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Water dispersible plant sterol formulation shows improved effect on lipid profile compared to plant sterol esters ☆

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ABSTRACT

While the cholesterol-lowering efficacy of plant sterols (PS) is known, issues surrounding reduced PS solubility of some dietary formulations remain to be elucidated. This study determined the efficacy of a water dispersible formulation of free plant sterols (WD-PS) versus plant sterol esters (PS-esters). Forty-seven mild-to-moderately hypercholesterolemic individuals in a randomized, crossover study were provided for 4 wk with a single-dose daily regimen of PS-enriched yogurt (2 g/d of PS from WD-PS or PS-esters) or placebo. Yogurt enriched with WD-PS or PS-esters induced similar decreases in serum total (7.7% and 6.3%, respectively) and LDL cholesterol levels (11.7% and 11.6%, respectively), as percentage relative to the control ($p < 0.001$; all). Ratios of total to HDL cholesterol and non-HDL to HDL cholesterol decreased more ($p < 0.05$) with WD-PS (10.6% and 15.2%, respectively) than with PS-esters (7.0% and 10.8%, respectively) compared with control. Consumption of WD-PS reduced serum triglyceride levels (13.9%, $p < 0.05$) compared to consumption of PS-esters (0.6%). Both WD-PS and PS-esters contributed effectively to LDL cholesterol lowering, however, the formulation of WD-PS yield additional effects on preventing cardiovascular diseases by improving serum TG and the ratio of total to HDL cholesterol.

Trial registration (clinicaltrials.gov): NCT01478789.

1. Introduction

According to World Health Organization, cardiovascular diseases (CVD) are the leading causes of death globally (WHO, 2011). The lowering of LDL cholesterol is central in the prevention of CVD and can be achieved through dietary modification and therapy (NCEP, 2001). Hence, due to the established cholesterol-lowering effect of PS, several advisory

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Abbreviations: CRP, C-reactive protein; PS, plant sterols; TC, total cholesterol; WD-PS, water dispersible plant sterols; PS-esters, esterified plant sterols

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bodies recommend intake of 2 g/day of PS as a component of modified diet, to optimize blood lipid levels (American Diabetes Association, 2008; Health Canada, 2010; NCEP, 2001). The major mechanism of action responsible for the cholesterol-lowering property of PS is the inhibition of intestinal cholesterol absorption (Trautwein et al., 2003). The competitive solubilization between cholesterol and PS in bile salt micelles purportedly decreases intestinal cholesterol absorption and thus reduces circulating levels of cholesterol, while partially increasing endogenous cholesterol biosynthesis (Abumweis, Barake, & Jones, 2008). Since PS decrease absorption of cholesterol, an important concern about PS use is that PS may reduce absorption of fat-soluble vitamins and carotenoids (Berger, Jones, & Abumweis, 2004; Law, 2000; Rudkowska, AbuMweis, Nicolle, & Jones, 2008b). However, results of different studies varied from no change in fat-soluble antioxidants (De Jong et al., 2008; Korpela et al., 2006; Thomsen, Hansen, Christiansen, Green, & Berger, 2004; Volpe et al., 2001), to a substantial decrease in tocopherol and/or β-carotene (Clifton et al., 2004; Mensink, Ebbing, Lindhout, Plat, & van Heugten, 2002; Noakes, Clifton, Doornbos, & Trautwein, 2005; Richelle et al., 2004), or decrease only in serum lutein and lycopene levels (Rudkowska et al., 2008b).

Owing to their chemical structure and poor solubility, PS must be properly formulated to achieve optimal health benefits. Indeed, PS possess crystalline properties, a high melting point, and low solubility in water and fats which complicate their incorporation into food matrices and limit their practical applications (Berger et al., 2004; Fornari, Torres, Torrelo, Senorans, & Reglero, 2009). Traditionally, the most common process is to convert PS to their esterified forms, with vegetable oil fatty acids, for subsequent integration into fat based foods such as margarine and spreads (Fornari et al., 2009). Furthermore, research in the nutraceutical industry has shown that the solubility and bioactivity of PS can be greatly enhanced by incorporating them within various emulsion-based delivery systems (Gremaud et al., 2002; Lin et al., 2009; Meguro et al., 2001; Ostlund, Spilburg, & Stenson, 1999). In some studies, formulating free PS with lecithin considerably reduced cholesterol absorption and circulating LDL-C, while less effect was seen with PS in crystalline form (Gremaud et al., 2002; Ostlund et al., 1999). Moreover, Lin et al. (2009) indicated that natural phytosterol glycosides, purified from soy lecithin, reduced cholesterol absorption by 37.6%, compared to the 30.6% reduction observed simultaneously with PS esters. Finally, the dose-dependent LDL-C–lowering efficacy of PS was shown by Demonty et al. (2009) in a meta-analysis of eighty-four trials, which confirmed that the efficacy of PS had no link with various treatment characteristics, including fat-based vs. non fat-based vehicles and/or free-PS vs. PS-esters forms.

