Cholesterol, bile acid and triglyceride metabolism intertwined

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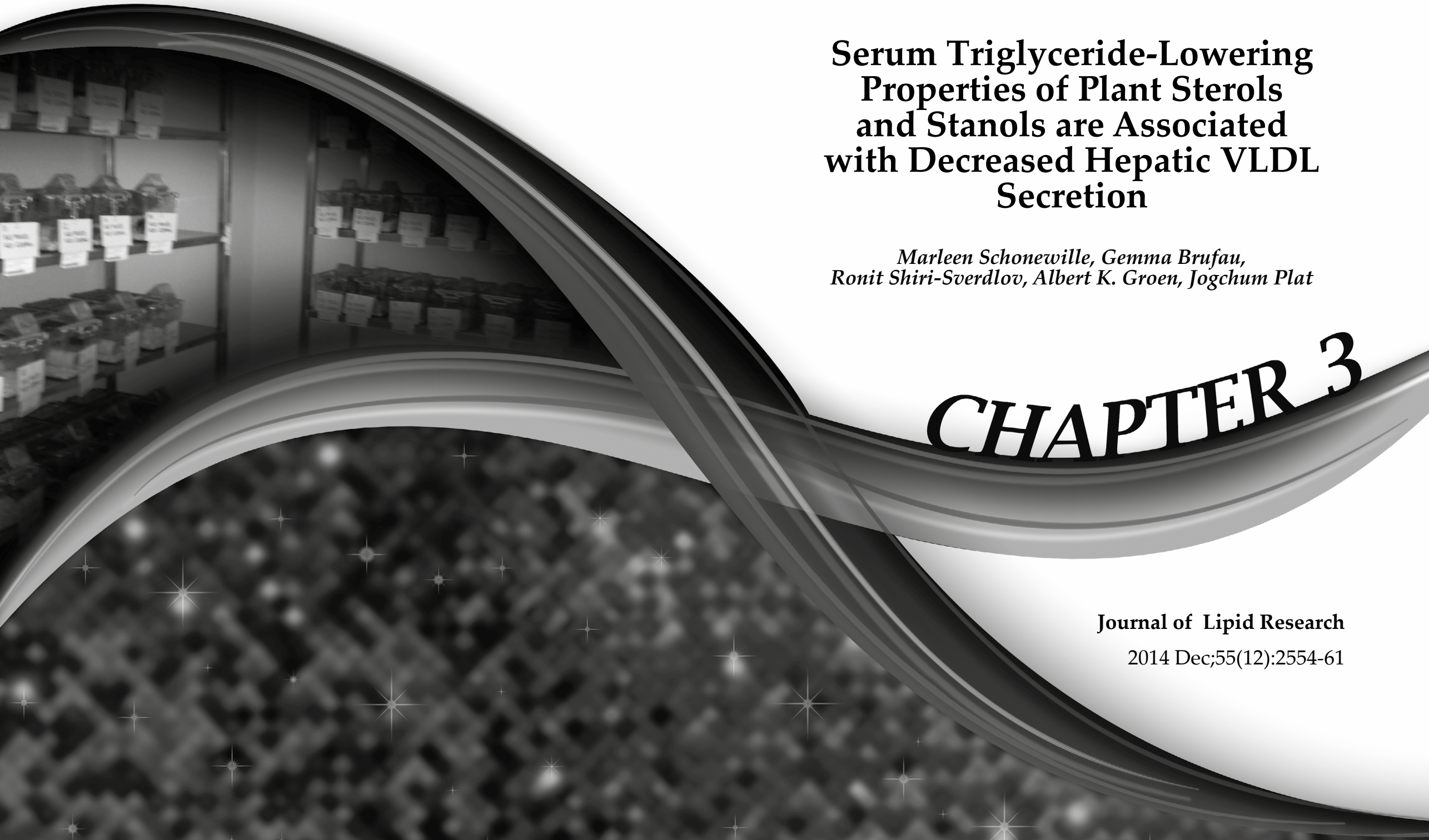

Serum Triglyceride-Lowering Properties of Plant Sterols and Stanols are Associated with Decreased Hepatic VLDL Secretion

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CHAPTER 3

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ABSTRACT

Introduction: Plant sterols and stanols are structurally similar to cholesterol and when added to the diet they are able to reduce serum total- and LDL-cholesterol concentrations. They also lower serum triglyceride concentrations in humans, particularly under conditions of hypertriglyceridemia.
The aim of this study was to unravel the mechanism by which plant sterols and stanols reduce serum triglyceride concentrations in high fat diet (HFD) fed mice.

