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COMMUNICATION

Plant Stanol Esters Lower Serum Triacylglycerol Concentrations via a Reduced Hepatic VLDL-1 Production

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Abstract Plant stanol esters not only lower low density lipoprotein cholesterol but also have previously been shown to lower serum triacylglycerol (TAG) concentrations, especially in subjects with elevated TAG concentrations. To find a possible explanation, we explored changes in serum lipoprotein profiles, as measured with nuclear magnetic resonance. For this, serum samples from two parallel-designed controlled studies were evaluated before and 8 weeks after the consumption of plant stanol esters. In the first study, dyslipidemic metabolic syndrome subjects participated and in the second study normolipidemic subjects. In metabolic syndrome subjects, plant stanol esters lowered concentrations of large (>60 nm) and medium (35–60 nm) VLDL particles as compared to controls. In normolipidemic subjects, the serum concentration of large VLDL-1 particles was also lowered, although less pronounced. Based on these findings, we hypothesize that the effect of plant stanol esters on serum TAG concentrations origins from a lowered hepatic production of large TAG-rich VLDL-1 particles.

Keywords Plant stanol esters · Diet · Triacylglycerol · Lipoproteins

Abbreviations

VLDL	Very low density lipoprotein
NMR	Nuclear magnetic resonance
TAG	Triacylglycerol
LDL	Low density lipoprotein
HDL	High density lipoprotein
LPL	Lipoprotein lipase
CETP	Cholesterol ester transfer protein
PPAR	Peroxisome proliferator activator receptor

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Introduction

It is well established that plant stanol esters lower intestinal cholesterol absorption, ultimately resulting in significantly reduced serum low density lipoprotein (LDL) cholesterol concentrations at a recommended daily intakes of 2.0–2.5 g [1–3]. Based on our meta-analysis [4], we recently suggested that plant stanol esters might also lower serum triacylglycerol (TAG) concentrations. These effects were in particular evident in subjects with elevated TAG baseline concentrations. This hypothesis was confirmed in a double-blind placebo-controlled intervention trial in subjects with the metabolic syndrome, who are characterised by elevated circulating TAG concentrations [5]. However, an explanation for the observed reduction in serum TAG concentrations is missing. Therefore, we monitored changes in serum lipoprotein profiles via nuclear magnetic resonance (NMR) analysis obtained in serum samples from the metabolic syndrome subjects. This NMR approach measures concentrations of the different lipoprotein subpopulations large-, medium- and small-VLDL, IDL, large-, small-, medium small- and very-small-LDL, large-, medium- and small-HDL. Large VLDL particles are further called VLDL-1 particles. In addition, VLDL-, LDL-, and HDL-size are calculated. This knowledge could provide new leads concerning effects of plan stanol esters on lipid and lipoprotein metabolism. For comparison, these parameters were also analysed in serum samples from another

double-blind placebo-controlled intervention study in normolipidemic subjects in which no effects of plant stanol esters consumption on serum TAG concentrations were found [6].

Methods

Study 1: Metabolic Syndrome Subjects, Diets and Design

Subject characteristics of this study have been reported before [5]. In short, we included 18 subjects, who had the metabolic syndrome according to the ATP III criteria [7]. All subjects gave their written informed consent before the start of the study. The study was approved by the medical ethical committee of Maastricht University. During a 3-week run-in period, all subjects were instructed to consume with the meals (lunch or diner, but not breakfast) a low-fat yogurt drink (Emmi, Lucerne, Swiss) containing no plant stanol esters. At the beginning of the experimental period, subjects were randomly allocated to one of the two treatment groups, stratified for gender and age. Baseline characteristics of the groups are listed in Table 1. The control group continued to use the placebo yogurt drink for another 8 weeks, while a second group used the same low-fat yogurt drink to which a vegetable oil based stanol ester mixture was added. Daily intake of plant stanols was 2 g provided as a one-shot yogurt drink. All products were coded with a coloured label to blind the subjects and the investigators.

