the study such as epilepsy, asthma, chronic obstructive pulmonary diseases, inflammatory bowel diseases, and rheumatoid arthritis. Finally, participants that had used medication such as corticosteroids, diuretics, or lipid-lowering drugs, including statins, 2 mo before the start of the study were excluded. During the screening procedures, fasting serum lipids, plasma glucose, blood pressure, and variables reflecting renal and hepatic function were determined on 2 separate occasions with an interval of at least 3 d. Blood pressure was measured 4 times after a 10-min rest and the last 3 values were averaged. All participants gave their written informed consent before the start of the study. The medical ethical committee of the Maastricht University approved the study.

Diets and design

The study had a 2 × 2 factorial design. During the 3-wk run-in period, participants were instructed to consume at lunch or at dinner, but not at breakfast, a low-fat yogurt drink (60 mL; Emmi) containing no plant stanol esters, and a placebo tablet in the evening. At the end of the run-in period, participants were required to return the empty yogurt tubs as well as the flasks containing the tablets that were left over, which were counted back to calculate yogurt and tablet usage during the run-in period. At the start of the experimental period, participants were randomly allocated to 1 of the 4 treatment groups, stratified for gender and age. Baseline characteristics of the 4 intervention groups are listed in **Table 1**. The control group continued to use the placebo yogurt drink and the placebo tablets for another 8 wk, while a second group (plant stanol group) used the same low-fat yogurt drink to which a vegetable oil-based plant stanol ester mixture (equivalent to 2 g of free plant stanols/d) was added and the placebo tablets. A third group (statin group) used the placebo yogurt drink plus a low-dose OTC statin (10 mg simvastatin), while the fourth group (combination group) received the low-fat plant stanol ester enriched yogurt drink plus the low-dose OTC statin. All products were coded with a color label so the participants and investigators were unaware of the treatments. The plant stanol ester mixtures in the yogurt drinks consisted of ~70% sitostanol ester and 30% campestanol ester. The nutritional composition of the yogurt beverages was 234 kJ, 3.2 g protein, 6.2 g carbohydrates, and 2.0 g fat in 100 mL. At the end of the experimental period, yogurt and tablet usage was again calculated as described for the run-in period.

To estimate their energy and nutrient intakes, participants recorded in the last week of both the run-in and the experimental periods their food intakes during the previous 4 wk by filling in validated food frequency lists consisting of 100 items (*7*). Food frequency lists were immediately checked by a dietician in the presence of a participant. Next, all 100 items in the list were coded according to daily, weekly, or monthly use and the composition of the diets was calculated using compositional data of all individual products as present in the Dutch food composition table. Items were coded and the composition of the diets was calculated according the Dutch food composition table. The participants recorded in diaries any signs of illness, medication used, and any deviations from the protocol. In addition, participants were asked to not change their habitual diet, level of physical exercise, use of alcohol, or oral contraceptives during the study. Body weight was recorded at each visit (6 times during the 11-wk study period).

Blood sampling and analyses

Blood sampling. Blood samples were taken twice during the run-in period (wk 2 and 3) and twice at the end of the experimental period (wk 10 and 11) after an overnight fast. In addition, participants were not allowed to drink alcohol the day preceding the morning of blood sampling. All venipunctures were performed by the same person at the same location and at approximately the same time of the same day of the

TABLE 1 Population characteristics before the start of the study[1]