Methods: Male C57BL/6J mice were fed HFD for four weeks. Subsequently, they received HFD, HFD supplemented with 2% plant sterol esters (PSE) or HFD supplemented with 2% plant stanol esters (PSA) for another three weeks.

Results: Both PSE and PSA feeding resulted in decreased plasma triglyceride concentrations compared to HFD, while plasma cholesterol levels were unchanged. Interestingly, hepatic cholesterol levels were decreased in the PSE/PSA groups compared to HFD and no differences were found in hepatic triglyceride levels between groups. To investigate the mechanism underlying the hypotriglyceridemic effects from PSE/PSA feeding we measured chylomicron and VLDL secretion. PSE and PSA feeding resulted in reduced VLDL secretion, while no differences were found between groups in chylomicron secretion.

Conclusion: In conclusion, our data indicate that plasma triglyceride-lowering resulting from PSE and PSA feeding is associated with decreased hepatic VLDL secretion.

Serum Triglyceride-Lowering Properties of Plant Sterols and Stanols are Associated with Decreased Hepatic VLDL Secretion

Introduction

Plant sterols and their saturated forms, plant stanols, are non-nutritive compounds that lower serum total and LDL-cholesterol concentrations in normolipidemic and hypercholesterolemic subjects without affecting serum HDL-cholesterol levels.[100] Plant sterols and plant stanols have a chemical structure that resembles cholesterol, and therefore it is generally believed that plant sterols and stanols reduce plasma cholesterol levels by competing with cholesterol for incorporation into mixed micelles in the small intestine.[101-103] Next to this, a new cholesterol-lowering mechanism of plant sterols has been suggested which involves an increased direct intestinal cholesterol excretion, by bypassing the "classical" hepato-biliary route.[27]

Besides lowering serum LDL cholesterol concentrations, it has been shown that, particularly in subjects with metabolic syndrome, plant sterols and stanols also lower serum triglyceride concentrations, where the efficacy was associated to baseline triglyceride levels.[47,104-106] Several mechanisms have been proposed to explain these effects in decreased triglyceride e.g., increased lipoprotein lipase activity (LPL), changes in cholesterol ester transfer protein (CETP) activity, reduced hepatic VLDL production or increased de novo lipogenesis and some related regulatory genes (such as Pparα) as a compensatory response.[47,104,107]

Traditionally, fasting triglycerides were considered to be an independent risk factor for cardiovascular diseases[108]. However, after correcting for several confounding factors, the association between fasting triglyceride levels and cardiovascular risk became very low or even absent.[108] Since humans are mostly in the fed state, the level of non-fasting triglycerides may be more relevant, indeed there is an increasing body of evidence showing non-fasting triglyceride levels as an independent cardiovascular risk factor.[109-111]

The goal of this study was to unravel the mechanism by which plant sterols and plant stanols lower plasma triglyceride concentrations in high fat diet fed wild-type mice by evaluating changes in processes that result in triglyceride appearance in the circulation. Prior to the start of the plant sterol/stanol intervention, all mice received a high fat diet (HFD) for four weeks, in order to induce hypertriglyceridemia. In the present study, we observed completely in line with the observations in humans that plant sterol and stanol ester consumption resulted in decreased plasma triglyceride levels, which was associated with and most likely due to a decreased hepatic VLDL secretion.

Material and Methods

Animals

Male C57BL/6J mice of 3 months of age were purchased from Charles River Laboratories (L'Abresle, Brussels, Belgium). All animals were housed individually in a temperature- and light- controlled facility. In all experiments, mice received a dietary treatment of 7 weeks. The dietary treatment started by feeding mice HFD containing 40% fat derived from beef for four weeks. After this period, mice were split in three groups (n= 6) and received either HFD, HFD supplemented with 3.1% (wt/wt) plant sterol esters (PSE) or HFD supplemented with 3.1% (wt/wt) plant stanol esters (PSA) for 3 more weeks (**Table 1**). The reason that the HFD contains more olive oil, soybean oil and linseed oil compared to the PSE and PSA diets is that plant sterols and stanols were not added at the expense of dietary fat since the plant sterols and stanols were esterified with a fatty acid and the exact amount of fat was added to the HFD to make sure that the three diets had a similar absolute fat content and similar fatty acid composition. Mice received food and water *ad libitum.* All experiments were approved by the Ethical Committee for Animal Experiments of the University of Groningen.