Study 2: Normolipidemic Subjects, Diets and Design

As described earlier [6], 112 non-hypercholesterolemic apparently healthy subjects participated in a double-blind placebo-controlled trial in which we compared the effects of vegetable oil based and pine-wood based plant stanol esters versus placebo. In brief, during a 4-week run-in period subjects were instructed to use margarines and shortenings with breakfast, lunch and for the preparation of dinner. At the beginning of the experimental period, subjects were randomly allocated to one of the three treatment groups, stratified for gender and age. Since there were no differences between the two plant stanol ester sources in their effects on cholesterol metabolism [6, 8, 9], the vegetable-oil based and pine-wood based groups were combined into one plant stanol ester group for further analysis. For the current analysis, we randomly selected 25 subjects form the control and 25 subjects from each plant stanol ester group, hereby taking gender distributions into account. Baseline characteristics of the control versus the intervention groups are listed in Table 1. The control group continued to use the placebo margarines and shortenings for another 8 weeks, while a second group used the same margarines and shortenings to which the plant stanol ester mixtures were added. Total daily plant stanol intake was 3.8–4.1 g/day, which is higher than the currently recommended intake. All products were coded with a coloured label to blind the subjects and the investigators. As in study 1, all subjects gave their written informed consent before the start of the study, and the study was approved by the medical ethical committee of Maastricht University.

Table 1 Baseline population characteristics of metabolic syndrome (study 1) and normolipidemic (study 2) subjects

	Metabolic syndrome subjects		Normolipidemic subjects	
	Controls ($N = 9$)	Plant stanols ($N = 9$)	Controls ($N = 25$)	Plant stanols ($N = 50$)
Age (years)	60 ± 7	60 ± 4	35 ± 17	34 ± 15
BMI (kg/m^2)	30.2 ± 1.9	28.1 ± 2.6	23.0 ± 3.2	22.9 ± 3.0
Blood pressure (mm Hg)				
Systolic BP	142 ± 14	138 ± 11	119 ± 12	123 ± 12
Diastolic BP	92 ± 10	94 ± 8	74 ± 8	75 ± 8
Serum lipid (mmol/L)				
Total cholesterol	6.50 ± 1.59	6.29 ± 1.19	4.81 ± 0.79	5.02 ± 0.69
Non-HDL cholesterol	5.47 ± 1.29	5.32 ± 1.29	–	–
LDL cholesterol	–	–	2.82 ± 0.73	2.89 ± 0.71
HDL cholesterol	1.03 ± 0.26	0.97 ± 0.15	1.61 ± 0.32	1.58 ± 0.39
Triacylglycerol	2.24 ± 1.26	2.21 ± 0.98	0.83 ± 0.34	1.02 ± 0.61

Values are means ± SD. To convert values for total, HDL and LDL cholesterol to mg/dL × 38.67. To convert values for triacylglycerols to mg/dL × 88.54

Blood Sampling and Analyses

Blood Sampling

In both studies, blood samples obtained at the end of the run-in period and at the end of the 8 weeks experimental periods, were used for further analysis. All blood samples were taken after an overnight fast; i.e., subjects were not allowed to eat and drink (except water) after 10 p.m. in the evening preceding blood sampling, to drink alcohol the day preceding blood sampling, or to smoke on the morning of blood sampling. Blood was sampled into clotting tubes (CORVAC, integrated serum separator tube, Sherwood Medical Company, St. Louis, USA). Serum was obtained by centrifugation of the clotting tubes at 2,000×*g* for 30 min at 4 °C, minimally 1 h after venipuncture. All serum samples were immediately stored in small portions, snap-frozen, and stored at −80 °C until further analysis.

Lipids, Lipoproteins and Lipoprotein Profiles

Serum lipids and lipoproteins were analysed as described [5]. All samples from one subject were analysed within the same run at the end of the study. In study 1, we reported serum non-HDL cholesterol concentrations instead of serum LDL cholesterol concentrations because we could not use the Friedewald equation to calculate serum LDL cholesterol, because of the increased serum TAG concentrations of our population. Lipoprotein profiles at the end of the run-in period and at the end of the 8 week intervention period were evaluated by NMR (Liposcience, Raleigh, USA). Before NMR analysis, two blood samples at the end of the run-in period and at the end of the experimental period (at least three and maximally 7 days between both blood sampling moments) were pooled to minimise the noise due to normal day-to-day variation in lipoprotein particle concentrations. Although the two intervention studies were performed 8 years apart, all NMR analysis were performed in samples stored at −80 °C in retrospect for both studies at the same time.

Statistics

Changes for all parameters were calculated for each subject as the difference between values of the intervention period and run-in periods and reported as means ± SD. The differences in changes between the groups were tested with an unpaired *t* test in which a $P < 0.05$ was considered significant. All statistical analyses were performed with Statview 4.5.