Similar to esters of PS, properly solubilized free sterols, have been shown in some studies, but not all, to induce a similar LDL-C–lowering effect when provided at the equivalent free sterol dose (Richelle et al., 2004). Decreased solubility of free PS, owing to the difficulty of formulating and delivering these relatively insoluble substances is one of the main causes for the inconsistency among the results of these studies (Berger et al., 2004; Moreau, Whitaker, & Hicks, 2002). Therefore, based on the importance of the form of PS in its bioactivity and efficacy, each new formulation ought to be assessed for value if they differ greatly from previously tested forms.

Therefore, the objective of this study was to examine the effects of a new formulation of a water dispersible PS (WD-PS) on serum lipids and fat soluble vitamins concentrations, compared against a positive control conventional PS-esters and placebo (vehicle only). In addition, safety parameters, defined as reported adverse events and/or undesirable changes in clinical chemistry parameters including liver enzymes, were examined during the 4 wk of each phase of the study. We hypothesized that WD-PS would be as effective as PS-esters in lowering blood total cholesterol and LDL cholesterol levels.

2. Materials and methods

2.1. *Treatment preparation*

WD-PS (Nutrartis SA, Santiago, Chile) and PS-ester (Arboris LLC, Savannah, GA, USA) are commercially available tall oil derived sterol products with the 2 major components distributed approximately as 70–80% and 15% for beta-sitosterol and campesterol by weight, respectively. The ester portion of the PS-ester corresponds to vegetable oil fatty acids. It also contains mixed tocopherols and ascorbyl palmitate as antioxidants (3000 ppm). The composition and nutritional information of WD-PS and PS-esters PS are shown in Tables 1 and 2.

The PS-ester was produced via esterification of pure free sterols (Arboris LLC, Savannah, GA, USA) with food fatty acids derived from edible vegetable oil. The food grade fatty acids and sterols were mixed and the combination was carried out at elevated temperature, without using any chemical catalyst. After the esterification, the excess free fatty acids were removed using high vacuum distillation and the refined PS-esters were cooled down. The WD-PS, sub-micron dispersion of free sterols with the targeted composition and particle size was prepared by Nutrartis S.A. (Santiago, Chile), using the patent application WO 2010/095067 25. Briefly, pure free sterols (Arboris LLC, Savannah, GA, USA) were melted to form an O/W (oil–water) emulsion of sterols in water and then cooled down to form dispersion. Particle size of the dispersion was determined using Horiba LA910 equipment giving an average particle size of 400 nm with no particles detected under 100 nm.

Plain yogurt (4% Fat Milk, Dairyland, Saputo, Canada) was used as a food carrier for both PS (WDPS and PS-esters) and also as the control. PS-esters were melted (at 60 °C/3 min) in a water bath (VWR 1227, San Diego, CA, USA) and then mixed into yogurt through gentle agitation (3.37 g PS-esters added to 100 g yogurt). WD-PS was a stable, non-decanting, readily-dispersible phytosterol dispersion that did not require high shear mixing or homogenization to be suitably formulated into food products and was incorporated into the yogurt through gentle agitation (20 g WD-PS added to 100 g yogurt). WD-PS and PS-esters were flavored and suspended in yogurt in the metabolic kitchen of the Richardson Centre for Functional Foods and Nutraceuticals (2 g free plant sterol added to 100 g yogurt for both treatments). No organoleptic differences were detected

Table 1 – Composition of plant sterols.

Component	% w/w
WD-PS	
Tall oil sterols	10.0
Vegetable oil fatty acids (Na + salts)	0.2
Polysorbate	0.3
Water	89.5
PS-esters	
Tall oil sterols	62.0
Fatty acids	38.0

Note: WD-PS, water dispersible plant sterol; PS-esters, plant sterol esters; W, weight.

Table 2 – Nutritional information of plant sterols per 100 g yogurt.

Component	WD-PS	PS-esters
Energy (kcal)	0.60	11.06
Total fat (g)	0.05	1.23
Saturated fat (g)	–	0.08
Trans fatty acids (g)	–	0.02
Monounsaturated fat (g)	0.04	0.79
Polyunsaturated fat (g)	–	0.28
Nitrogen-free extract (g)	0.04	–
Plant sterols (g)	2.00	2.00

Note: WD-PS, water dispersible plant sterol; PS-esters, plant sterol esters.

between the PS-enriched (WD-PS and PS-esters) and the placebo yogurts.

2.2. Study population

Individual men and women with above optimal serum LDL cholesterol concentrations were recruited in the Winnipeg metropolitan area by advertisements in newspapers. Potential study volunteers were initially screened by questionnaire regarding personal health information, medical conditions, and disease history. If subjects were determined to be potentially eligible, they underwent blood screening at the first visit where fasting blood was taken to test for general lipid profile including TC, HDL-C, LDL-C, and TG levels. Inclusion criteria included baseline LDL-C above 2.8 mmol/l, TG below 4.5 mmol/l, a body mass index (BMI) between 20 and 30 kg/m^2, and age 19–75 yr. Volunteers were excluded if they took statins, nicotinic acid, or fibrates. Subjects who were diagnosed to have diabetes mellitus, sitosterolemia, heart disease, liver disease, kidney disease, or who had recently undergone major surgery were also excluded from the study. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Biomedical Research Ethics Board at University of Manitoba. Written informed consent was obtained from all subjects.