	Control	Simvastatin	Plant stanols	Simvastatin + plant stanols
Males/females, *n*	5/4	6/4	7/2	5/3
Age, *y*	60 ± 7	61 ± 8	60 ± 4	60 ± 8
BMI, *kg/m^2*	30.2 ± 1.9	29.2 ± 3.3	28.1 ± 2.6	29.7 ± 7.5
Waist circumference, *cm*	101.7 ± 6.7	94.7 ± 7.0	95.7 ± 6.7	100.4 ± 4.3
Systolic BP, *mm Hg*	142 ± 14	139 ± 7	138 ± 11	150 ± 30
Diastolic BP, *mm Hg*	92 ± 10	89 ± 7	94 ± 8	96 ± 21
Serum total cholesterol,[2] *mmol/L*	6.50 ± 1.59	6.12 ± 1.03	6.29 ± 1.19	7.08 ± 1.05
Non-HDL-C,[2] *mmol/L*	5.47 ± 1.29	5.04 ± 1.09	5.32 ± 1.29	5.82 ± 1.21
Serum HDL-C,[2] *mmol/L*	1.03 ± 0.26	1.08 ± 0.25	0.97 ± 0.15	1.26 ± 0.39
Serum TAG,[3] *mmol/L*	2.24 ± 1.26	2.27 ± 0.73	2.21 ± 0.98	2.38 ± 1.02
Plasma glucose, *mmol/L*	6.02 ± 1.07	5.97 ± 0.78	6.33 ± 0.56	6.43 ± 0.62

[1] Values are means ± SD. Groups did not differ significantly.
[2] To convert values for total, HDL and non-HDL-C to mg/dL, multiply by 38.67.
[3] To convert values for TAG to mg/dL, multiply by 88.54.

week, with participants in a recumbent position after lying down for 10 min. No blood was sampled on Mondays to exclude effects of possible deviating behavior during the weekends. Blood was collected into EDTA tubes (Monoject sterile, Sherwood Medical) and clotting tubes (CORVAC, integrated serum separator tube, Sherwood Medical). EDTA blood was used for hematological variables and for preparing EDTA plasma by centrifugation at 2000 × *g*; 30 min at 4°C directly after sampling. Serum was obtained by centrifugation of the clotting tubes at 2000 × *g*; 30 min at 4°C, for at least 1 h after venipuncture. All plasma and serum samples were immediately snap-frozen and stored in small portions at −80°C until further analysis. EDTA plasma was used for analysis of all inflammatory markers, glucose, apolipoprotein (apo) CII, apoCIII, FFA, and cholesterol ester transfer protein (CETP) mass, whereas serum was used for apoA-I, apoB, TAG, and cholesterol measurements.

Lipids and (apo)lipoproteins. Total cholesterol (CHOD/PAP method; Roche Diagnostics Systems, Hofmann-La Roche), HDL-C (CHOD/PAP method; Roche Diagnostics Systems, Hofmann-La Roche) after precipitation of apoB-containing lipoproteins by adding phosphotungstic acid and magnesium ions (precipitation method; Monotest cholesterol, Boehringer Mannheim), and TAG (GPO-Trinder; Sigma Diagnostics) were analyzed in serum enzymatically. apoB and apoA-1 were measured using an immunoturbidimetric reaction (UNI-KIT apoB and UNI-KIT apoA-I, Roche). All samples from 1 participant were analyzed within 1 analysis at the end of the study using a semiautomatic COBAS Mira analyzer (Roche). The lipid and (apo)-lipoprotein concentrations of samples from wk 2 and 3 and from wk 10 and 11 were averaged before data analysis. We could not use the Friedewald equation to calculate serum LDL-C, because of the increased serum TAG concentrations of our population. Instead, we report non-HDL-C concentrations.

Apo-CII, apoC-III, and FFA concentrations and CETP mass. ApoC-II, apoCIII, and FFA concentrations as well as CETP mass were determined at the end of the run-in period (wk 2 and 3) and the experimental period (wk 10 and 11). Analysis was performed in samples that were pooled before analysis (wk 2 and 3) or wk 10 and 11. Samples from 1 participant were always analyzed within 1 analysis. ApoC-II and apoCIII concentrations were determined using a commercially available immunonephelometric assay (Wako) using a cobas mira autoanalyzer (Roche Switzerland). FFA concentrations were analyzed by using a NEFA C-kit (Wako Biochemicals) and CETP mass was determined as described (8).

Liver x receptor and PPARα mRNA expression in peripheral mononuclear blood cells. Peripheral mononuclear blood cells (PBMC) were isolated from EDTA blood by using lymphoprep (Nycomed Pharma) according to the manufacturer's instructions. From these PBMC, total mRNA was isolated by using a Qiagen RNeasy mini kit combined with on-column DNase treatment (Qiagen). Liver x receptor (LXR)-α and PPARα mRNA expression levels were determined using assays on demand (Hs00172885 m1 for LXRα and Hs00231882 m1 for PPARα and Hs99999903 m1 for β-actin, all from Applied Biosystems).

