Triglyceride-Lowering Response To Plant Sterol and Stanol Consumption

Todd C Rideout[1], **Christopher PF Marinangeli**[1], and **Scott V Harding**[2]

[1]Department of Exercise and Nutrition Sciences, School of Public Health and Health Professions, University at Buffalo, Buffalo, NY, 14214

[2]Diabetes and Nutritional Sciences Division, School of Medicine, King's College London, London SE1 9NH, United Kingdom

Abstract

Phytosterols (PS) have long been recognized for their cholesterol-lowering action, however, recent work has highlighted triglyceride (TG)-lowering responses to PS that may have been overlooked in previous human interventions and mechanistic animal model studies. This review assesses the current state of knowledge regarding the effect of dietary PS supplementation on blood TG concentrations by examining the average therapeutic response, potential mechanisms, and metabolic and genetic factors that may contribute to inter-individual variability. Data from human intervention trials demonstrates that, compared to baseline concentrations, PS supplementation results in a variable TG-lowering response ranging from 0.8 to 28%. It is evident that hypertriglyceridemic individuals (>1.7 mmol/L) have a greater TG-lowering response to PS (11–28%) than subjects with normal plasma TG concentrations (0.8–7%). Although a genetic basis for the variable TG-lowering effects of PS is probable, there are only limited studies to draw on. The available data suggest that polymorphisms in the apolipoprotein E (apoE) gene may affect responsiveness, with PS-induced reductions in TG more readily evident in apoE2 than apoE3 or E4 subjects. Although only a minimal number of animal model studies have been conducted to specifically examine the mechanisms whereby PS may reduce blood TG concentrations, it appears that there may be multiple mechanisms involved including interruption of intestinal fatty acid absorption and modulation of hepatic lipogenesis and VLDL packaging and secretion.

In summary, the available data suggest that PS may be an effective therapy to lower blood TG, particularly in hypertriglyceridemic individuals. However, before PS can be widely recommended as a TG-lowering therapy, studies that are specifically powered and designed to fully access therapeutic responses and the mechanisms involved are required.

Introduction

Since discovery of the association between elevated blood cholesterol and increased cardiovascular disease (CVD) risk with early animal model [1] and epidemiological investigations [2], diet-based and pharmacological cholesterol-lowering therapies have become integral components of primary and secondary CVD prevention programs. Although

Correspondence to: Todd C Rideout (rideout@buffalo.edu).

these therapies have largely reduced the prevalence of high LDL-C amongst Americans, close to 33% of US adults still have elevated LDL-C and there is concern that high-risk individuals often fail to meet their LDL-target goals.

Phytosterols (PS), plant-based sterols that structurally resemble mammalian cholesterol, are arguably the best-defined nutraceutical approach to reduce blood cholesterol concentrations by interfering with intestinal cholesterol absorption. PS have a proven track record as 'natural' cholesterol-lowering agents with consistent LDL-cholesterol reductions in the range of 10–16% in numerous well-controlled clinical interventions [3] and pre-clinical studies that have defined the molecular mechanisms involved [4–6]. Although PS are highly regarded as effective for reducing circulating total- and LDL-C, they were traditionally believed to have no effect on triglyceride (TG) concentrations, an important independent CVD risk factor. However, recent animal and human studies have highlighted a potential role for PS in regulating blood TG concentrations (Tables 1 and 2). That the TG-lowering properties of PS are just now surfacing may seem unexpected given that their health benefits have been actively researched in controlled human studies since the 1950's. However, a close assessment of previous clinical PS interventions reveals TG-lowering responses that may have been overlooked for a variety of reasons. First, the lipid hypothesis placed such a major emphasis on cholesterol as the major CVD risk factor that PS interventions were specifically designed and statistically powered to detect movement in the primary endpoint of LDL-C, not other lipid risk factors. Furthermore, recent work suggests that the TG lowering responses of PS are most clearly observed in hypertriglyceridemic individuals, however, the vast majority of PS interventions were designed with hypercholesterolemia as the main study inclusion criteria. Finally, the TG-lowering action of PS may have been difficult to discern as metabolic and genetic factors may contribute to a relatively variable response compared with the more consistent reductions observed in circulating cholesterol levels.