Results and Discussion

Serum Lipid and Lipoproteins

In both studies, plant stanol ester consumption improved serum lipoprotein profiles. Compared with the control group, consumption of plant stanol esters for 8 weeks lowered serum non-HDL cholesterol concentrations by 0.73 ± 0.52 mmol/L or 13.8% ($P = 0.012$) in the metabolic syndrome subjects. In addition, plant stanol esters lowered TAG concentrations by 0.23 ± 0.36 mmol/L or 27.5% ($P = 0.044$). No effects on serum HDL cholesterol concentrations were found.

In normolipidemic subjects, plant stanol esters also significantly lowered serum LDL cholesterol concentrations. Compared with the control group, this decrease was 0.44 ± 0.29 mmol/L or 13.7% ($P < 0.001$) for the plant stanol ester group. There were no effects on serum TAG and HDL cholesterol concentrations. The finding that serum TAG concentrations were unchanged in this study is in line with all other studies in normolipidemic subjects [1].

We now aim to find an explanation for the observed effects on serum TAG concentrations and here postulate the hypothesis that the TAG lowering effect of plant stanol esters originates from a reduced hepatic production of large TAG-rich VLDL-1 particles. We had earlier excluded that the effects of plant stanol ester consumption on serum TAG concentrations could be explained by changes in serum apoCII and apoCIII concentrations, which are both determinants of lipoprotein lipase (LPL) activity [5]. In line with the unaffected HDL cholesterol concentrations, we have also shown that CETP activity was not changed [5]. The hypothesis postulating effects on hepatic VLDL-1 production is based on the present finding that in metabolic syndrome subjects plant stanol esters significantly reduced concentrations of large (>60 nm) and medium (35–60 nm) VLDL particles, as compared to the control group. We are aware of the fact that we did not measure hepatic VLDL particle production and VLDL kinetics (i.e., referring to LPL activity) by using stable isotope approaches. Therefore it remains speculative whether the suggested hypothetical mechanism is correct. No effects were found on small (27–35 nm) VLDL particles or any of the other lipoprotein fractions (Table 2). The fact that there was no change in the number of small sized LDL particles was somewhat unexpected, since the large VLDL-1 particles—of which the numbers were strongly reduced after plant stanol ester consumption—are precursors for the smaller denser LDL particles. This might also imply that the effects on VLDL-1 concentrations are not caused by a reduced hepatic VLDL-1 production.

Table 2 Effects of plant stanol esters on lipoprotein particle concentrations in metabolic syndrome (study 1) and normolipidemic (study 2) subjects

	Metabolic syndrome subjects		Normolipidemic subjects	
	Controls ($N = 9$)	Plant stanols ($N = 9$)	Controls ($N = 25$)	Plant stanols ($N = 50$)
Large VLDL particles, >60 nm (nmol/L)				
Run-in	5.9 ± 7.1	6.7 ± 8.2	1.9 ± 1.6	2.5 ± 4.1
Test period	8.1 ± 6.8	2.9 ± 3.2	2.0 ± 2.1	1.8 ± 2.8
Change	2.2 ± 5.1	−3.8 ± 6.7[a]	0.1 ± 2.7	−0.7 ± 3.1[a]
Medium VLDL particles, 35–60 nm (nmol/L)				
Run-in	33.4 ± 21.5	36.6 ± 20.9	21.8 ± 10.6	22.7 ± 10.6
Test period	39.2 ± 20.8	27.5 ± 17.8	20.3 ± 12.1	22.0 ± 14.7
Change	5.8 ± 17.5	−9.1 ± 17.7[a]	−1.5 ± 15.5	−0.7 ± 17.3
Small VLDL particles, 27–35 nm (nmol/L)				
Run-in	54.3 ± 14.8	53.6 ± 13.8	41.4 ± 11.6	44.9 ± 17.4
Test period	51.9 ± 17.1	55.5 ± 20.7	35.3 ± 11.9	38.1 ± 19.2
Change	−2.5 ± 9.9	1.9 ± 17.6	−6.1 ± 13.7	−6.8 ± 23.1
IDL particles, 23–27 nm (nmol/L)				
Run-in	107.9 ± 68.4	76.3 ± 72.4	27.0 ± 25.9	49.4 ± 48.5
Test period	107.7 ± 80.1	84.0 ± 54.0	35.8 ± 35.1	36.3 ± 35.5
Change	−0.2 ± 45.1	7.6 ± 54.3	8.7 ± 50.5	−13.1 ± 48.9[a]
Large LDL particles, 21.2–23 nm (nmol/L)				
Run-in	358 ± 154	416 ± 285	378 ± 115	388 ± 131
Test period	248 ± 210	348 ± 167	351 ± 87	372 ± 116
Change	−110 ± 192	−69 ± 213	−26 ± 133	−16 ± 155
Small LDL particles, 18–21.2 nm (nmol/L)				
Run-in	1,303 ± 633	1,319 ± 456	521 ± 195	589 ± 328
Test period	1,449 ± 652	1,388 ± 363	548 ± 214	567 ± 326
Change	146 ± 315	68 ± 338	27 ± 279	−22 ± 411
Large HDL particles, 8.8–13 nm (μmol/L)				
Run-in	3.7 ± 2.0	5.0 ± 2.3	7.7 ± 2.6	8.3 ± 3.9
Test period	3.6 ± 2.0	5.2 ± 1.7	7.8 ± 2.9	8.2 ± 3.5
Change	−0.1 ± 1.2	0.3 ± 2.7	0.1 ± 3.9	−0.2 ± 3.7
Medium HDL particles 8.2–8.8 nm (μmol/L)				
Run-in	4.1 ± 3.3	2.8 ± 3.0	3.2 ± 2.4	3.7 ± 3.0
Test period	3.9 ± 3.4	2.7 ± 3.3	3.2 ± 2.4	4.4 ± 3.5
Change	−0.2 ± 1.8	−0.1 ± 3.7	−0.0 ± 3.9	0.6 ± 3.7
Small HDL particles, 7.3–8.2 nm (μmol/L)				
Run-in	23.6 ± 2.4	21.8 ± 2.7	21.3 ± 3.8	19.5 ± 4.8
Test period	23.8 ± 2.7	22.6 ± 3.4	20.6 ± 5.5	20.2 ± 4.7
Change	0.2 ± 2.0	0.8 ± 2.6	−0.7 ± 6.6	0.6 ± 5.0