2.3. Experimental protocol

Fifty-three participants (19–75 yr) were recruited. The study was a free-living, randomized, crossover trial consisting of three 29 d treatment phases each separated by 4 wk washout intervals. Subjects were assigned to receive the treatments, WD-PS-enriched yogurt, PS-esters-enriched yogurt, or yogurt without PS (placebo), in a random order. During each treatment period subjects were instructed to follow their normal diet and consume their standardized supper meal at RCFFN followed by ingestion the treatment, under supervision on a daily basis to monitor compliance.

2.4. Blood sampling and analysis

Twelve-hour fasting blood samples were collected on d 1, 2, 28 and 29 of each of the 3 phases of the trial. Blood samples obtained on d 1 and 2 were used to measure baseline values for different study measurements, whereas blood samples obtained on the 2 last days were used to measure final values. Blood samples were collected and centrifuged for 20 min at 3000*g*. The separated aliquots were frozen at −80 °C until analysis. Plasma TC, TG, HDL-C, and liver enzymes (AST, ALT, LDH, ALKP, GGT) levels were analyzed using a VITROS 350 chemistry autoanalyser (Ortho-Clinical Diagnostics, Markham, ON, Canada). Plasma LDL-C concentrations were calculated using the Friedewald equation (Friedewald, Levy, & Fredrickson, 1972). Plasma CRP was analyzed using an Adiva 1800 clinical chemistry system (Siemens Healthcare Diagnostic INC, ON, Canada).

2.5. Plasma cholesterol precursor and plant sterol analyses

Plant sterols and precursor sterols concentrations in serum were measured using gas chromatography GC (Bruker 430; Billerica, MA, USA) based on previously established methods (Jones et al., 2005). Briefly, 5α-cholestane as an internal standard (Sigma–Aldrich Canada Ltd, Oakville, ON, Canada) was added to each plasma sample (100 μg/0.5 ml plasma). Samples were saponified with 0.5 M methanol-KOH for 2 h at 100 °C, and the unsaponifiable portion extracted with petroleum ether. The extracted non-saponifiable materials were used to determine PS concentration levels. Samples were derivatized with 500 μl TMS reagent (pyridine–hexamethyldisilazane–trimethylchlorosilane; 9:3:1) (Jones et al., 2005). After preparation samples were injected into the GC equipped with a flame ionization detector and an auto-injector system. Separation was achieved on a 30 m capillary column with an internal diameter of 0.25 mm and film thickness of 0.25 μm (SAC-5, Supelco, Sigma–Aldrich Canada Ltd., Oakville, ON, Canada). The column temperature was initiated at 130 °C for 2 min. The temperature was then increased to 270 °C (rate: 30 °C/min) for 10 min and was augmented again to 290 °C (rate: 10 °C/min). After 8 min the temperature was increased to 310 °C (rate: 20 °C/min) for 2 min, (total time was 30 min). The injector and detector temperatures were set at 295 °C and 300 °C, respectively. The carrier gas (helium) flow rate was set for 1 ml/min with the inlet splitter set at 100:1. Peaks of interest were identified using authentic standards (Sigma–Aldrich Canada Ltd, Oakville, ON, Canada).

Cholesterol synthesis was assessed based on the previously established surrogate method (Jones et al., 2005; Mackay & Jones, 2011). Briefly, the ratio of absolute amount of lathosterol to cholesterol (μmol/mmol of cholesterol) was used to

determine cholesterol biosynthesis (Miettinen, Strandberg, & Gylling, 2000; Simonen, Gylling, & Miettinen, 2008; Vanstone & Jones, 2004).

2.6. Plasma fat-soluble vitamin and carotenoid analyses

An isocratic high-performance liquid chromatography HPLC (1100 HPLC, Agilent Technologies, Palo Alto, CA, USA) with UV–VIS spectrophotometer detector (SPD-6A) was used for simultaneous determination of plasma α-tocopherol, γ-tocopherol, retinol, lutein, lycopene, and β-carotene. The extraction procedure was as described by Gueguen, Herbeth, Siest, and Leroy (2002). In brief, an internal standard (Sigma–Aldrich, St. Louis, MO, USA), a solution of retinol acetate and β-apo-8′-carotenal in methanol was added to 200 ul serum to quantify vitamin and carotenoid levels, respectively. Serum samples were deproteinized with ethanol and extracted twice with hexane. Duplicates of each sample were prepared, then resulting extracts injected onto a C18 reversed-phase column (30 cm × 3.9 mm id) (Zorbax EclipseXDB, Agilent Technologies, Palo Alto, CA, USA) with column guard eluted with methanol–acetonitrile–tetrahydrofuran (75:20:5, v/v/v). The flow rate was set at 1.0 ml/min. Full elution of all the analytes was realized isocratically within 38 min. The detection wavelengths were set at 290 nm, 325 nm, 450 nm, and 475 nm for α and γ-tocopherol, retinol, lutein and lycopene analyses, respectively.

