Original article

Potential risks associated with increased plasma plant-sterol levels

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Abstract

The consumption of plant sterols is associated with a decrease in LDL cholesterol. However, it is also associated with an increase in plasma plant-sterol (sitosterol, campesterol) levels that may be detrimental. Indeed, the genetic disease sitosterolaemia, which is characterized by elevated plasma levels of plant sterol, is associated with premature atherosclerosis. Yet, although plasma plant-sterol levels are recognized markers of cholesterol absorption, the relationship between such levels and atherosclerosis is not clear. Several studies have analysed the association between plasma plant-sterol levels and cardiovascular disease (CVD), but have found conflicting results. Although the largest prospective trials and genome-wide association studies suggest that high plasma levels of plant sterols are associated with increased CV risk, other studies have reported no such association and even an inverse relationship. Thus, the available data cannot confirm an increased CV risk with plant sterols, but cannot rule it out either. Only a prospective interventional trial to analyse the effects of plant-sterol-enriched food on the occurrence of CV events can exclude a potential CV risk linked with their consumption.

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The consumption of plant sterols is associated with a decrease in low-density lipoprotein cholesterol (LDL-C). However, their use raises concerns in the scientific and medical community because they also induce an increase in plasma plant-sterol (sitosterol, campesterol) levels that may be detrimental. Their toxicity is illustrated by the rare genetic disorder sitosterolaemia, caused by a mutation of ABCG5/ABCG8 transporters, and characterised by very high levels of sitosterol and campesterol in plasma and in atherosclerotic plaque. Such high plant-sterol levels are associated with tendon and tuberous xanthelasmas and accelerated atherosclerosis [1].

It is also well established that increased consumption of plant-sterol-enriched food induces a significant elevation in plasma plant-sterol levels, which nevertheless remain far below levels observed in sitosterolaemia [2,3]. However, the possibility that such an increase in plasma plant-sterol levels following the consumption of plant-sterol-enriched food may be harmful in the long-term cannot be totally excluded [4,5]. In fact, our group has reported on the case of a woman who developed extravascular lipid deposits (xanthelasmas) associated with increased plasma phytosterol levels 18 months after starting a regimen that included margarine enriched with plant sterols at the usual level of consumption [6]. This clinical case raises concerns over the potential cardiovascular (CV) risk after long-term consumption of these margarines. In addition, as discussed below, some but not all population-based studies have reported associations between elevated plasma concentrations of plant sterols and cardiovascular disease (CVD). Thus, the potentially detrimental CV effect of plant-sterol-enriched food is a critical issue.

1. Plasma plant-sterol concentrations and CV risk

Several studies have analyzed the association between plasma plant-sterol concentrations and CVD, but the results have been discordant. Some reported a positive association between plasma...
However, this study did not correct plant-sterol levels to account for total plasma cholesterol concentrations.

Two case-control studies in postmenopausal women also showed a positive association between plasma plant-sterol and CVD risk. In one case, plasma levels of campesterol/cholesterol and sitosterol/cholesterol ratios were independent predictors of CV events [8]. In the other, campesterol/cholesterol and sitosterol/cholesterol ratios were significantly higher (+26%, P < 0.05) in women with coronary artery disease (CAD) than in those without [9].

Another study observed that individuals with a family history of coronary heart disease (CHD) had significantly higher plasma levels of sitosterol and campesterol, and sitosterol/cholesterol and campesterol/cholesterol ratios, than those without a family history of CHD [10].

In the Prospective Cardiovascular Münster (PROCAM) study, a positive association between plasma levels of sitosterol and risk for coronary events was reported [11]. In this study, the odds ratio (OR) for coronary events was 1.8 (P = 0.014) in subjects in the highest quartile of plasma sitosterol levels than in those in the other three quartiles.

In 569 subjects from the Framingham Offspring study, plasma levels of sitosterol and campesterol were significantly higher in those with CHD and, on multivariable analyses, campesterol (OR = 2.47, 1.71–3.56) and sitosterol (OR = 1.86, 1.38–2.50) were independently associated with CVD [12].

In a case-control study involving 177 non-diabetic individuals, it was shown that the plasma campesterol/cholesterol ratio (OR = 1.60, 1.06–2.46) was independently associated with CVD [13].

Finally, the Finnish Scandinavian Simvastatin Survival Study (4S) evaluated 868 patients with CAD, and concluded that those treated with statins had higher plasma concentrations of plant sterols and also experienced a recurrence of coronary events [14].

1.1.2. Prospective studies

The MONICA/KORA study, which followed 1186 men for up to 10 years, demonstrated that the risk of CV events in individuals in the highest quartile of plasma campesterol level was three times greater (hazard ratio [HR] = 3.00, 1.16–7.78) than in those in the lowest quartile [15].