Table 1. Composition of the diets.

Composition (%)	HFD	PSE	PSA
Sucrose	39.75	38.97	38.97
Casein	23.64	23.18	23.18
Beef fat	15.78	15.47	15.47
Cellulose	5.91	5.79	5.79
Olive Oil	2.94	2.07	2.07
Soybean oil	2.27	2.07	2.07
Corn Starch	2.59	2.54	2.54
Vitamin Mix[1]	0.58	0.58	0.58
Mineral Mix[2]	5.44	5.33	5.33
Choline	0.47	0.46	0.46
DL Methionine	0.24	0.23	0.23
Cholesterol	0.20	0.20	0.20
Linseed Oil	0.19	-	-
Plant sterol esters	-	2.0	-
Plant stanol esters	-	-	2.0

[1]Vitamin mix: vitamins premix, trace elements premix; [2]Mineral mix: calcium hydrogen phosphate, calcium carbonate, potassium chloride, potassium dihydrogen phosphate, magnesium sulphate heptahydrate, sodium chloride, magnesium oxide.

Experimental procedures

Biochemical parameters

Body weight and food intake were determined at the start of the experiment and repeated weekly until the end of the experiment. Feces were collected during the last three days of the experiment. At the end of the dietary treatment, mice were sacrificed by heart puncture under isofluorane anesthesia, followed by cervical dislocation. Plasma was stored at -20°C until analyzed. The liver was removed, weighted and snap-frozen in liquid nitrogen. The intestine was excised, flushed with (4°C) PBS (phosphate buffered saline) and subsequently snap-frozen in liquid nitrogen. Both liver and intestine were stored at -80°C until biochemical analysis and RNA isolation. Hepatic lipids were extracted according to Bligh & Dyer.[112] Plasma parameters were measured by using commercial available kits (Roche Diagnostics, Mannheim, Germany and DiaSys Diagnostic Systems, Holzheim, Germany).

Determination of VLDL secretion

In a separate experiment, after the dietary treatment, overnight fasted mice (n=6) were injected intraperitoneally with Poloxamer 407 (1 g/kg body weight) in saline, as previously described.[113] Blood samples were drawn by retro-orbital bleeding into heparinized tubes at 0, 30, 60, 120 and 240 minutes after injection under isofluorane anesthesia. Animals were sacrificed by cervical dislocation. Blood samples were centrifuged (10 min, 4000xg, 4°C) to obtain plasma. TG production rates were determined as previously described.[113] Nascent VLDL (d<1.006) was isolated from the final plasma sample of each animal from experiment 2 using a Optima TM LX tabletop ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA) at 508.000 g for 150 minutes. VLDL composition was determined by using commercial kits (for triglycerides and cholesterol: Roche Diagnostics, Mannheim, Germany and DiaSys Diagnostic Systems, Holzheim, Germany; for phospholipids: Wako Chemicals).

Determination of chylomicron secretion

In a third experiment, after the dietary treatment, mice (n=6) received an intragastric load of 200 µl olive oil after an overnight fasting. Subsequently, blood samples were drawn by retro-orbital bleeding into heparinized tubes at 0, 30, 60, 120 and 240 minutes under isofluorane anesthesia. Animals were sacrificed by cervical dislocation. Plasma was stored at -20°C until analysis of plasma triglycerides.

Plasma lipoprotein analysis

Plasma lipoproteins were separated by fast protein liquid chromatography (FPLC) as described previously.[114] Values are presented from a pool of n=6 mice/group. Total cholesterol and triglyceride levels of the collected fractions were determined using a commercially available kit (Roche Diagnostics, Mannheim, Germany).

β-Hydroxybutyrate

The concentration of β-hydroxybutyrate was determined using a commercial kit (Diasys Diagnostic Systems, Holzheim Germany) using a VITALAB Selectra E instrument (VWR ser.Nr.:0-2496).

RNA isolation

RNA isolation, cDNA synthesis and real-time quantitative PCR were performed as described previously.[115] PCR results of liver and intestine were normalized to *36B4 mRNA* levels. Primer and probe sequences for the genes tested are deposited at the primer database (rtprimerdb.org) and in **Supplemental Table I**.

