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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