The Ludwigshafen Risk and Cardiovascular Health (LURIC) study, with a 7.3-year follow-up of 1257 individuals not treated with statins, showed that subjects from the highest tertile of campesterol/cholesterol ratio had, after adjustments for potential confounding factors, significantly higher total mortality (HR = 1.37, 1.03–1.83; P = 0.03) and CV mortality (HR = 1.43, 1.00–2.04; P = 0.05) than those in the lowest tertile [16].

1.1.3. Genetic studies

Polymorphisms in the ABCG8 gene that contribute to variations in plasma concentrations of plant sterols have been identified. In a genome-wide association study (GWAS), it was reported that certain specific ABCG8 genotypes were associated with increased plasma plant-sterol levels as well as increased CVD [17]. In this study, the association between ABCG8

Table 1
Human studies of the association between plasma plant-sterol (PPS) levels and cardiovascular disease (CVD) risk.

<table>
<thead>
<tr>
<th>Study type</th>
<th>Population studied</th>
<th>Association between PPS and CVD risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case-control</td>
<td>585 hypercholesterolaemic subjects</td>
<td>Positive</td>
</tr>
<tr>
<td>Case-control</td>
<td>48 women CAD+, 61 women CAD–</td>
<td>Positive</td>
</tr>
<tr>
<td>Case-control</td>
<td>47 women CAD+, 62 women CAD–</td>
<td>Positive</td>
</tr>
<tr>
<td>Case-control</td>
<td>26 subjects with CVD family history, 27 with no CVD family history</td>
<td>Positive</td>
</tr>
<tr>
<td>Case-control</td>
<td>159 men CAD+, 318 men CAD–</td>
<td>Positive</td>
</tr>
<tr>
<td>Case-control</td>
<td>155 subjects CAD+, 414 subjects CAD–</td>
<td>Positive</td>
</tr>
<tr>
<td>Case-control</td>
<td>Non-diabetics: 66 CAD+, 111 CAD–</td>
<td>Positive</td>
</tr>
<tr>
<td>Case-control</td>
<td>868 patients with CAD (high vs low PPS levels)</td>
<td>Positive</td>
</tr>
<tr>
<td>Prospective</td>
<td>1186 men, 10-year follow-up</td>
<td>Positive</td>
</tr>
<tr>
<td>Prospective</td>
<td>1257 subjects, 7.3-year follow-up</td>
<td>Positive</td>
</tr>
<tr>
<td>GWAS</td>
<td>13,766 subjects CAD+, 13,630 subjects CAD–</td>
<td>Positive</td>
</tr>
<tr>
<td>GWAS</td>
<td>17,121 subjects CAD+, 40,473 subjects CAD–</td>
<td>Positive</td>
</tr>
<tr>
<td>GWAS</td>
<td>2012 patients with HeFH</td>
<td>Positive</td>
</tr>
<tr>
<td>GWAS</td>
<td>9427 subjects with angiography-verified CAD</td>
<td>Positive</td>
</tr>
<tr>
<td>GWAS</td>
<td>143,677 subjects</td>
<td>Positive</td>
</tr>
<tr>
<td>Atherosclerotic lesions</td>
<td>2440 patients undergoing angiography</td>
<td>Positive</td>
</tr>
<tr>
<td>Atherosclerotic lesions</td>
<td>82 patients with aortic valve replacement</td>
<td>Positive</td>
</tr>
<tr>
<td>Atherosclerotic lesions</td>
<td>21 patients undergoing valve surgery, 10 controls</td>
<td>Positive</td>
</tr>
<tr>
<td>Atherosclerotic lesions</td>
<td>25 patients undergoing carotid endarterectomy</td>
<td>Positive</td>
</tr>
<tr>
<td>Case-control</td>
<td>2542 subjects</td>
<td>No</td>
</tr>
<tr>
<td>Case-control</td>
<td>82 subjects CAD+, 213 subjects CAD–</td>
<td>No</td>
</tr>
<tr>
<td>Case-control</td>
<td>186 subjects CAD+, 231 subjects CAD–</td>
<td>No</td>
</tr>
<tr>
<td>Case-control</td>
<td>373 subjects CAD+, 758 subjects CAD–</td>
<td>No</td>
</tr>
<tr>
<td>Case-control</td>
<td>299 subjects CAD+, 584 subjects CAD–</td>
<td>Inverse</td>
</tr>
<tr>
<td>Case-control</td>
<td>125 subjects CAD+, 1117 subjects CAD–</td>
<td>Inverse</td>
</tr>
<tr>
<td>Prospective</td>
<td>232 high-CVD-risk men, 22-year follow-up</td>
<td>Inverse</td>
</tr>
<tr>
<td>Prospective</td>
<td>623 subjects aged ≥ 75, 17-year follow-up</td>
<td>Inverse</td>
</tr>
</tbody>
</table>

CAD: coronary artery disease; GWAS: genome-wide association study; HeFH: heterozygous familial hypercholesterolaemia.

plant-sterol levels and CVD, whereas others showed no association or even an inverse relationship (Table 1).