This review will provide a thorough assessment of the effects of PS on TG metabolism with discussion of the TG-lowering effects reported in previous clinical interventions and what is known regarding the potential molecular mechanisms that may underlie these responses. We review the extent of our knowledge regarding the metabolic and genetic factors that are thought to influence these responses and discuss future research priorities that must be addressed to more fully evaluate PS as a potential TG-lowering therapy.

Clinical Assessment of TG Lowering in Response to PS

A review of the clinical trial database demonstrates a TG-lowering effect of PS ranging from 0.8 to 28% compared to baseline values [7–19]. The TG-lowering response of selected studies are presented in Table 1.

A purview of this data demonstrates that, for the most part, the TG-lowering efficacy of PS increases as baseline TG levels become more prominent. For example, in studies where subjects were hypertriglyceridaemic (>1.7 mmol/L), 1.6–4 g/d PS lowered circulating TG levels 11%–28% [9–14,18]. Conversely, in studies where baseline TG levels were < 1.7 mmol/L, 1.6–4.1 g/d PS lowered TG levels by 0.8–7% [7,11,12,15,16,19]. Pooled analysis

of five clinical studies showed that subjects with baseline TG concentrations of 1.0 mmol/L experienced a 1.0%, 3.8% and 4.7% reduction in circulating TG levels with 2.0 g/d, 3.0 g/d or 4.0 g/d plant stanols, respectively [20]. However, across the same dosages of PS, when baseline TG were 2.0 and 3.0 mmol/L, TG levels were decreased by ~1.5% and ~2.0%, ~5.8% and ~7.8%, and, ~7.0% and ~9.7%, respectively [20]. Using data from 12 clinical trials, Demonty et al. [21] showed PS facilitated a mild decrease in circulating TG of 6.0%. However, with the exception of two subjects with mildly elevated baseline TG levels (1.73 mmol/L and 1.93 mmol/L), baseline TG's were relatively normal. Therefore, the 6.0% reduction in circulating TG aligns with data demonstrated in Table 1. In the same study, when the data were stratified by baseline TG levels, PS lowered TG concentrations by 0.18 mmol/L in subjects with TG levels within the 75th percentile (1.9 mmol/L) [21]. This reduction is substantially greater than the 0.0006 mmol/L and 0.08 mmol/L decrease in circulating TG concentrations among subjects with baseline TGs within the 25th (0.99 mmol/L) and 50th percentile (1.36 mmol/L), respectively [21]. To date, only one clinical trial by Theuwissen et al.[10] has sought to delineate PS TG-lowering efficacy. Using subjects with baseline TG levels of at least 1.73 mmol/L, PS had no effect on circulating TG concentrations. However, among subjects with moderate-to-high baseline TG levels (> 2.3 mmol/L), 2.5 g/d PS lowered TG concentrations by 11% compared with baseline. It is noted that, among normotriglyceridemic subjects (1.2 mmol/L), Davidson et al. [17] observed a 13.3% decrease in TG levels with 3.0 g/d. However, no effect was demonstrated with 6.0 g/d and 9.0 g/d PS [17]. While the TG-lowering results outlined in Table 1 are promising, a more focused approach for delineating PS effects on circulating TG is required. Overall, data suggest that 2.0–4.0 g/PS/d facilitate significant reductions in circulating TG levels in humans. However, the degree of PS-induced TG-lowering could be dependent on the presence and magnitude of hypertriglyceridemia.

In addition to applying hypertriglyceridemia as inclusion criteria in future clinical trials, future studies should also consider the consistency as to how the TG-lowering response is reported. Table 1 shows that the TG-lowering efficacy of PS are reported as comparisons to baseline, the control group or both. This is problematic when deciphering the true of effect PS on circulating TG levels. For example, Jones et al. [18], reported that, compared to baseline, hypertriglyceridemic subjects receiving 1.84 g/d PS decreased circulating TG by 18.9% and 17.4%, respectively. However, compared to the control group, TG levels were modestly decreased by 1.0% [18]. Shaghaghia et al. [19] demonstrated the opposite effect, where 2.0 g/d PS decreased TG relative to baseline and the control group by 0.8% and 13.9%, respectively. Large disparities in relative reductions in TG between baseline and controls values could suggest that background diets, or other lifestyle factors, are imposing a substantial effect on the TG response. Perhaps consistent reporting of absolute TG reductions would address the abovementioned discrepancies in data that support the effects of PS on elevated TG concentrations.