2.7. Statistical analyses

The sample size was calculated to detect an anticipated difference in LDL-C levels due to PS treatment of 12% (0.54 mmol/l) using a standard deviation of 0.732 mmol/l. The alpha and power were 0.05 and 0.8, respectively. The sample size was determined taking into account the block size and an estimated 10% premature subject withdrawal rate.

All data are presented as mean ± SEM. Statistical significance was set at $p < 0.05$ for all the analyses. Statistical analysis was performed using SPSS 16.0 (SPSS, Inc., Chicago, IL, USA). Baseline variables were analyzed by using 1-factor ANOVA. For all data, endpoint values were reported as averages of d 28 and 29. Endpoint, absolute change, and percentage change values were compared using analysis of variance (ANOVA) followed by a Dunnett's multiple comparison test, with treatment and phase as fixed factors, and subject as a random factor in the model. Baseline values were inserted into the model as covariates for serum lipid measurements. To standardize plasma PS, as well as serum fat-soluble vitamins and carotenoids, serum concentrations of each compound were divided by plasma TC or LDL-C concentrations, respectively.

3. Results

3.1. Subject characteristics

Fifty-three participants were initially recruited while 47 subjects (25 males and 22 females) completed the entire trial. Inability to complete the trial included not being able to consume yogurt ($n = 2$), relocation to another city ($n = 1$), and personal reasons ($n = 3$). Baseline characteristics of the subjects who completed the study are shown in Table 3. All participants exhibited good tolerance to the experimental treatments and no side effects were reported. Subjects reported no change in physical activity, and no significant differences were observed in body weight after the consumption of the three treatments.

3.2. Plasma lipid concentrations in response to PS treatments

Baseline and endpoint plasma lipid concentration responses to each treatment are shown in Table 4. No significant differences at baseline among the three treatments in any of the lipid parameters assessed were observed.

In the present study, supplementation of both WD-PS and PS-esters improved serum lipoprotein profiles. In particular, reductions ($p < 0.001$) were observed in endpoint total cholesterol and LDL cholesterol levels compared with control. Consumption of the WD-PS-enriched yogurt and PS esters-enriched yogurt similarly lowered ($p < 0.001$ for all) total cholesterol concentrations on average by 7.7% and 6.2%, respectively, compared with the control product. Likewise, consumption of WD-PS and PS-esters in yogurt lowered ($p < 0.001$ for all) LDL-cholesterol on average by 11.7% and 11.6%, respectively (Table 4). Although no treatment effects were observed in HDL-C levels at endpoint across the three treatments, ratios of TC to HDL-C and Non-HDL-C to HDL-C showed significant reductions (10.5%, 15.2%, $p < 0.05$, respectively) for the supplementation of WD-PS, but not for supplementation of the PS-esters compared to control (Table 4). Plasma TG concentrations did not differ as a function of treatment between endpoints. Over the treatment period, however, TG levels were reduced (13.9%, $p < 0.05$) from baseline due to consumption of WD-PS-enriched yogurt, compared to PS-esters-enriched yogurt, but not compared to control (Table 4). Further analysis was done to examine the potential relationship between positive effect of WD-PS on TG levels and baseline TG concentrations. There was no relationship between the reductions in plasma TG levels with the baseline TG circulations.

3.3. Plasma carotenoid and vitamin level in response to treatment

Table 5 provides the fat-soluble vitamin and carotenoid concentrations at the end of each phase, as well as the serum fat-soluble vitamin and carotenoid concentrations, expressed relative to LDL-C levels. Neither intervention, WD-PS and/or PS-esters, had an effect on fat-soluble vitamins or carotenoids.

3.4. Plasma plant sterol concentrations in response to treatments

Plasma PS concentrations and PS concentrations expressed relative to TC are provided in Table 6 at the end of each phase. Plasma concentrations of campesterol and β-sitosterol were

Table 3 – Baseline characteristic of subjects.

Variable	Mean	SEM
Age (yr)	50	2.1
Sex		–
Male	25	–
Female	22	–
Body wt (kg)	80.4	2.7
Height (cm)	168.2	1.4
BMI (kg/m^2)	28.2	0.7
Total cholesterol (mmol/l)	6.0	0.2
LDL-cholesterol (mmol/l)	3.8	0.2
HDL-cholesterol (mmol/l)	1.4	0.1
TG (mmol/l)	1.7	0.1
CRP (mg/l)	1.5	0.3
Blood pressure		
Systolic (mmHg)	121	2.4
Diastolic (mmHg)	78	1.8
Liver enzymes		
AST (U/l)	22.9	2.0
ALT (U/l)	38.2	4.7
LDH (U/l)	529.7	10.7
ALKP (U/l)	76.9	3.0
GGT (U/l)	37.4	3.4