Markers of low-grade inflammation and endothelial (dys)function

At the end of the run-in period and the experimental period, markers reflecting low-grade inflammation and endothelial (dys)function were measured in EDTA plasma. Samples from wk 2 and 3 and from wk 10 and 11 were pooled before analyses. Samples from 1 participant were analyzed on 1 plate or in 1 analysis. sE-selectin and sICAM were measured as described (9,10). sVCAM-1, monocyte chemoattractant protein-1, interleukin-6 (IL-6), matrix metallopeptidase-9, and CD40 ligand concentrations were measured using commercially available kits (R&D Systems Europe) according to the manufacturer's instructions. hsCRP was measured on Cobas Mira with a commercially available kit (Kamiya Biomedical). Serum amyloid A (SAA) was analyzed as described (11). For all assays, samples were analyzed in duplicate.

Clinical safety variables and hematological measurements

In wk 3 and 11, concentrations of variables reflecting kidney and liver function, i.e. total bilirubin, aspartate aminotransferase, alanine transaminase, alkaline phosphatase, γ-glutamyl transpeptidase, and creatinine were determined on a Beckman Synchron CX7 Clinical systems at the Department of Clinical Chemistry, University Hospital Maastricht, Maastricht, the Netherlands. Hematological variables (white blood cell count, percentages and numbers of lymphocytes, mononuclear cells and granulocytes, RBC count, hemoglobin concentration, hematocrit, mean corpuscular volume, and platelet count and volume) were determined on a Coulter Counter (Coulter MD series, Beckmann Coulter). None of these variables were affected by the treatments (data not shown).

Statistics. Baseline characteristics were tested for significant differences among the 4 groups with ANOVA. Changes for all variables were calculated for each participant as the difference between values of the experimental period and run-in period. Equality of variance for changes of all variables was tested. Because all changes showed an equal variance, the differences in changes among the groups were tested with factorial ANOVA consistent with the 2 × 2 factorial design. We calculated the individual contribution of plant stanols and simvastatin to the response variables as well as the possible interaction between plant stanols and simvastatin on these response variables. When a significant diet effect was found ($P < 0.05$), the 4 treatments were compared pair wise and corrected for multiple group comparisons using the Bonferroni multi comparison test. To exclude the possibility that nonsignificant differences in concentrations at the end of the run-in period had influenced the outcome of the study, ANCOVA analysis using values at the end of the run-in period as covariates were performed as well. All statistical analyses were performed with SPSS 11.0 for Mac Os X. Values in the text are means ± SD.

Results

Baseline characteristics. Participants had a clear atherogenic phenotype as defined by the National Cholesterol Education Program adult treatment panel III consisting of an increased BMI, elevated blood pressure, low HDL-C, and elevated TAG and or glucose concentrations (Table 1). Population characteristics did not differ significantly among the 4 groups.

Dietary composition and body weight. During the run-in period, the mean daily intake of energy for all participants was 8.3 ± 2.4 MJ, total fat was 32 ± 5 energy percent (en%), SFA was 12 ± 2 en%, monounsaturated fatty acids was 10 ± 2 en%, PUFA was 6 ± 1 en%, protein was 19 ± 3 en%, carbohydrates was 48 ± 6 en%, cholesterol was 218 ± 88 mg, and total fiber was 25 ± 8 g. The reported intake of monounsaturated fatty acids (en%) was somewhat low in relation to the intake of SFA and total fat intake, for which we do not have a clear explanation. However, this occurred in all 4 groups, and for all other variables, the 4 groups did not differ during the run-in period. Dietary intakes did not change during the experimental period (data not shown). Counting back capsules and empty yogurt drink cups that were left over indicated excellent compliance throughout the entire study. Body weight did not change during the study.