Pre-Clinical Assessment of TG Lowering in Response to PS

Several animal species, namely hamsters, mouse and rat models, demonstrate fairly consistent reductions in both blood and hepatic TG concentrations following dietary PS incorporation (Table 2). It is of interest to note that these TG reductions are evident despite

drastic differences in study design including diverse animal models with distinct lipid metabolism and different background diets (chow vs. semi-synthetic) with variable types of fat, and wide-ranging sources and supplementation levels of PS. With a few exceptions, most of these studies were designed to specifically examine cholesterol-lowering responses and associated mechanisms, however, results of these studies do shed light on potential pathways by which PS may directly or indirectly modulate TG metabolism.

There are multiple lines of evidence to suggest that at least part of the TG-lowering response to PS is related to alterations in TG and/or FA metabolism within the intestine. In what we believe to be one of the first studies explicitly designed to examine the TG-lowering mechanisms of PS, we fed male C57BL/6J mice a semi-synthetic 'Western' diet supplemented with 2% PS (Reducol) for 6 weeks. Wild type C57BL/6J mice are a unique model as they exhibit reductions in blood and hepatic TG following PS supplementation but are considered non-responders to the cholesterol-lowering action of PS due their high cholesterol synthetic capacity [22]. Compared with unsupplemented animals, PS-fed mice exhibited reductions in plasma (28%) and hepatic (30%) TG concentrations. At least part of this TG lowering response was associated with changes in intestinal fat metabolism including increased fecal fatty acid excretion, specifically fecal palmitate and stearate. Supporting a potential role of PS in reducing intestinal TG absorption, Tomoyori et al. (2004) reported that PS reduced the postprandial lymphatic transport of TG (5–7 hours following a meal) in thoracic duct–cannulated Sprague-Dawley rats [23]. In our mouse study, we detected no difference in the expression of a host of intestinal genes related to FA absorption and chylomicron assembly, however, PPARα mRNA expression was reduced compared with control animals (discussed below). These results may suggest a physical interference of PS on intestinal FA absorption rather than a direct effect on the expression of genes and proteins that regulate FA uptake, similar to the interference of PS with the incorporation of cholesterol into bile salt micelles. Alternatively, Liang et al. (2011) reported that male Syrian golden hamsters exhibited a 28% reduction in blood TG which was associated with reduced mRNA expression of intestinal microsomal triglyceride transfer protein (MTP) following dietary supplementation with β-sitosterol or stigmasterol (0.1%) [24]. As MTP plays a pivotal role in the assembly and secretion of apolipoprotein B (apoB)-containing chylomicron particles, PS-mediated reductions in the expression of this gene could conceivably be linked to TG lowering and should be confirmed in future mechanistic studies. Although many studies (both animal and human) have directly examined intestinal cholesterol absorption in response to PS consumption, we are not aware of any studies that have employed stable-isotope methodology to directly measure FA absorption following PS intervention. This is a major knowledge gap in our understanding of the potential mechanisms involved in PS-mediated TG reductions and should be addressed in future animal and human studies.

As mentioned above, in a previous study designed to examine the TG lowering responses of PS, we have observed reductions in intestinal peroxisome proliferator-activated receptor alpha (PPARα) mRNA expression [25]. PPARα is a nuclear receptor highly expressed within intestinal enterocytes that mediates the effects of nutrients, specifically fatty acids, on gene expression. PPARα activation has been shown to regulate a whole-host of intestinal functions including nutrient transport, fatty acid oxidation, oxidative stress, and

inflammation and its expression patterns mimics that of other critical genes involved in fatty acid absorption [26–28]. The broad scope of its regulation and constant exposure to its fatty acid ligands makes PPARα an intriguing therapeutic target for obesity and dyslipidemia. At this point, it is difficult to say whether the observed reduction in PPARα mRNA expression is a direct effect of PS or an indirect consequence, possibly from a direct interference of PS with intestinal TG absorption.