Values are means ± SD

[a] $P < 0.05$ versus control

In the normolipidemic subjects we also found a reduction in the number of large VLDL-1 particles (Table 2) in serum from the plant stanol ester group, as compared to controls. The reduction was however, smaller as compared to the reduction observed in subjects with the metabolic syndrome, which is in line with the lack of effects on serum TAG concentrations. There are however, substantial differences between both studies (i.e., plant stanol intake, age, etc.) not allowing a direct comparison between the outcomes from both studies.

Assuming that plant stanol esters lower hepatic VLDL-1 production in humans, the mechanism underlying this effect is still open. In this respect, Ikeda et al. [11] have recently shown that campest-5-en-3-one (campestenone), an oxidised derivative of campesterol, was a potent hepatic PPARalpha activator in mice. Consumption of this plant

sterol derivative resulted in a lower concentration of TAG in serum and liver. These changes in mice fit very well with our suggestion of a lowered hepatic large TAG-rich VLDL particle production after plant stanol ester consumption. However, in our studies we provided plant stanol esters and not plant sterol esters. This introduces the question whether intermediates with similar effects can be formed from plant stanol esters in vivo in humans.

In apoE*3-Leiden mice, Volger et al. [10] have shown that plant stanol ester consumption lowered hepatic TAG (−38%), free cholesterol (−31%), and cholesterol ester (−62%) content. Since hepatic TAG concentrations are thought to be a driving force behind large sized VLDL particle production—at least in humans—[12], the observed reduction in hepatic TAG in these mice suggest that the number of larger sized VLDL particles was probably lowered. However, only cholesterol incorporation into nascent VLDL particles was lowered, while total VLDL-TAG and total VLDL-apoB production rates were surprisingly unchanged. Unfortunately, VLDL production rates of the different VLDL-particle subpopulations were not measured.

This data also introduces a final discussion point: the functional consequences of a lowered VLDL-1 production. In humans, it is generally recognised that the amount of fat in the liver is an important factor determining hepatic VLDL-1 production [12]. Therefore, the lowered hepatic VLDL-1 production may indicate a reduced ectopic fat accumulation in the liver. If this is indeed true, plant stanol ester consumption may be even more beneficial than currently anticipated. This latter assumption warrants, however, further study.

In conclusion, we have earlier reported that plant stanol esters not only lower serum LDL cholesterol concentrations, but also serum TAG concentrations in subjects with elevated TAG concentrations at baseline. We here present data supporting the hypothesis that the effect is caused by a reduction in the hepatic production of large TAG-rich VLDL-1 particles. Effects are most pronounced in metabolic syndrome subjects, resulting in subsequent reductions in serum TAG concentrations.

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