Serum lipid and (apo)lipoproteins. Simvastatin lowered serum total cholesterol concentrations by 1.43 mmol/L ($P <$ 0.001 vs. control). Plant stanol esters lowered serum total cholesterol by 0.75 mmol/L ($P = 0.015$ vs. control), whereas their combination lowered serum total cholesterol concentrations by 1.89 mmol/L ($P < 0.001$ vs. control and $P = 0.001$ vs. plant stanol) (**Table 2**). Reductions in serum total cholesterol could be entirely ascribed to changes in non-HDL-C concentrations. Simvastatin lowered non-HDL-C by 1.50 mmol/L ($P < 0.001$ vs.

TABLE 2 Effects of simvastatin (10 mg/d), plant stanol esters (2.0 g/d) or simvastatin (10 mg/d) + plant stanol esters (2.0 g/d) on serum lipid and (apo)lipoprotein concentrations in patients with metabolic syndrome[1]

	Control	Simvastatin	Plant stanols	Simvastatin + plant stanols	Plant stanol effect, *P*-value	Simvastatin effect, *P*-value	Simvastatin × plant stanol interaction
n	9	10	9	8			
Total cholesterol, *mmol/L*							
Run-in	6.47 ± 1.49	6.40 ± 1.13	7.45 ± 1.27	7.25 ± 1.23			
Change	0.06 ± 0.47[a]	−1.43 ± 0.6[b]	−0.75 ± 0.47[c]	−1.89 ± 1.01[b]	0.008	<0.001	0.441
Non-HDL-C, *mmol/L*							
Run-in	5.29 ± 1.40	5.27 ± 1.04	6.28 ± 1.31	6.08 ± 1.11			
Change	0.12 ± 0.45[a]	−1.50 ± 0.61[b]	−0.73 ± 0.52[c]	−2.02 ± 1.00[b]	0.004	<0.001	0.459
HDL-C, *mmol/L*							
Run-in	1.18 ± 0.20	1.12 ± 0.23	1.16 ± 0.19	1.17 ± 0.42			
Change	−0.07 ± 0.09[a]	0.06 ± 0.60[b]	−0.02 ± 0.14[a]	0.12 ± 0.15[b]	0.159	<0.001	0.879
Total-:HDL-C							
Run-in	5.55 ± 1.13	5.85 ± 1.23	6.54 ± 1.65	6.77 ± 2.23			
Change	0.37 ± 0.48[a]	−1.57 ± 0.54[b]	−0.60 ± 0.12[c]	−2.15 ± 0.90[b]	0.007	<0.001	0.477
TAG, *mmol/L*							
Run-in	1.97 ± 0.91	2.30 ± 0.85	1.45 ± 0.50	2.00 ± 0.92			
Change	0.23 ± 0.54[a]	−0.23 ± 0.52[b]	−0.23 ± 0.36[b]	−0.42 ± 0.24[b]	0.042	0.041	0.399
ApoB, *g/L*							
Run-in	1.17 ± 0.27	1.12 ± 0.19	1.26 ± 0.18	1.29 ± 0.19			
Change	−0.02 ± 0.09[a]	−0.25 ± 0.10[b]	−0.07 ± 0.10[a]	−0.36 ± 0.17[b]	0.018	<0.001	0.540
ApoA-I, *g/L*							
Run-in	1.52 ± 0.14	1.49 ± 0.20	1.46 ± 0.13	1.51 ± 0.26			
Change	0.00 ± 0.03[a]	0.03 ± 0.05[a,b]	−0.03 ± 0.06[a]	0.07 ± 0.09[b]	0.640	0.001	0.692

[1] Values are means ± SD. Means in a row with superscripts without a common letter differ, $P < 0.05$.