There is also evidence to suggest that PS directly or indirectly influence hepatic FA and TG metabolism. The liver is central to whole-body FA and TG metabolism as a primary site for the *de novo* lipogenesis of FA, the synthesis and secretion of nascent VLDL particles, and the clearance of fatty acids (FA) from TG-rich remnant lipoprotein particles and HDL species. PS supplementation is regularly associated with a reduction in hepatic TG concentrations in hamster, mouse, and rat models (Table 2). In addition to tissue TG concentrations, there is also evidence to suggest that PS may modulate the hepatic FA profile. We observed a shift in hepatic fatty acid composition toward increased saturated 16:0 (~50%) and reduced monounsaturated FA (16:1, ~40%; and 18:1, ~24%) in PS-supplemented hamsters compared with un-supplemented animals [29]. Similarly, Brufau et al. (2007) also observed an increase in the hepatic incorporation of lauric (12:0) and myristic (14:0) acids in PS-supplemented female Dunkin Hartley guinea pigs vs. animals fed a saturated fat diet [30]. The implications of this apparent shift in hepatic FA toward a more saturated profile is not yet known, although it may be a secondary effect due to a reduction in intestinal TG absorption or modulation of hepatic lipogenesis and/or lipoprotein synthesis. In support of a TG-lowering mechanism of hepatic origin, Plat et al. (2009) reported a reduction in large and medium plasma VLDL particles in dyslipidemic metabolic syndrome subjects consuming 2 g of PS provided in a yogurt drink matrix [11].

We have investigated the modulation of hepatic *de novo* lipogenesis as a potential mechanism that may underlie TG reductions following PS-supplementation in both mouse and hamster models [25,29]. However, the results of these studies highlight differential model-specific responses that preclude any clear consensus regarding the impact of PS on hepatic lipogenesis. We observed an increase in hepatic *de novo* lipogenesis in PS-fed C57BL6 that we interpreted to be a compensatory response to interference with intestinal FA absorption as evidenced by increased fecal FA excretion. We have recently identified a similar response in a PS-fed Zucker rat model that demonstrated an increase in the ratio of hepatic 16:0/18:2n-6, an indirect measure of hepatic *de novo* lipogenesis (Rideout et al; unpublished). However, in a separate study, PS fed Syrian golden hamsters exhibited a reduction in *de novo* lipogenesis that was supported by a decrease in the protein abundance of fatty acid synthase (FAS), a rate-limiting enzyme in the lipogenic pathway. Given the similarity in the design factors between the two studies, including background diet, PS supplementation level, and stable isotope analysis, this discrepancy highlights the underlying differences in lipid metabolism between mouse and hamster species and clearly demonstrates the need for estimates of *de novo* lipogenesis as part of a mechanistic human intervention.

It is of interest to note that lipid reductions (both cholesterol and TGs) have been reported following intraperitoneal and subcutaneous PS injections in various animal models [31–33].

Vanstone et al. [34] observed TG reductions in Syrian golden hamsters subcutaneously injected with PS at a dose of 5mg/kg/BW. These results suggest that PS may mediate blood lipid concentrations independent of their direct effects within the intestine, possibly by modulating the expression of genes that regulate hepatic TG balance.

Factors Affecting Responsiveness

Recent work has highlighted a variety of subject specific metabolic and genetic factors that predict the magnitude and direction of the cholesterol response to PS [22,35–37]. Although less work has specifically examined the heterogeneity of responses in blood TG, its range has been estimated to be between 6–20% [3]. As discussed previously, the TG-lowering efficacy of PS seems to depend on the presence and magnitude of hypertriglyceridaemia. This stands to reason as TG reductions in response to ezetimibe, the well-characterized intestinal cholesterol absorptive inhibitor, have also been shown to be dependent on baseline TG concentrations [38].

Although a genetic basis for the variable TG-lowering effects of PS is probable, we are only aware of two studies that have examined a potential genetic link, both with polymorphisms in the apolipoprotein E (apoE) gene. ApoE is an apolipoprotein component of lipoproteins including chlyomicrons, VLDL, LDL, and HDL and is therefore heavily involved in directing lipoprotein metabolism and remodeling with the plasma compartment. Apolipoprotein E is polymorphic with three common alleles in the population, namely ε4, ε3 and ε2, that are thought to underlie the lipid-lowering responses to drug and diet-based therapies. Sanchez-Muniz et al. (2009) observed TG reductions in apoE2 but not in apoE3 or E4 subjects following PS intervention in hypercholesterolemic adults [8]. Although not significant, Geelen et al. (2002) reported that E4 subjects tended (p=0.13) to have a greater TG-lowering response compared with E3/3 subjects consuming 3.2 g of daily PS intake in margarine (difference of 0.08 mmol/L) [39]. There is a need to conduct further studies to understand the genetic basis of responsiveness with a focus on genes that are known to modulate plasma TG concentrations and lipoprotein metabolism. Furthermore, there has yet to be any studies to examine more readily identifiable patient characteristics such as ethnicity, age, gender, and BMI that may underlie the TG-lowering response to PS.