Statistics

Data are shown as means ± SD with n=6 mice/group. Statistical analysis was assessed by using Kruskal-Wallis H test followed by Conover post-hoc comparisons using the Brightstat.[116] Levels of significance were set at * $p<0.05$. compared to HFD.

Table 2. Basal parameters in C57BL/6J fed HFD, PSE and PSA diet.

	HFD	PSE	PSA
Basal parameters			
Body weight			
Start weight	28.4 ± 2.2	28.3 ± 0.9	27.9 ± 1.6
Final weight	30.7 ± 2.2	28.2 ± 1.2	29.1± 1.5
Change from start[1](%)	13.2 ± 5.0	5.9 ± 4.2*	10.7 ± 3.4
Food intake (g/d)	3.6 ± 0.5	5.2 ± 0.3*	5.9 ± 0.3*
Calorie intake (kcal/day)	16.8 ± 2.1	23.4 ± 1.6*	26.7 ± 1.3*
Feces production (g/d)	0.41 ± 0.04	0.68 ± 0.05*	0.73 ± 0.07*
Plasma			
TC (mmol/l)	3.9 ± 0.3	3.8 ± 0.2	3.9 ± 0.2
TG (mmol/l)	0.96 ± 0.23	0.59 ± 0.03*	0.65 ± 0.10*
NEFA (mmol/l)	1.08 ± 0.10	0.89 ± 0.12*	0.83 ± 0.14*
Liver			
Liver (% BW)	4.8 ± 0.2	4.7 ± 0.3	4.8 ± 0.4
TG (nmol/mg)	15.3 ± 2.4	16.6 ± 5.0	17.5 ± 5.5
TC (nmol/mg)	7.0 ± 0.6	4.8 ± 0.3*	5.4 ± 1.2*
PL (nmol/mg)	25.4 ± 2.6	25.8 ± 1.8	25.0 ± 1.6

[1]Start feeding diets with or without supplementation of PSE or PSA

Results

Effects of plant sterol and plant stanol feeding on plasma and hepatic lipids and sterols

Prior to start with the diets, every group was matched by body weight. PSE treatment did result in an attenuated increase in body weight compared to HFD-fed mice. Similar results were found in the PSA group, although it did not reach significance (**Table 2**). Interestingly, the attenuated increase in body weight was parallel to significant higher food intake (g/d) and a concomitant higher feces production (**Table 2**). The high fat diet (HFD) contains slightly but negligible more calories than the plant sterol (PSE) and plant stanol (PSA) ester enriched diets (**Supplemental Table II**), but this difference is compensated in the experiment because the mice consumed more of the plant sterol/ stanol supplemented diet (**Table 2**). PSE and PSA treatments resulted in a 25% decrease in hepatic cholesterol content as compared to HFD, whereas hepatic triglyceride and phospholipid levels did not differ between the treatments (**Table 2**).
Although the liver TG content did not change after plant sterol or stanol ester consumption, plasma triglyceride levels decreased. Similarly, the groups with PSE and PSA supplementation had the lowest concentrations in plasma non-esterified fatty acids (NEFA) (**Table 2**). Plasma cholesterol concentrations were similar in the HFD, PSE and PSA fed mice.

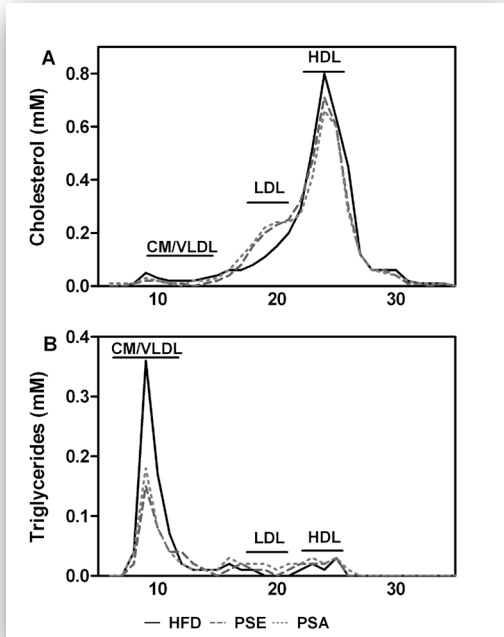

Figure 1. FPLC profiles in C57BL/6J mice fed HFD, PSE and PSA diet.
(A) cholesterol concentrations;
(B) triglyceride concentrations.