control), plant stanol esters by 0.73 mmol/L ($P = 0.012$ vs. control), and the combination treatment by 2.02 mmol/L ($P < 0.001$ vs. control). Only simvastatin and the combination, but not plant stanol esters only, changed serum HDL-C concentrations. Simvastatin increased serum HDL-C by 0.06 mmol/L and the combination treatment by 0.12 mmol/L (both $P < 0.001$ vs. control). Consistent with the effects on serum total and HDL-C concentrations, the ratio of total-:HDL-C was lowered by 1.57 ($P < 0.001$ vs. control) in the simvastatin group, 0.60 in the plant stanol ester group ($P < 0.05$ vs. control), and 2.15 in the combination group ($P < 0.001$ vs. control). Interestingly, 10 mg simvastatin significantly lowered serum TAG concentrations by 0.23 mmol/L ($P = 0.034$ vs. control), but also the plant stanol esters lowered serum TAG concentrations by 0.23 mmol/L ($P = 0.044$ vs. control). The combination was even more effective, because serum TAG concentrations were lowered by 0.42 mmol/L ($P < 0.01$ vs. control). Compared with the control group, the simvastatin as well as the combination groups had reduced apoB concentrations of 0.27 g/L ($P < 0.001$) and 0.38 g/L ($P < 0.001$), respectively. Finally, in the simvastatin and combination groups, apoA-I concentrations increased with 0.03 g/L ($P < 0.05$) and 0.07 g/L ($P < 0.05$), respectively. For all lipids and (apo)lipoproteins, we also conducted ANCOVA analysis using values at the end of the run-in period as covariates; however, this did not change the outcome and conclusions. Factorial ANOVA analysis to calculate the effect of the factor plant stanol esters or simvastatin had the same results as the ANOVA analysis (Table 2). The interaction term of the factorial model was not significant, indicating that there was no synergism between plant stanol ester consumption and simvastatin treatment.

Apo-CII, apoCIII, FFA, and CETP mass. Plant stanol esters lowered the absolute apoCII concentrations by 7.9 mg/L ($P = 0.024$ vs. control). The combination of simvastatin and plant stanol esters lowered absolute apoCII concentrations by 8.0 mg/L ($P = 0.022$ vs. control). Changes in apoCIII concentrations and the apoCII:CIII ratio did not differ between the groups (**Table 3**). There were also no significant changes in serum CETP mass or FFA concentrations by the interventions compared with the controls.

LXR and PPARα mRNA expression in PBMC. Levels of LXRα and PPARα mRNA in PBMC did not change in any group from the end of the run-in period through the end of the experiment (data not shown).

Markers of low-grade inflammation and endothelial (dys)-function. None of the interventions lowered hsCRP or SAA concentrations (**Supplemental Table 1**). In fact, hsCRP concentrations tended to be greater than in the control group in the plant stanol ester ($P = 0.062$) and combination ($P = 0.086$) groups. In the 3 treatment groups, the change in hsCRP correlated with the change in SAA concentrations ($r = 0.484$; $P = 0.011$). The treatments did not affect markers of endothelial function (**Supplemental Table 2**).

Discussion

Plant stanol esters have gained a prominent position in strategies to lower CHD risk because of their serum LDL-C–lowering effects (1), as consistently demonstrated in numerous placebo-controlled trials in different population and patient groups (12). Our results are fully consistent with these observations. Interestingly, we show here that plant stanols also lower serum TAG concentrations in metabolic syndrome patients. These effects are

TABLE 3 Effects of plant stanol esters on apoCIII, apoCII, CETP, and FFA concentrations, and the apoCII:apoCIII ratio in patients with metabolic syndrome[1]

	Control	Simvastatin	Plant stanols	Simvastatin + plant stanols	Plant stanol effect, *P*-value	Simvastatin effect, *P*-value	Simvastatin x plant stanol interaction, *P*-value
n	9	10	9	8			
apoCIII, *mg/L*							
Run-in	123.5 ± 49.9	151.4 ± 36.6	14.9 ± 49.8	147.1 ± 39.8			
Change	6.7 ± 26.8	−17.5 ± 30.0	13.1 ± 35.9	19.6 ± 6.4	0.243	0.105	0.347
apoCII, *mg/L*							
Run-in	59.3 ± 33.9	78.4 ± 21.9	63.9 ± 23.3	76.0 ± 21.7			
Change	8.0 ± 13.7a	−2.0 ± 16.4[a,b]	7.9 ± 16.3[b]	8.0 ± 7.4[b]	0.028	0.295	0.305
apoCII:apoCIII ratio							
Run-in	0.47 ± 0.20	0.52 ± 0.10	0.56 ± 0.10	0.52 ± 0.09			
Change	0.03 ± 0.10	0.06 ± 0.11	0.04 ± 0.13	0.00 ± 0.08	0.101	0.321	0.851
CETP mass, *mg/L*							
Run-in	3.46 ± 1.01	2.67 ± 0.88	2.44 ± 0.84	2.54 ± 0.68			
Change	0.37 ± 0.64	0.31 ± 0.51	0.37 ± 0.53	0.41 ± 0.66	0.857	0.549	0.647
FFA, μmol/L							
Run-in	306 ± 107	399 ± 142	299 ± 102	299 ± 84			
Change	−28 ± 145	−100 ± 91	−40 ± 89	−37 ± 137	0.518	0.382	0.340