Summary

Although PS are well-substantiated for their LDL-C lowering effects in hypercholesterolemic patients, the efficacy of PS as a TG-lowering therapy has only recently gained momentum within the nutrition community through a limited number of animal and human studies specifically designed to examine this response and the potential mechanisms involved. Nonetheless, clinical trial data has shown that, among subjects with hypertriglyceridemia (TG > 1.73 mmol/L), 2–4 g/PS/d can facilitate a decrease in TG concentrations of ≈ 11%. Results from a number of different animal studies suggested that the TG-lowering mechanisms of PS may be multifactorial including interference with FA absorption within the intestinal lumen, modulation of hepatic *de novo* lipogenesis, and a reduction in circulating medium and large VLDL particles. The effects of PS on the expression of a variety of gene and protein targets, including FAS and PPARα, suggest that

there may be a molecular component to the TG lowering response. However, to fully substantiate the utility of PS as a TG-lowering therapy, human clinical trials that are specifically powered to detect an effect of PS on TG concentrations in hypertriglyceridemic subjects are required. Furthermore, human interventions should explore a mechanistic basis for the TG-lowering response with a direct examination of FA absorption and whole-body lipogenesis in response to PS supplementation. Finally, responsiveness studies that identify both metabolic and genetic factor that determine the magnitude of PS-induced TG reductions will be critical in defining the clinical utility of PS as a TG-lowering therapy.

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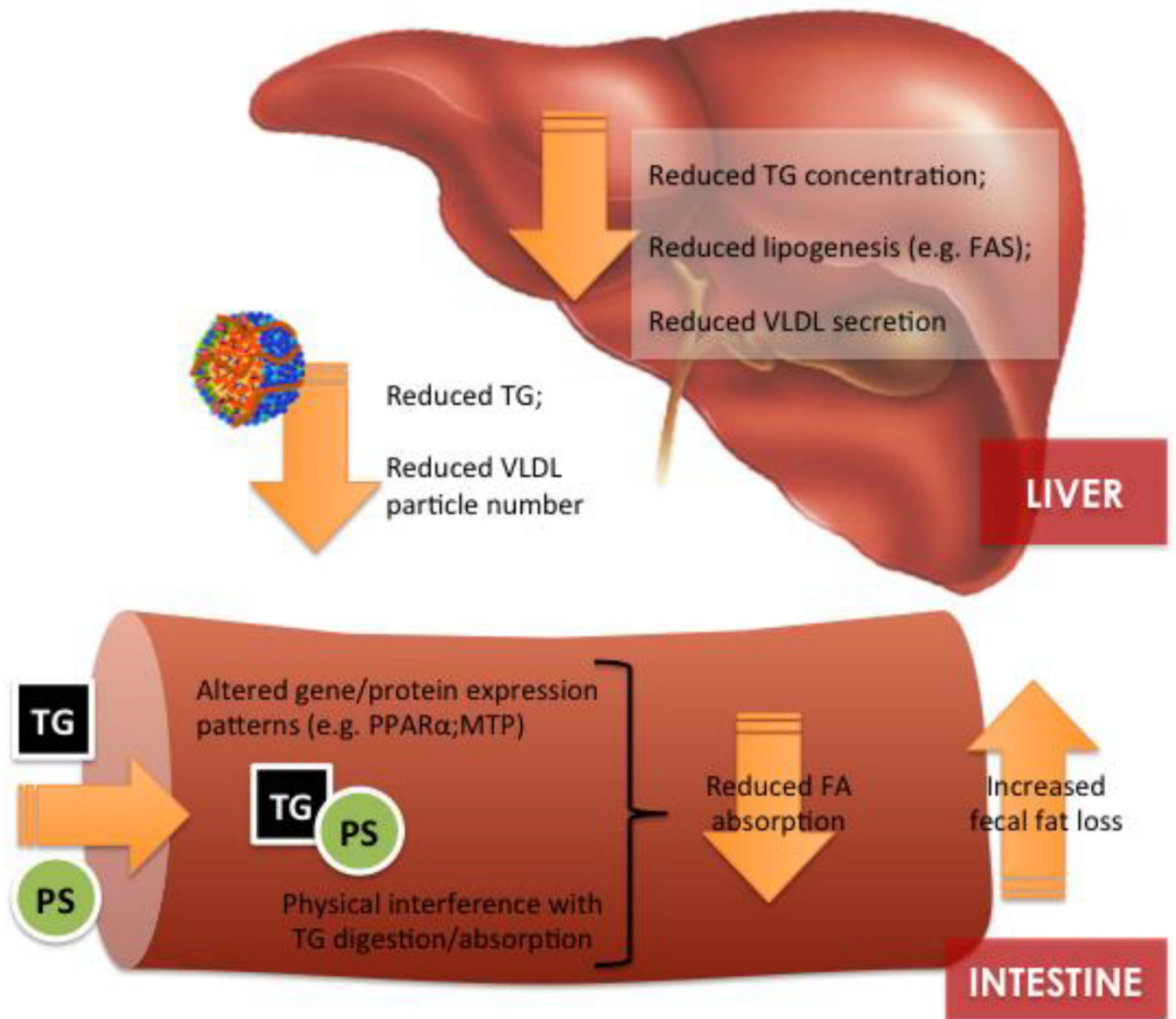
Figure 1.
Potential mechanisms involved in the triglyceride-lowering response to physterols/ phytostanols.