Plant sterols and plant stanols supplemented to a high fat diet results in decreased VLDL secretion in mice

The reduction in plasma triglyceride levels was associated with a reduction in the VLDL/chylomicron fraction in the PSE and PSA fed mice (**Figure 1**). To determine the rate of hepatic VLDL secretion, lipases were inactivated by intraperitoneal administration of poloxamer 407 and subsequent measurement of plasma triglycerides over a period of 4 hours. PSE and PSA feeding resulted in respectively a 12.0% and 15.1% ($p<0.05$) lower VLDL-TG

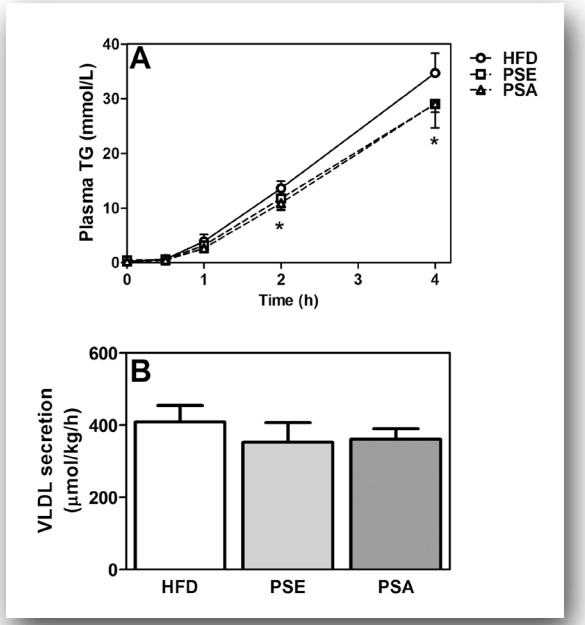

Figure 2. Hepatic VLDL secretion in mice fed HFD, PSE and PSA diet.
(A) Plasma triglyceride concentrations in mice after an intraperitoneal administration of poloxamer 407 (1 g/kg) i.p.; (B) VLDL-triglycerides production rate.

secretion compared to HFD fed mice ($p<0.01$) (**Figure 2**). Next to this, the hepatic expression of the most important genes involved in VLDL secretion and assembly were measured. No differences between treatments were found in the expression of *Pparα*, *ApoB, and Mttp* whereas hepatic *Cd36* expression was decreased and *ApoAIV* expression was elevated in the PSE and PSA groups as compared to the HFD group (**Table 3**). *Dgat2* and *Hepatic lipase* were unchanged after PSE and PSA feeding (**Table 3**). To investigate a potential anti-inflammatory effect of plant sterols/stanols, we also determined the expression of several hepatic pro-inflammatory genes.[117] Interestingly, PSE/PSA treatments did not result in changes in the expression of any pro-inflammatory genes (**Table 4**) suggesting that the observed effect of PSE and PSA on hepatic VLDL production occurred independent of potential anti-inflammatory effects of these compounds.

Plant sterol and plant stanol feeding results in down regulation of Lxr target genes in the liver

Hepatic VLDL secretion and *de novo* lipogenesis have been reported to be regulated by several transcription factors, including LXR.[114,118-120] To study whether PSE and PSA feeding may influence VLDL secretion via down regulation of LXR signaling we determined expression levels of LXR target genes. As shown in T**able 5**, LXR target genes were indeed down regulated by PSE and PSA supplementation.

Plant sterols and plant stanols supplemented to a high fat diet did not interfere in chylomicron secretion

To determine whether absorption of lipids was impaired after PSE and PSA feeding, we determined the rate of chylomicron production by administration of an oral fat bolus to the mice after overnight fasting. No differences were found in chylomicron secretion or in the postprandial excursion for serum TGs between the three different dietary conditions (**Figure 3**). Next, we evaluated intestinal mRNA expression of *Pparα* (a nuclear receptor involved

Table 3. mRNA expression of genes involved in fat absorption in the intestine and in the liver in C57BL/6J mice fed HFD, PSE and PSA diet.