Note: Values are mean ± SEM; N = 47.
ALKP, alkaline phosphatase; ALT, alanine aminotransferase; AST, asparagine aminotransferase; GGT, g-glutamyltransferase; LDH, lactate dehydrogenase; CRP, C-reactive protein.

increased ($p < 0.001$) for both WD-PS and PS-esters relative to control. Similarly, endpoints adjusted campesterol and β-sitosterol by TC levels were increased ($p < 0.001$) after both the WD-PS and PS-esters phases, compared to control. Plasma stigmasterol levels increased ($p < 0.001$) after consumption of the WD-PS-enriched yogurt compared to control yogurt. Likewise, the endpoint adjusted stigmasterol, to TC ratios, were increased ($p < 0.05$) for WD-PS phase, not for PS-esters, compared to control. No effects of WD-PS or PS-esters were observed on the cholesterol synthesis markers desmosterol and lathosterol.

3.5. *Clinical chemistry measures as a function of treatment*

Clinical chemical parameters were analyzed including AST, ALT, ALKP, GGT, LDH, and CRP (Table 7). Although variations were found in the parameters, these differences were of no clinical significance. No treatment effect was observed with any of these parameters.

4. Discussion

The present study is the first report comparing the efficacy of a commercial sub-micron dispersion of free sterols and PS-esters within a yogurt-based medium. This head to head comparison between two different formulations of PS showed that consumption of both WD-PS and PS-esters resulted in a similar reduction of plasma total and LDL-cholesterol concentrations, compared to control. Moreover, consumption of WD-PS was associated with additionally optimizing the blood lipid profile of the participants. More specifically, consumption of WD-PS lowered ratios of TC:HDL-C and also NHDL-C:HDL-C while consumption of PS-esters did not effectively alter either of these ratios. In addition, it was shown that consumption of WD-PS helped to regulate circulating TG levels compared to PS-ester.

Although recent analysis demonstrated that free PS and PS-ester incorporated into foods have similar cholesterol lowering action (Abumweis et al., 2008; Demonty et al., 2009; Katan et al., 2003), in some studies consumption of PS showed reduced or no LDL-C lowering effect (Denke, 1995; Jones, Vanstone, Raeini-Sarjaz, & St-Onge, 2003). The lack of efficacy of PS in these studies may have been caused by the reduced PS solubility and formation of crystalline sterols in the preparations used. If not properly formulated, PS form highly stable crystals in which are not readily soluble in bile, the hydrophilic hydroxyl groups are requisitioned inside the matrix and are not available to solubilizing fluids, hence do not reduce cholesterol absorption (Ostlund et al., 1999).

In present study, the comparable cholesterol lowering effect of new formulation of WD-PS with PS-esters supports the notion that proper solubilization of every new PS formulations is essential for proper efficacy. The magnitude of the WD-PS and PS-ester induced reductions in plasma TC and LDL-C concentrations, found in the present study, were comparable with the changes reported in the literature in which 2–3 g/d PS was consumed in conjunction with various background diets.

In addition, consumption of WD-PS-enriched yogurt, but not PS-esters-enriched yogurt, effectively lowered TC/HDL-C and NHDL/HDL-C ratios, compared to control. The ratio of TC/HDL-C as an index combining the proportion of atherogenic to anti-atherogenic lipids and lipoproteins, has therapeutic target value for CVD risk in patients on LDL-C–lowering therapy (Kastelein et al., 2008; Ridker, Rifai, Cook, Bradwin, & Buring, 2005) and also considers as a better predictor of CVD events than is total cholesterol or any individual lipid measurement (Assmann, Schulte, von Eckardstein, & Huang, 1996; Kinosian, Glick, Preiss, & Puder, 1995; Stampfer, Sacks, Salvini, Willett, & Hennekens, 1991). According to the global cardiovascular risk assessment guidelines reaching LDL-C goals remains to be the first line therapy for CVD, however, TC/HDL-C ratio and triglyceride levels are also defined as important to better stratify intermediate risk patients for primary prevention of CVD (Genest et al., 2009).

Consumption of PS is not traditionally allied with modulation of TG levels. However, more recently, a positive relationship between supplementation of plant stanol and TG circulation was established, especially in individuals with hypertriglyceridemia (Naumann, Plat, Kester, & Mensink, 2008; Plat, Brufau, Dallinga-Thie, Dasselaar, & Mensink, 2009; Theuwissen, Plat, van der Kallen, van Greevenbroek, & Mensink, 2009). The relationship between plant stanol consumption and TG levels origins from a cutback in the hepatic production of large TG-rich VLDL-1 particles which may be more voluntarily detected as a reduction in serum TG circulations in individuals with baseline elevated TG levels (Plat & Mensink, 2009; Theuwissen et al., 2009). In the current study, over the treatment period, consumption of WD-PS-enriched yogurt was associated with a reduction in plasma TG levels compared to PS-esters-enriched yogurt (13.9%, $p < 0.05$).