Table 1

Selected clinical studies reporting changes in plasma triglyceride concentrations in response to phytosterol/phytostanol supplementation.

Study	n		Design	Duration	Dose	Vehicle	Baseline TG (mmol/L)	Effect on TG Level (% change from baseline)
Maki et al. [7]	28 7M 21F	Primary hypercholesterolaemia	Randomized, single-blinded, placebo-controlled, crossover NCEP TLC weight maintenance diet	6 wks	1.8 g/d(81% PS & 19% PSA)	Capsule	1.41	vs. Base: ↓4% vs. Ctl:↓ 9.1%
Sialvera et al. [9]	101 60M 48F	Metabolic syndrome	Randomized, single-blinded, placebo-controlled, parallel-arm Western-type diet	2 mo	4 g/d PS	Yogurt beverage	2.20	vs. Base: ↓24% vs. Ctl: ↓12.5%
Sanchez-Muniz et al. [8]	217	Hypercholesterolaemic, stratified by carrier of the Apo E2 (E2E2), E3 (E3E3) and E4 (E3E4, E4E4) alleles	Randomized, double-blinded, placebo-controlled parallel-arm NCEP Step 1 diet	5 wks	1.1–2.2 g/d PS	Margarine	E2: 1.66 E3: 1.33 E4: 1.69	E2 vs. Ctl: ↓15.5% E3 vs. Ctl: ns E4 vs. Ctl: ns
Theuwissen et al. [10]	28 16M 12F	Elevated triglyceride levels	Randomized, double-blinded, placebo-controlled parallel-arm Habitual diet	3 wks	2.5 g/d PSA	Margarine	2.63	vs. Base: ↓11% (subjects with baseline TG > 2.3 mmol/L)
Plat et al. [11]	18	Dyslipidemic metabolic syndrome	Randomized, double-blinded, Placebo-controlled, parallel-arm	8 wks	2.0 g/d PSA	Yogurt beverage	2.21	vs. Base: ↓28%
	75	Normolipidemic	Randomized, double-blinded, Placebo-controlled, parallel-arm	8 wks	3.8–4.1 g/d PSA	Margarine and shortenings	1.02	ns
Rideout et al. [12]	33 15M 18F	Normolipidemic	Randomized, placebo-controlled, crossover Controlled feeding trial (50% CHO, 35% fat, 15% protein)	28 d	1.95 g/d PS	Low fat soy beverage	1.65	ns
	23 10M 13F	Hypercholesterolaemic	Randomized, placebo-controlled, crossover Controlled feeding trial (50% CHO, 35% fat, 15% protein)	28 d	1.95 g/d PS	Moderate fat soy beverage	2.06	vs. Ctl: ↓9.4%
Plat et al. [13]	36 23M 13F	Metabolic syndrome	Randomized, placebo-controlled parallel-arm	8 wks	2.0 g/d PSA	Yogurt beverage	2.21	vs. Base: ↓15.9% vs. Ctl: ↓44.5%
Clifton et al. [14]	151 77M 74F	Hypercholesterolemic	Randomized, double-blinded, placebo-controlled parallel-arm Habitual diet	6 wks	Wk 0–3: 1.6 g/d PS Wk 3–6: 3.0 g/d PS	Margarine	1.92	Wk 0–3 vs. Base: ↓15.9% vs. Ctl: ↓17.4% Wk: 3–6 vs. Base: ↓12.5%