	HFD	PSE	PSA
Small intestine			
Ppara (Pparα)	1.0 ± 0.2	0.6 ± 0.2*	0.7 ± 0.2*
Cd36 (Fat)	1.0 ± 0.2	0.3 ± 0.2*	0.5 ± 0.3*
Fatp4	1.0 ± 0.2	0.8 ± 0.1	0.8 ± 0.2
Apob	1.0 ± 0.4	0.6 ± 0.2	0.7 ± 0.2
Mttp	1.0 ± 0.1	0.8 ± 0.2*	0.7 ± 0.1*
Apoa4	1.0 ± 0.2	0.7 ± 0.2	0.8 ± 0.2
Liver			
Ppara (Pparα)	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.2
Cd36 (Fat)	1.0 ± 0.1	0.5 ± 0.1*	0.6 ± 0.2*
Apob	1.0 ± 0.3	1.2 ± 0.4	1.2 ± 0.1
Mttp	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.2
Apoa4	1.0 ± 0.3	2.7 ± 0.9*	3.5 ± 1.2*
Dgat2	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.3
Lipc (Hepatic Lipase)	1.0 ± 0.1	1.0 ± 0.2	0.9 ± 0.1

in lipid metabolism) and several genes known to be involved in intestinal fat absorption and chylomicron assembly. Supplementing plant sterols and stanols to HFD resulted in decreased expression of *Pparα*, Cd36 and *Mttp* in proximal small intestine compared to HFD fed mice (**Table 3**).

Plant sterols and stanols feeding changes expressions of β-oxidation related genes in liver

To investigate whether increased hepatic β-oxidation might explain the unchanged hepatic triglyceride levels while VLDL secretion is decreased, we measured hepatic gene expression of genes involved in β-oxidation and plasma levels of β-hydroxybutyrate.

Table 4. Hepatic expression of inflammatory genes in C57BL/6J mice fed HFD, PSE and PSA diet.

	HFD	PSE	PSA
Tnf (Tnfa)	1.0 ± 0.5	0.9 ± 0.3	0.5 ± 0.2
Ilbβ	1.0 ± 0.2	1.0 ± 0.4	1.0 ± 0.3
Cd68	1.0 ± 0.2	1.2 ± 0.4	1.3 ± 0.3
Ccl2 (Mcp1)	1.0 ± 0.9	0.6 ± 0.2	0.7 ± 0.4
Icam	1.0 ± 0.5	0.7 ± 0.2	0.7 ± 0.2

Table 5. Hepatic expression of LXR target genes in C57BL/6J mice fed HFD, PSE and PSA diet.

	HFD	PSE	PSA
Abca1	1.0 ± 0.2	0.8 ± 0.2	0.7 ± 0.1*
Abcg5	1.0 ± 0.1	0.5 ± 0.1*	0.5 ± 0.0*
Abcg8	1.0 ± 0.2	1.5 ± 0.1*	0.6 ± 0.1*
Lpl	1.0 ± 0.2	0.5 ± 0.1*	0.5 ± 0.1*
Srebf1(Srebp1c)	**1.0 ± 0.2**	**0.6 ± 0.1***	**0.7 ± 0.1**

PSE or PSA feeding resulted in a slight increase in Acacb (Acc2) and Cpt1a (both genes involved in mitochondrial β-oxidation) while Acox1 (gene involved in peroxisomal β-oxidation), was decreased (**Table 6)**. Concentrations of β-hydroxybutyrate, a ketone body, in plasma were unchanged after supplementation of plant sterols or stanols to HFD.

Discussion

Plant sterols and stanols are well known for their ability to reduce plasma cholesterol levels. In our study we found a lack of change in plasma cholesterol however this result was expected since in earlier studies using C57BL/6J mice both our own group as well as others observed a lack of effect of plant sterol/stanol consumption on plasma cholesterol concentrations.[107,121] However we did see a decrease of hepatic cholesterol concentrations, which indicates that plant sterols affect hepatic lipid and lipoprotein metabolism independent from changes in serum cholesterol concentrations.
Besides their cholesterol-lowering properties, plant sterols/stanols are also able to reduce triglyceride levels.[105,106] In our study, we observed that plant sterols and stanols feeding led to a decrease in plasma triglycerides, while hepatic triglyceride levels remain unchanged. The latter is a surprising observation since previous results from Rideout et al. in C57BL/6J show a decrease in hepatic triglycerides after plant sterol feeding and similar results were found in ApoE -/- mice and ApoE3*-Leiden transgenic mice.[122,123] The different diet composition used in these studies may explain the variation in hepatic triglyceride levels observed.