Table 4 – Plasma lipid concentrations at the baseline and endpoint of each treatment phases.

Lipid variables	Control	WD-PS yogurt	PS-esters yogurt
Total cholesterol			
Start (mmol/l)	5.85 ± 0.15	5.98 ± 0.15	5.89 ± 0.14
End (mmol/l)	6.04 ± 0.15	5.73 ± 0.13**	5.72 ± 0.13**
Change (mmol/l)	0.20 ± 0.08	−0.26 ± 0.07**	−0.18 ± 0.08**
Change (%)	3.85 ± 1.25	−3.85 ± 1.12**	−2.41 ± 1.35*
Change relative to control (%)		−7.69 ± 1.46	−6.25 ± 1.93
LDL-Cholesterol			
Start (mmol/l)	3.67 ± 0.14	3.84 ± 0.14	3.75 ± 0.13
End (mmol/l)	3.82 ± 0.15	3.58 ± 0.13**	3.50 ± 0.12**
Change (mmol/l)	0.17 ± 0.08	−0.27 ± 0.06**	−0.25 ± 0.07**
Change (%)	5.5 ± 2.1	−6.22 ± 1.46**	−6.05 ± 1.91**
Change relative to control (%)		−11.72 ± 2.52	−11.56 ± 2.94
Non-HDL Cholesterol			
Start (mmol/l)	4.40 ± 0.15	4.58 ± 0.15	4.48 ± 0.14
End (mmol/l)	4.60 ± 0.15	4.30 ± 0.13	4.32 ± 0.13
Change (mmol/l)	0.20 ± 0.09	−0.28 ± 0.07	−0.17 ± 0.07
Change (%)	5.69 ± 1.96	−5.44 ± 1.42**	−3.03 ± 1.56*
Change relative to control (%)		−11.13 ± 2.34	−8.72 ± 2.60
HDL-Cholesterol			
Start(mmol/l)	1.44 ± 0.06	1.40 ± 0.05	1.41 ± 0.05
End(mmol/l)	1.44 ± 0.05	1.43 ± 0.05	1.40 ± 0.05
Change (mmol/l)	−0.01 ± 0.04	0.03 ± 0.02	−0.02 ± 0.02
Change (%)	1.18 ± 1.85	2.65 ± 1.79	−0.05 ± 1.58
Change relative to control (%)		2.66 ± 1.79	−0.04 ± 1.58
Triacylglycerol			
Start (mmol/l)	1.70 ± 0.12	1.68 ± 0.11	1.63 ± 0.11
End (mmol/l)	1.80 ± 0.13	1.62 ± 0.12	1.80 ± 0.13
Change (mmol/l)	0.09 ± 0.09	−0.05 ± 0.05***	0.17 ± 0.06
Change (%)	13.16 ± 7.71	−0.76 ± 3.40***	13.74 ± 4.49
Change relative to control (%)		−13.92 ± 8.63***	0.58 ± 9.05
TC/HDL-C			
Start (mmol/l)	4.32 ± 0.19	4.50 ± 0.18	4.41 ± 0.17
End (mmol/l)	4.46 ± 0.19	4.21 ± 0.16*	4.33 ± 0.18
Change(mmol/l)	0.13 ± 0.11	−0.30 ± 0.09**	−0.09 ± 0.06
Change (%)	5.16 ± 3.61	−5.39 ± 1.53*	−1.85 ± 1.34
Change relative to control (%)		−10.55 ± 3.75	−7.01 ± 3.97
NHDL/HDL-C			
Start (mmol/l)	3.32 ± 0.19	3.5 ± 0.18	3.41 ± 0.17
End (mmol/l)	3.46 ± 0.19	3.21 ± 0.16	3.32 ± 0.18
Change (mmol/l)	0.13 ± 0.11	−0.29 ± 0.09**	0.09 ± 0.06
Change (%)	8.47 ± 5.83	−6.74 ± 1.94*	−2.36 ± 1.74
Change relative to control (%)		−15.21 ± 5.90	−10.82 ± 6.23

Note: Treatment effects were examined by one-way ANOVA. Values are mean ± SEM; N = 47.
Change and percentage change are based on individual data.
* $p < 0.05$ Comparison of WD-PS and PS-esters with Control are significant.
** $p < 0.001$ Comparison of WD-PS and PS-esters with Control are significant.
*** $p < 0.05$ Comparison of WD-PS with PS-esters is significant, but not significant from Control.

However, no relationship was observed between the reductions in plasma TG levels with the baseline TG circulations. Moreover, previous clinical trials incorporating free sterols in yogurt found no effect on TG levels (Rudkowska et al., 2008b; Volpe et al., 2001). To the best of our knowledge, the current study is the first study observing a positive effect of intake of free plant sterols on TG levels. The fact that there was no relationship between the effect of WD-PS and baseline TG levels might imply the potential importance of formulations of plant sterol (plant stanols vs. PS as well as WD-PS vs. PS-esters) also on their possible TG lowering efficacy. Further studies may need to prove this hypothesis.