Study	n		Duration	Design	Dose	Vehicle	Baseline TG (mmol/L)	Effect on TG Level (% change from baseline)
								vs. Ctl: ↓21.5%
Plana et al. [15]	83	Hypercholesterolaemic	6 wks	Multi-centre, randomized, double-blind, placebo-controlled, parallel-arm	1.6 g/d PS	Yogurt beverage	1.55	vs. Base: ↓7% vs. Ctl: ↓14%
Judd et al. [16]	53 26M 27F	Normocholesterolaemic and mildly hypercholesterolaemic	3 wks	Randomized, placebo-controlled, double-blind, crossover	3.6 g/d PS	Salad dressing	1.46	vs. Ctl: ↓7.3%
Davidson et al. [17]	84 46M 38F	Healthy	8 wks	Randomized, double-blind, placebo-controlled, parallel-arm Habitual diet	3.0 g/d PS 6.0 g/d PS 9.0 g/d PS	Reduced fat spread and/or salad dressing	1.20 1.18 1.47	3.0 g/d, vs. Base: ↓13.3% 6.0 g/d, vs. Base: ns 9.0 g/d, vs. Base: ns
Jones et al. [18]	15M	Hypercholesterolaemic	21 d	Randomized, double-blind, placebo-controlled, crossover Controlled feeding trial (50% CHO, 35% fat, 15% protein)	1.84 g/d PS 1.84 g/d PSA	Margarine	2.52 2.39	PS vs. Base: ↓18.9% vs. Ctl: ↓1.0% PSA vs. Base: ↓17.4% vs. Ctl: ↓0.9%
Shaghaghi et al. [19]	47 25M 22 F	Mild to moderately hypercholesterolaemic	29 d	Randomized, placebo-controlled, crossover Habitual diet	2.0 g/d water dispersible PS	Yogurt	1.68	vs. Base: ↓0.8% vs. Ctl: ↓13.9%

Table 2

Selected pre-clinical studies reporting plasma and or tissue triglyceride responses following phytosterol/phytostanol supplementation.

Author	Model	Diet & Design	Result	Notes
Hamster model				
Rideout et al. (2013)[29]	Male Syrian Golden	Semi-synthetic 'Western' diet2% PS supplementation (Reducol) 6 wks feeding	↓ blood TG (49%) Shift in hepatic FA (↑ 16:0; ↓16:1 and 18:1 ↓ de novo lipogenesis (44%) ↓ intestinal SREBP1c, hepatic PPARα, and FAS mRNA	Comparable TG reductions to ezetimibe supplementation Reductions in blood and hepatic cholesterol levels also observed
Ntanios et al. (1998)[40]	Male and female Syrian Golden	Semi-synthetic diet supplemented with phytosterols (0.5 or 1%) from tall oil or soybean oil ~13 wks feeding	↓ blood TG in males fed 1% soybean oil-phytosterols (no % given)	No effect in female hamsters
Ntanios et al. (2003)[41]	Male F_1B Syrian Golden	Semi-synthetic diet enriched with graded doses of phytosterol ester (0.24–2.84).	↓ blood TG in animals fed >0.96% phytosterol ester (0.96, 1.92, 2.84%)	
Vanstone et al. (2001)[34]	Male Syrian Golden	Semi-synthetic diet; tall oil or soybean oil derived PS supplemented in diet or subcutaneously injected (matched to 5 mg/kg BW/day) ~9 wks feeding	↓ blood TG (−42%) in animals injected with soybean oil derived PS	
Jain et al. (2008)[42]	Male Syrian Golden	Semi-synthetic, cholesterol-enriched diet with sitostanol (0.5%)	↓ blood TG (−22%) in sitostanol group compared with control	
Liang et al. (2011)[24]	Male Syrian Golden	Semi-synthetic, cholesterol-enriched diet supplemented with β-sitosterol or stigmasterol (0.1%)	↓ blood TG (−28%) in both groups	↓ in intestinal mRNA expression of microsomal triglyceride transfer protein in both groups
Ebine et al. (2006)[43]	Male Syrian Golden	Semi-synthetic, cholesterol-enriched diet supplemented with 0.7 and 1.4% disodium ascorbyl phytostanyl phosphate*	↓ blood TG (−45%) in 1.4% supplemented group	
Mouse model				
Rideout et al. (2010)[25]	Male C57BL/6J	Semi-synthetic 'Western' diet2% PS supplementation (Reducol) 6 wks feeding	↓ blood TG (−28%) ↓ hepatic TG (30%) ↑ fecal 16:0 and 18:0 excretion ↑ hepatic SREBP1c and FAS mRNA; ↓ intestinal PPARα mRNA ↑ de novo lipogenesis (23%)	Blood total cholesterol not altered No change in a host of intestinal FA absorption & metabolism gene expression
Volger et al. (2001)[44]	Female apolipoprotein E*3-Leiden transgenic	Semi-synthetic containing 0.25% cholesterol and 0.0%, 0.25%, 0.5%, 0.75%, or 1.0% plant stanols 8 wk feeding	↓ hepatic TG (−38%) in the 1% dietary stanol group	No effect on serum TG No effect on hepatic VLDL-TG secretion
Plosch et al. (2006)[45]	Male C57BL/6J	Semi-synthetic cholesterol supplemented diet with 0.5% plant sterols or stanols 4 wks feeding	↑ hepatic TG in both sterol and stanol groups	No change in plasma TG concentrations