Since plant sterols and stanols are non-nutritional compounds and very poorly absorbed in the intestine, we first hypothesized that these compounds exert their primary effects at the intestinal level.[124] Gene expression levels of CD36, involved in fatty acid absorption, was decreased after PSE and PSA treatment. However, this hypothesis could not be confirmed since chylomicron secretion was unaltered after plant sterol and stanol feeding.

In this study, we show that the triglyceride-lowering properties of plant

Table 6. Hepatic expression of genes involved in β-oxidation and plasmic β-hydroxybutyrate in C57BL/6J mice fed HFD, PSE and PSA diet.

	HFD	PSE	PSA
Liver			
Acox1 (Aox)	1.0 ± 0.1	0.7 ± 0.1*	0.6 ± 0.3*
Acacb (Acc2)	1.0 ± 0.4	1.5 ± 0.3*	1.5 ± 0.2*
Cpt1a	1.0 ± 0.2	1.1 ± 0.1	1.2 ± 0.1*
Plasma			
BHB (mmol/L)	0.33 ± 0.22	0.40 ± 0.08	0.22 ± 0.17

sterol/stanols feeding are associated with changes in VLDL secretion. After administration of poloxamer 407, we observed a significant decrease in VLDL-TG secretion in plasma in PSA and PSE fed mice respectively. Hepatic triglyceride levels have been described to determine for the number and size of VLDL-TG particles.[125-127] However, in our study, hepatic triglyceride concentrations did not differ between the HFD and PSE/PSA fed mice. Similarly, several authors have reported a lack of association between hepatic triglyceride content and VLDL-TG production rate.[128-130]

Since there is a potential link between hepatic inflammation and VLDL production, we investigated whether plant stanols/stanols could also act as anti-inflammatory agents, as has been previously suggested (see review[117]) and whether this could explain the decrease in hepatic VLDL production. We did not find any differences in hepatic mRNA levels of several inflammatory markers. However, expression levels of these pro-inflammatory genes were rather low suggesting that there was hardly inflammation in the liver of C57BL/6J mice on a HFD for this duration. In addition, the HFD fed animals showed a higher VLDL-TG production rate as compared to chow fed animals (data not

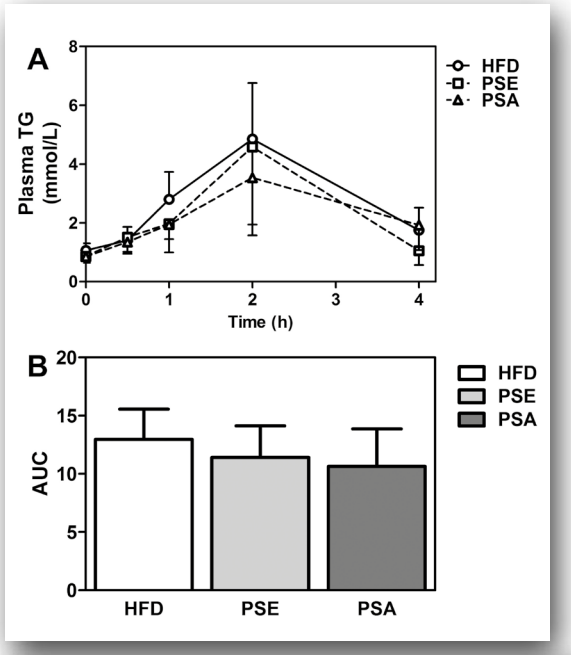

Figure 3. Chylomicron formation in mice fed HFD, PSE and PSA diet.
(A) Plasma triglyceride concentrations in mice after an intragastric load (200 μL olive oil); (B) Area under the curve (AUC).

Supplemental Table I: Primer sequences.

Name	Forward	Reverse
Tnfα	CATCTTCTCAAAATTCGAGTGACAA	TGGGAGTAGACAAGGTACAACCC
Cd68	TGACCTGCTCTCTCTAAGGCTACA	TCACGGTTGCAAGAGAAACATG
Mcp1	GCTGGAGAGCTACAAGAGGATCA	ACAGACCTCTCTCTTGAGCTTGGT
Icam	CTACCATCACCGTGTATTCGTTTC	CGGTGCTCCACCATCCA
Il-1b	AAAGAATCTATACCTGTCCTGTGTAATGAAA	GGTATTGCTTGGGATCCACACT

shown) without showing a clear hepatic inflammatory phenotype, which also suggests that these two entities are not directly linked. Therefore, it seems unlikely that the decrease in VLDL secretion after PSE/PSA treatment is due to changes in hepatic inflammation in these C57BL/6J mice.