[1] Values are means ± SD. Means in a row with superscripts without a common letter differ, $P < 0.05$.

in line with the results of our recent meta-analysis (4), from which we concluded that plant stanol esters lowered serum TAG concentrations, especially in participants with elevated baseline serum TAG levels. What was lacking until now was a confirmation study in a population with elevated TAG concentrations such as we have presented here.

The effects on serum TAG concentrations can be explained by at least 3 different underlying mechanisms, i.e. an enhanced lipoprotein lipase (LPL)-mediated lipolysis and FFA uptake, an increased CETP activity, or a reduced hepatic VLDL production. Effects of plant stanol esters on LPL activity have not been studied before to our knowledge. Therefore, we first analyzed effects of plant stanol esters on serum apoCII and apoCIII concentrations. ApoCII and apoCIII are, respectively, the activator and inhibitory ligands for LPL. However, the unchanged apoCII:CIII ratio suggests that LPL activity was most likely unaffected. In addition, we were unable to show an effect on CETP mass during plant stanol ester consumption. This absence is consistent with the observation in human intervention studies that serum HDL-C concentrations are not increased during plant stanol ester consumption. However, 2 earlier studies (13,14) reported a reduction in CETP concentrations by plant stanol esters in healthy Japanese individuals or for plant sterol esters in hypercholesterolemic individuals. Currently, we cannot explain the discrepancy between these 2 studies and ours. Altogether, this suggests that the reduced serum TAG concentrations during plant stanol ester consumption are not attributable to the remodeling of TAG-rich lipoprotein particles within the circulation.

Concerning the third possibility, i.e. a lowered hepatic VLDL production, the finding that an oxidation product of campesterol (campestenone) could activate in rats hepatic expression of PPARα target genes, among which genes encoding for enzymes involved in the β-oxidation of fatty acids, is interesting (15). In fact, hepatic fat accumulation in the treated rats decreased, which may have resulted in decreased VLDL production (15). However, campestanone cannot be synthesized from a stanol molecule. Therefore, it remains to be answered whether plant sterols or plant sterol metabolites also affect hepatic PPARα gene expression in humans and whether the effect on hepatic PPARα target genes as described for campestanone is also true for plant stanols or a metabolite formed from plant stanols. We evaluated effects of plant stanol ester consumption on PPARα mRNA expression in isolated PBMC. It has been shown that mRNA expression of PPARα and especially LXRα in these cells is related to serum cholesterol concentrations and CHD risk (16). However, we did not find any change in the expression of both transcription factors in PBMC. Although disappointing, this does not exclude the possibility that there was a change in PPARα activity in the liver.

Interestingly, the reduction in serum TAG concentrations in the combination group was almost the sum of the separate effects induced by 10 mg simvastatin and the plant stanol esters. This suggests that effects on TAG were additive, which might imply that simvastatin and plant stanol esters lower serum TAG concentrations by different mechanisms. As already discussed, the mechanism for the serum TAG-lowering effects of plant stanols is not known, whereas statins lower hepatic production of VLDL1 particles (17,18). Alternatively, it is possible that the mechanisms are similar and that effects at the present intakes of stanols or simvastatin had not reached their maximal effects. In fact, the results from our meta-analysis (4) did not suggest that effects of plant stanol esters on TAG leveled off at higher intakes. For simvastatin, it has also been shown that increasing the dose from 20 to 40 mg/d lowered serum TAG by an additional 9% (19). Therefore, it is possible that consumption of plant stanol esters also decreases hepatic VLDL1 production. Therefore, the finding that plant stanol esters did not lower apoB concentrations in this population of metabolic syndrome patients is not supportive. On the other hand, it might also be that there has been a shift from VLDL1 to VLDL2 particles produced by the liver, explaining the effects on serum TAG concentrations without significantly lowering apoB concentrations.