Long term consumption of PS has shown to decrease plasma fat soluble vitamins and carotenoid concentrations (Hansel et al., 2007; Law, 2000). In the present study, supplementation of WD-PS and PS-ester did not adversely influence fat soluble vitamin or carotenoid levels before or after adjustment for LDL-C levels, as compared to control. The present results are in agreement with those of Raeini-Sarjaz, Ntanios, Vanstone, and Jones (2002) who showed no effect of sterol

Table 5 – Endpoint plasma carotenoid and vitamin levels in response to treatment.

Analyte	Control	WD-PS		PS-esters	
	Wk 4	Wk 4	Absolute change	Wk 4	Absolute change
α-Tocopherol (μmol/l)	63.87 ± 2.64	62.66 ± 2.51	−1.22 ± 3.28	62.36 ± 4.11	−1.51 ± 3.28
α-Tocopherol-LDL (μmol/mmol)	10.54 ± 0.43	10.9 ± 0.45	0.37 ± 0.51	10.79 ± 0.63	0.25 ± 0.51
γ-Tocopherol (μmol/l)	9.62 ± 0.65	9.87 ± 0.72	0.25 ± 0.84	9.61 ± 0.41	−0.30 ± 0.84
γ-Tocopherol/LDL (μmol/mmol)	1.60 ± 0.11	1.73 ± 0.13	0.13 ± 0.13	1.62 ± 0.07	0.03 ± 0.13
Retinol(μmol/l)	3.40 ± 0.19	3.88 ± 0.18	−0.02 ± 0.19	3.89 ± 0.26	−0.00 ± 0.19
Retinol/LDL (μmol/mmol)	0.65 ± 0.03	0.67 ± 0.03	0.02 ± 0.03	0.67 ± 0.04	0.03 ± 0.03
Lutein (μmol/l)	0.36 ± 0.02	0.36 ± 0.02	0.00 ± 0.02	0.32 ± 0.02	−0.04 ± 0.02
Lutein/LDL (μmol/mmol)	0.06 ± 0.00	0.06 ± 000	0.00 ± 0.00	0.06 ± 0.00	−0.00 ± 0.00
β.Carotene (μmol/l)	0.63 ± 0.07	0.62 ± 0.05	−0.01 ± 0.05	0.59 ± 0.06	−0.04 ± 0.05
β.Carotene/LDL (μmol/mmol)	0.10 ± 0.00	0.10 ± 0.00	0.00 ± 0.01	0.10 ± 0.00	0.00 ± 0.01
Lycopene (μmol/l)	0.69 ± 0.05	0.62 ± 0.04	−06 ± 0.07	0.63 ± 0.07	−0.06 ± 0.07
Lycopene/LDL (μmol/mmol)	0.12 ± 0.00	0.11 ± 0.00	−0.01 ± 0.01	0.11 ± 0.01	0.01 ± 0.01

Note: Values are mean ± SEM; $N = 47$.
Treatment effects were examined at the end of the three different phases by one-way ANOVA ($p < 0.05$).Absolute change was calculated for each subject as follow: [Wk 4 (WD-PS, PS-esters)]-[Wk 4 (control)].

Table 6 – Plasma plant sterol, stanol, and cholesterol precursor concentrations at the end of each treatment phases.

Plant sterol variables (μmol/l)	Control	WD-PS		PS-esters	
	WK 4	WK 4	Absolute change	WK 4	Absolute change
Desmosterol	2.80 ± 0.37	2.95 ± 0.35	0.15 ± 0.33	3.06 ± 0.29	0.26 ± 0.33
Brassicasterol	0.18 ± 0.14	0.22 ± 0.09	0.03 ± 0.15	0.19 ± 0.09	0.01 ± 0.15
Lathosterol	3.69 ± 0.36	4.03 ± 0.37	0.34 ± 0.27	3.86 ± 0.36	0.18 ± 0.27
Campesterol	6.47 ± 0.62	8.94 ± 0.67	2.48 ± 0.37**	8.75 ± 0.37	2.25 ± 0.37**
Stigmasterol	0.11 ± 0.06	0.65 ± 0.28	0.54 ± 0.26**	0.30 ± 0.15	0.18 ± 0.26
β sitosterol	3.52 ± 0.47	6.06 ± 0.62	2.55 ± 0.46**	5.51 ± 0.56	2.00 ± 0.46**
Stigmastanol	0.35 ± 0.14	0.58 ± 0.46	0.23 ± 0.41	0.49 ± 0.33	0.14 ± 0.41
Ratios (μmol/mmol)					
Desmosterol/cholesterol	0.46 ± 0.06	0.51 ± 0.06	0.05 ± 0.06	0.54 ± 0.05	0.07 ± 0.06
Brassicasterol/cholesterol	0.02 ± 0.02	0.04 ± 0.02	0.13 ± 0.02	0.03 ± 0.02	0.01 ± 0.02
Lathosterol/cholesterol	0.61 ± 0.06	0.70 ± 0.06	0.09 ± 0.05	0.67 ± 0.06	0.06 ± 0.05
Campesterol/cholesterol	1.07 ± 0.10	1.56 ± 0.12	0.49 ± 0.06**	1.53 ± 0.11	0.46 ± 0.06**
Stigmasterol/cholesterol	0.02 ± 0.01	0.12 ± 0.05	0.10 ± 0.05*	0.06 ± 0.03	0.04 ± 0.05
β sitosterol/cholesterol	0.58 ± 0.08	1.05 ± 0.10	0.47 ± 0.14**	0.96 ± 0.10	0.37 ± 0.13**
Stigmastanol/cholesterol	0.06 ± 0.02	0.10 ± 0.08	0.04 ± 0.07	0.08 ± 0.06	0.03 ± 0.07