Since VLDL secretion rates are decreased while hepatic triglyceride concentration remain unchanged, the decrease in plasma triglyceride concentrations could be due to increased β-oxidation. We observed a slight increase in the gene expression of *Acacb (Acc2)* and *Cpt1a*, which are involved in mitochondrial β-oxidation, in contrast *Acox1* gene expression, involved in peroxisomal β-oxidation, was decreased. Next to this, plasma levels of β-hydroxybutyrate, a ketone body, was measured and no changes were observed. Altogether, our data on β-oxidation is not conclusive enough to say that β-oxidation is increased.

The question how the hepatic VLDL secretion is decreased after PSE and PSA feeding could be answered by a decrease in hepatic LXR. LXR (NR1H3) is a transcription factor involved in lipid metabolism and is an intracellular sensor for sterols.[131] According to Basciano et al, activation of LXR leads to increased plasma triglycerides and VLDL.[132] In our study, we have observed opposite effects i.e. decreased plasma TG and VLDL production in combination with down regulation of hepatic LXR target genes after supplementation of PSE and PSA to HFD. Next to this, the expression of the LXR target gene Cd36 (Fat) in the liver, a transporter involved in fatty acid uptake, was examined.[133] Koonen et al. have investigated the role of hepatic Cd36 and have shown that increased Cd36 leads to increased fatty acid uptake, hepatic triglyceride storage and secretion via VLDL.[134] After PSE and PSA feeding, hepatic TG remained unchanged and VLDL secretion was decreased, which could be explained by a decrease in fatty acid uptake by Cd36. Expression of the LXR target gene Apoa4 was increased after PSE and PSA feeding. Verhage et al. observed that hepatic Apoa4 expression was associated with increased VLDL secretion and decreased hepatic triglyceride content, which is in contradiction with our data.[135] This contradiction could be explained by the observed steatosis in the study of Verhage et al., which was not observed in our study.[135]

Studies from our group using LXR knockout mice indicated that disruption of LXRα induced a decrease in VLDL production while on the other hand, treatment with

a LXR agonist increased VLDL production.[114,136] Our study is in line with these observations since we observed a decreased expression of hepatic LXR target genes together with decreased VLDL production. According to earlier studies of our group plant sterols can activate LXR in vitro in Caco2 cells.[137] (Plat, Nichols, & Mensink, 2005). In the current study, however, we show a decrease in the expression of hepatic LXR target genes. We hypothesize that the concentration of plant sterols in the liver is not high enough to activate hepatic LXR. This is also in line with data from our own group that LXR target gene expression was lower in liver samples from plant sterol fed C57BL/6J mice[121] and the lack of hepatic LXR activation is therefore rather consistent.

In summary, similarly as observed in humans, plant sterol and stanol ester consumption resulted in decreased plasma triglyceride levels in HFD fed mice. This decrease was associated with decreased hepatic VLDL secretion.

For many years, it has been accepted that chylomicrons and its remnants are the main carrier of triglycerides in the non-fasting state, since these lipoproteins are increased after ingestion of a meal.[138] Recently, it has been proven that the major triglyceride carriers in the postprandial state are VLDL and its remnants.[139] Future studies investigating the association between triglyceride metabolism and plant sterol/stanol intake, should include a full kinetic characterization of VLDL, chylomicrons and their remnants.

Supplemental Table II: Fatty acid composition in the diets

	HFD	PSE	PSA
Fatty Acid Composition			
C8-C12:0 (g/kg)	0.68	0.61	0.61
C14:0 (g/kg)	5.53	5.38	5.38
C16:0 (g/kg)	46.70	44.49	44.49
C16:1 (g/kg)	5.60	5.38	5.38
C18:0 (g/kg)	34.63	33.62	33.62
C18:1 (g/kg)	84.86	76.63	76.63
C18:2 (g/kg)	20.47	18.19	18.16
C18:3 (g/kg)	4.21	2.96	2.96
C20-C22 (g/kg)	1.95	1.87	1.87
Total Caloric Content (kcal/kg)	4719.69	4519.40	4519.40

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