The reduction in serum non-HDL-C concentrations is in the upper range of what could be expected for this daily plant stanol intake (12). This might suggest that metabolic syndrome patients, or maybe overweight persons in general, are more susceptible to interventions aimed at lowering intestinal cholesterol absorption. However, the opposite has also been suggested, i.e. that metabolic syndrome patients are rather cholesterol synthesizers than absorbers (20), which makes it difficult to explain the

13.8% reduction in non-HDL-C. It should, however, be realized that we are reporting here changes in serum non-HDL-C concentrations instead of serum LDL-C concentrations. Serum non-HDL-C includes serum VLDL- and intermediate density lipoprotein-C concentrations. Because we report a change in serum TAG concentrations in this population of metabolic syndrome patients, we should acknowledge a reduction in serum VLDL-C concentrations in the experimental groups compared with control. This means that the changes in serum LDL-C concentrations, when, e.g., analyzed by using LDL direct assay, would have been somewhat lower.

Besides serum lipoprotein concentrations, markers related to the process of low-grade chronic inflammation and endothelial dysfunction are also valid predictors of future CHD risk (21). Whether plant stanol esters affect inflammatory profiles and/or markers of endothelial function has scarcely been studied. Some studies have reported that plant stanol or sterol ester consumption lowered hsCRP concentrations (6,22), but this was not confirmed by other studies (5,23,24). In the present study, we also did not find an effect of plant stanol esters on hsCRP concentrations. In addition, hsCRP concentrations were not lowered in the group receiving the low-dose statins, which is consistent with earlier studies using 10 mg/d simvastatin with a comparable duration of 12 wk (25,26). Because studies using a higher simvastatin dose or combining 10 mg simvastatin with, e.g., ezetimibe (25) lowered hsCRP levels, it seems that the dose and/or the concomitant reduction in serum non-HDL-C in the present study was not large enough to find an effect on hsCRP. With respect to markers reflecting endothelial dysfunction, we have in earlier studies not shown effects of plant stanol esters on sICAM, sVCAM, sE-selectin, and monocyte chemoattractant protein-1, although LDL-C was significantly lowered (5). Because that study was performed in participants who had already received stable statin treatment for several years, it was speculated that the absence of an effect of plant stanol esters could be ascribed to the overruling pleiotropic effects of the statins. Another possible explanation is that the duration of our intervention period in the present study was too short to induce effects on endothelial function. This assumption is very likely, because the groups receiving the low-dose statins also did not show any changes in these circulating markers of low-grade inflammation (hsCRP, SAA, and IL-6) or endothelial function (sICAM, sVCAM, sE-selectin, matrix metallopeptidase-9, and CD40 ligand). This is consistent with an earlier 6-wk study (27) showing no effects on sVCAM, IL-6, or SAA in patients with mixed hyperlipidemia, whereas 1 y of 20 mg/d simvastatin lowered sICAM, sE-selectin, and sP-selectin in patients with established CHD (28).

In conclusion, we have shown that in addition to lowering serum non-HDL-C concentrations, consumption of a plant stanol ester-enriched yogurt drink for 8 wk lowered serum TAG concentrations by 27% in metabolic syndrome patients. Effects on TAG concentrations were also evident when plant stanol esters were consumed in combination with a low-dose (10 mg) simvastatin treatment. Although we realize that the sample size of this study is relatively small and findings should be confirmed in larger studies, it seems fair to conclude that consumption of plant stanol esters alone or in addition to OTC statin intake has beneficial effects on improvement of the atherogenic lipoprotein profile in metabolic syndrome patients.

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