Note: Values are mean ± SEM; $N = 47$.
Treatment effects were examined at the end of the three different phases by one-way ANOVA.
Absolute change was calculated for each subject as follow: Wk 4 (WD-PS, PS-esters)]-[Wk 4 (control)].
** $p < 0.001$ compared with the control.
* $p < 0.05$ compared with control.

Table 7 – Liver function test parameters and C-reactive protein levels in response to treatment.

Parameters	Control	WD-PS		PS-esters	
	Wk 4	Wk 4	Absolute change	Wk 4	Absolute change
AST (U/l)	20.6 ± 1.33	22.5 ± 1.72	1.9 ± 1.30	21.3 ± 1.48	0.7 ± 1.30
ALT (U/l)	35.2 ± 2.71	38.1 ± 3.54	2.9 ± 3.13	40.5 ± 4.54	5.4 ± 3.13
LDH (U/l)	504.5 ± 7.96	509.7 ± 9.92	5.2 ± 11.02	514.8 ± 13.17	10.4 ± 11.02
ALKP (U/l)	80.3 ± 6.39	73.7 ± 2.52	−6.6 ± 6.93	72.8 ± 2.62	−7.5 ± 6.93
GGT (U/l)	35.3 ± 2.84	36.8 ± 3.08	1.6 ± 1.51	36.6 ± 2.82	1.5 ± 1.51
CRP (mg/l)	1.75 ± 0.35	1.50 ± 0.29	−0.26 ± 0.44	1.60 ± 0.31	−0.15 ± 0.44

Note: Treatment effects were examined by one-way ANOVA ($p < 0.05$). Values are mean ± SEM; $N = 47$.
ALKP, alkaline phosphatase; ALT, alanine aminotransferase; AST, asparagine aminotransferase
GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; CRP, C-reactive protein
Absolute change was calculated for each subject as follow: WK 4 (WD-PS, PS-esters)]-[Wk 4 (control)].

(1.9 g/d) or stanol ester (1.8 g/d) enriched diets on serum retinol, α- and γ-tocopherol, vitamin D and K concentrations, or their change relative to baseline. Similarly, Hallikainen and Uusitupa (1999) reported no changes in serum retinol concentrations after 8 wk of consumption of 2.3 g/d stanol esters, while serum β-carotene and α-tocopherol concentrations were reduced. Therefore, we can conclude that the 2-g/d dose of WD-PS or PS-esters appears to provide minimal reductions in other plasma fat-soluble components.

In the present study, consumption of both forms of PS–enriched yogurt (WD-PS and PS-esters) increased plasma campesterol and sitosterol levels. This finding is consistent with existing knowledge that an increase in PS intake promotes plasma concentrations of sitosterol and campesterol by 20–100% and 40–100%, respectively (Derdemezis, Filippatos, Mikhailidis, & Elisaf, 2010; Fransen et al., 2007; Jones et al., 2000). Furthermore, in the present study consumption of WD-PS or PS-esters showed no increase in plasma markers of cholesterol synthesis. Similar to this result, Gremaud et al. (2002) observed that lecithin-solubilized stanols, in an oil–water emulsion, decreased cholesterol absorption without a corresponding increase in cholesterol synthesis. The lack of change in cholesterol synthesis, as in this present study, however, is in contrast with the effects seen in other study (Fransen et al., 2007) where PS was incorporated into traditional matrices, such as margarine. The difference in magnitude of the change in cholesterol synthesis may also be due to the dose of PS consumed or to the frequency of PS consumption, either of which could potentially alter cholesterol synthesis to a greater or lesser degree (Rudkowska, Abumweis, Nicolle, & Jones, 2008a).

In summary, both WD-PS and PS-ester-enriched yogurt favorably modified blood lipid profiles without altering plasma liver enzymes or CRP concentrations. However, in comparison with PS-esters-enriched yogurt, consumption of WD-PS-enriched yogurt, in hypercholesterolemic subjects, not only similarly decreased total and LDL cholesterol but also decreased triglycerides and TC/HDL-C ratio. Hence, our study supports the fact that there may be added advantages of a more highly solubilized WD-PS form over traditional PS-esters in terms of overall lipid level management.

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