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Effects of phytosterols on markers of inflammation: A systematic review and meta-analysis

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ABSTRACT

Background and aims: Regular intake of phytosterols (PS) is proven to dose-dependently lower LDL-cholesterol (LDL-C). Whether PS consumption can also impact low-grade inflammation is unclear. Considering the low feasibility of outcomes studies involving PS consumption, investigation of surrogate markers of atherosclerosis represents a valuable approach. This study assessed the anti-inflammatory effect of PS consumption, according to inflammatory biomarkers, mainly C-reactive protein (CRP).

Methods and results: A systematic search of *Medline, Cab Abstracts,* and *Food Science & Technology Abstracts* was conducted through January 2015. Our study selection included randomized controlled trials (RCT), involving intake of PS-enriched foods as active treatment, and measurement of plasma inflammatory biomarkers. Random-effects meta-analyses were performed using average baseline and end-of-intervention concentrations and control-adjusted absolute changes in CRP and blood lipids. There were 20 eligible RCTs including a total of 1308 subjects. The absolute change of plasma CRP levels with PS consumption was −0.10 mg/L (95%CI -0.26; 0.05), a non-significant change, and heterogeneity had borderline significance ($I^2 = 29.1$; p-value = 0.073). The absolute reduction of LDL-C was −14.3 mg/dL (95%CI -17.3; -11.3). Meta-regression analyses showed that both the dose and duration of PS intake significantly influenced the absolute changes in plasma CRP ($\beta = -0.35$, $p = 0.0255$ and $\beta = -0.03$, $p = 0.0209$, respectively).

Conclusions: In this meta-analysis, regular intake of PS-enriched foods did not significantly change CRP, whilst LDL-C concentrations were significantly reduced. Further studies with higher PS doses may provide more definite conclusions on a potential anti-inflammatory effect of PS intake.

1. Introduction

Elevated plasma concentrations of low-density lipoprotein cholesterol (LDL-C) constitute a cardiovascular risk factor, and reduction of its levels results in significant decrease in cardiovascular events and mortality [1]. Regular intake of plant sterols and stanols, which are natural compounds found in plant-based foods, often referred to as phytosterols (PS), presents a significant LDL-C-lowering effect [2,3]. Meta-analyses have demonstrated a dose-dependent LDL-C-lowering efficacy of PS [3,4], increasing up to consumption of approximately 3 g/d with an average effect of −12% [4].

Contemporary evidence has demonstrated that the decrease in cardiovascular event risk is proportional to LDL-C reduction, even beyond statin use. Indeed, the recently presented IMPROVE-IT trial has shown a superior cardiovascular benefit in post-acute coronary syndrome patients who received ezetimibe, a cholesterol absorption inhibitor, in addition to simvastatin, in comparison with those on statin monotherapy [5]. The evidence that non-statin LDL-C-lowering interventions can lower cardiovascular risk strengthens a potential decrease in cardiovascular risk with PS regular intake, a hypothesis that still warrants confirmation [6]. Despite the clear LDL-C lowering effect of regular PS intake, the safety of this intervention has been debated. One potential health concern that has been raised relates to elevated plasma PS levels following PS-enriched food consumption, considering the widespread belief that phytosterolemia (also known as sitosterolemia) associates with premature onset of atherosclerosis [7]. However, not all

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studies support a causal association between very high levels of plasma PS and premature atherosclerosis. A recent publication reported 5 patients with phytosterolemia and mutations in the ABCG5/ABCG8 transporter complex, but no evidence of cardiovascular disease [8].

Recent data also oppose a substantial increase of plasma PS after consumption of plant sterols from enriched foods. A meta-analysis including 41 randomized studies demonstrated that after intake of an average 1.6 g/d of plant sterols from enriched foods, total plasma plant sterols, although increased, persist in concentrations that are considerably lower than those observed in homozygous phytosterolemics [9].

The lack of studies on clinical hard outcomes and the concerns about possible unwanted effects of PS have motivated us to study potential non-lipid-lowering actions of PS consumption, such as vascular function effects [10] and a potential anti-inflammatory effect [11]. Low-grade systemic inflammation has been implicated in the pathophysiology of atherosclerosis, and multiple inflammatory biomarkers associate with future cardiovascular events [12]. High-sensitivity C-reactive protein (CRP), a liver-produced acute phase protein, represents the most extensively studied plasma inflammatory biomarker. CRP levels present continuous associations with subsequent risk of coronary heart disease, ischemic stroke, and vascular death [13]. Contemporary evidence has also suggested the potential usefulness of CRP as a therapeutic target. Indeed, statin use lowers both LDL-C and CRP, with its greatest benefit observed among subjects achieving the lowest levels of both biomarkers [14]. Large-scale phase III studies with agents that lead to marked reductions in CRP levels are also underway [15].