Author	Model	Diet & Design	Result	Notes
Brufau et al. (2011)[46]	Male C57BL/6J	Semi-synthetic cholesterol supplemented diet with 1, 2, 4, or 8% plant sterols 2 wks feeding	↓ blood TG (−26%) in 4% supplemented group ↑ hepatic TG (59%) in the 2% supplemented group	
Looije et al. (2005)[47]	Male C57BL/6J	Low fat semi-synthetic diet supplemented with 2% FM-VP4*	↓ blood TG (% reduction not specified)	
Lukic et al. (2003)[48]	Male apoE knockout	Cholesterol-supplemented chow diet with 0.1, 0.5, 1.0, and 2.0% FM-VP4* 12 wks feeding	↓ blood TG in 2% supplemented group at 4 and 8 weeks	
Rat model				
Matasuoka et al. (2008)[49]	Male Sprague-Dawley rats	Semi-synthetic diet supplemented with 0.5% free PS (FPS) or free PS egg yolk lipoprotein complex (PSY) 3 wks feeding	↓ hepatic TG in both groups (FPS, −33%; PSY, −22%)	No change in serum TG concentrations
Awaisheh et al. (2012)[50]	Male Sprague-Dawley rats	Semi-purified high fat/ cholesterol chow; daily gavage with non-fermented milk with and without PS (5 mg/mL) 8 wks feeding	↓ blood TG (−16%) ↓ hepatic TG (−92%)	
Ikeda et al. (2006)[51]	Male Sprague-Dawley rats	Semi-synthetic diet supplemented with campestenone (0.5%)	↓ blood TG (−76%) ↓ hepatic TG (−69%) ↑ expression of β-oxidation genes ↓ hepatic SREBP1c expression	
Tomoyori et al. (2004)[23]	Male Sprague-Dawley rats	Semi-synthetic supplemented with 0.25% PS	↓ lymphatic transport of TG	
Pig model				
Brufau et al. (2006)[30]	Female Dunkin Hartley guinea pigs	Cholesterol enriched (0.33%), isocaloric diets, chow vs semi-synthetic not specified; Supplemented with 3 doses of PS (0, 1.27, 2.45%); Diets with combination pectin and PS were also examined 4 wks feeding	↑ apparent absorption of saturated FA in PS-supplemented animals including lauric (12:0) and myristic (14:0) acids ↑ hepatic incorporation of lauric (12:0) and myristic (14:0) acids in PS-supplemented animals vs. animals fed saturated fat diet	
Brufau et al. (2007)[52]	Female Dunkin Hartley guinea pigs		↓ fecal excretion of lauric (12:0) and myristic (14:0) acids compared with high saturated fat diet ↑ fecal excretion of arachidic (20:0) and behenic (22:0) acids compared with high saturated fat diet	
Brufau et al. (2008)[53]	Female Dunkin Hartley guinea pigs		No change in plasma TG	

*A semi-synthetic esterified phytostanols-ascorbic acid derivative