Whether PS supplementation associates with an anti-inflammatory effect is not clear. Although multiple studies have shown reduction in CRP plasma levels in humans after PS supplementation [16–19], others reported no CRP changes [20–27]. Moreover, studies examining the effect of PS intake on inflammation markers as a primary outcome are still lacking.

The objective of this study was to perform a systematic review of randomized controlled studies to assess the effect of PS intake on inflammatory markers. As part of the systematic review, we performed a meta-analysis to estimate the average effect of PS on CRP concentrations, which is the inflammation marker most frequently reported in intervention studies.

2. Methods

2.1. Search strategy

Three databases were systematically searched through January 2015: *Medline, Cab Abstracts, and Food Science & Technology Abstracts*. A search strategy was developed including the following terms: (phytosterol* or phytostanol* or plant sterol* or plant stanol* or sitosterol* or sitostanol* or campesterol* or campestanol* or stigmasterol* or stigmastanol* or brassicasterol*) and (inflammat* or CRP or C-reactive protein or hs-CRP or interleukin* or cytokine* or chemokine* or IL-6 or TNF or VCAM or ICAM or selectin*) and (trial* or stud* or intervention*). The search was limited to humans, English language publications, and intervention studies. We also used hand searching to include studies that were not captured by our search.

2.2. Study selection

The following criteria for selecting eligible studies were predefined: a) controlled human intervention studies; b) intake of PS from enriched foods or supplements as active treatment; c) absence of co-intervention from which consumption of PS-enriched foods could not be isolated; d) no special populations included (i.e. conditions primarily involving a high-grade systemic inflammatory or immune activation); e) duration $\geq$2 weeks; f) dose of PS < 10 g/day; g) no unusual formulation of PS (i.e., no ferulated PS from rice bran oil or shea nut oil); h) measurement of plasma inflammatory biomarkers; i) no duplicates.

The selection of eligible studies was performed in two phases. In the first selection phase, study titles and abstracts were screened, and those studies that clearly did not meet the predefined selection criteria were excluded, such as *in vitro* or animal studies, reviews, or studies with no PS intervention. In the second selection phase, the publications were fully read for adequate analysis concerning their eligibility.

2.3. Data extraction

The parameters extracted from the selected studies included: a) publication characteristics (author name and year of publication); b) study characteristics (parallel or crossover design, control and intervention sample sizes, study duration); c) subject characteristics (mean age, mean body mass index (BMI), gender distribution, health status of subjects); d) treatment characteristics (PS dose, form of PS (free or esterified PS), PS type (sterol or stanol), food format; e) baseline and final outcome variables (CRP, total cholesterol (TC), LDL-C, HDL-cholesterol (HDL-C), and triglycerides). As non-CRP inflammatory markers were not consistently reported, a meta-analysis using those data was not possible. Instead, reported results on non-CRP inflammatory markers were captured in the supplementary table for descriptive purposes.

A meta-analysis to estimate the average PS effects on blood lipids was also performed. Confirming the lipid-lowering effect of regular PS intake in this study would suggest that the selection of included studies is representative of the total body of evidence as summarized in previous meta-analyses [3,4]. Additionally, the concomitant examination of effects of PS intake on both lipids and on inflammation allows a comparison between these two effects.

2.4. Data transformation

To convert the unit of blood lipid concentrations and accompanying variance measures from mmol/L to mg/dL, the values of TC, LDL-C and HDL-C were multiplied by 39, and the triglyceride values by 88. CRP concentrations and variance measures were all expressed in mg/L. Because CRP data were often not normally distributed, some studies reported the CRP data using the median and the first and third quartiles, or the median and the minimum and maximum values [17,21,22,26,28–30]. To be able to use these data in our meta-analysis, we estimated, for these studies, the sample mean and SD based on the method presented by Wan et al. [31]. This method is based on the assumption that the data are normally distributed, which we know is not the case. Nevertheless, to avoid exclusion of valuable data on CRP upon PS intervention, we decided to transform the median data and to do sensitivity analysis to check whether exclusion of these data would change the outcomes of our meta-analysis.

Control-adjusted absolute changes and accompanying SEs in CRP and blood lipids were calculated based on the formulas presented previously by Ras et al. [9]. For crossover studies, changes were calculated based on the end-of-intervention data whereas, for parallel studies, data at baseline were taken into account as well.

2.5. Statistical analysis

Average baseline concentrations, end-of-intervention concentrations and control-adjusted absolute changes in CRP and blood