1.1. Studies reporting a positive association between plasma plant-sterols and CVD

1.1.1. Case-control studies

The Dallas Heart Study reported, in a subgroup of hypercholesterolaemic individuals, a significant increase in CV risk in those with elevated plasma levels of either stigmasterol or campesterol, independently of plasma cholesterol levels [7].
genotypes and CVD persisted even after adjusting for age, gender and body mass index (BMI) [17]. In another GWAS, this one involving 2012 patients with heterozygous familial hypercholesterolaemia (HeFH), genetic variations in the ABCG8 gene were associated with CVD after adjusting for relevant CV risk factors [18]. Nevertheless, in this study, the variant that was weakly associated with increased CV risk (D19H) was later shown to be associated with lower levels of plasma plant-sterols, lower cholesterol absorption and higher cholesterol synthesis rate [19]. A large GWAS involving 17,121 patients with CAD and 40,473 controls demonstrated an independent association between an ABCG8 genetic variant known to be coupled with increased plasma plant-sterol levels and CVD [20]. Furthermore, some genetic variants of the ABO genes, known to be associated with increased plasma plant-sterol concentrations, were also shown to be independent predictors of CV events in both a study of 9427 individuals with angiography-verified CAD [21] and one involving 143,677 subjects [22]. Nevertheless, in these studies, the variants associated with CAD were also associated with higher LDL-C levels, which preclude assigning a causal role to phytosterol levels in the association with CAD.

As ABCG8 is a sterol exporter in enterocytes, its function influences cholesterol absorption. Indeed, plasma plant-sterol levels correlate with sterol (including cholesterol) absorption. Although the positive association between plasma plant-sterols and increased CV risk reported in the literature is apparently independent of plasma cholesterol levels, it cannot be totally ruled out that the positive association between plant-sterol concentrations and CVD might be related to an underlying causal association between increased cholesterol absorption and CVD risk.

1.1.4. Atherosclerosis studies

In the LURIC study, which included 2440 patients who underwent angiography, the campesterol/cholesterol ratio correlated positively with the severity of CAD [23]. In another study of 82 patients who underwent aortic valve replacement, those who ate plant-sterol-enriched margarine showed increased plasma concentrations and fivefold higher sterol concentrations in their aortic valve tissue [24]. In yet another study, increases in concentrations of sitosterol and campesterol in plasma and in aortic valve tissue were observed in CVD patients who were undergoing valve surgery [25].

In addition, in a study of 25 patients undergoing carotid endarterectomy, a significant positive correlation was found between the campesterol/cholesterol ratio in plasma and the campesterol/cholesterol ratio in the carotid artery wall ($r = 0.68$) [26].

1.2. Studies reporting no association between plasma plant-sterols and CVD

Some case-control studies have shown no association between plasma plant-sterol levels and CVD risk. Although the Dallas Heart Study reported a positive association in hypercholesterolaemic patients [7], no such association was found in the whole population ($n = 2542$) of that study [27]. Also, in patients with type 1 diabetes, plasma levels of campesterol in patients with CAD ($n = 82$) were no different from those in patients without CAD ($n = 213$) [28]. Likewise, the case-control CORA study found no association between plasma levels of sitosterol or campesterol and CAD [29], while the European Prospective Investigation of Cancer (EPIC)-Norfolk study also showed no correlation between plasma plant-sterols and CVD in healthy individuals [30].

1.3. Studies reporting an inverse relationship between plasma plant-sterols and CVD

1.3.1. Case-control studies

Data from the EPIC study conducted in Spain demonstrated a lower CHD risk among people who had higher plasma concentrations of plant sterols [31]. In this study, however, a higher plasma plant-sterol concentration reflected a greater consumption of healthy foods, including fruit, vegetables and polyunsaturated fatty acids, and this may have introduced a major bias.

In the Longitudinal Aging Study Amsterdam (LASA), conducted in 1242 individuals aged 65 and over, plasma sitosterol levels were associated with a reduction in CAD risk on multivariate analysis [32].

1.3.2. Prospective studies

In a Finnish prospective study following 232 men with high CV risk for 22 years, baseline plasma levels of sitosterol were significantly higher in those who survived and, on multivariate analysis, baseline sitosterol/cholesterol ratios were associated with lower total mortality [33]. However, it must be noted that the number of patients was not large enough for a prospective study and, therefore, no firm conclusions can be drawn from these data.

More recently, a prospective study of 623 subjects, aged 75 and over and followed for 17 years, showed that those with the lowest plasma sitosterol levels had the highest total mortality (OR = 1.34, 1.12–1.61), but not CV mortality (OR = 1.05, 0.77–1.45) [34].

1.3.3. Meta-analyses

Two recent meta-analyses—one based on CVD risk ratios comparing the highest vs lowest tertiles of sterol distribution, and the other based on standardized mean differences between CVD cases and controls—showed no significant association between plant sterols (either campesterol or sitosterol) and CVD risk [35]. However, the studies included in these meta-analyses were extremely heterogeneous and, therefore, their findings were prone to bias due to uncontrolled confounders. Thus, it is very difficult to draw any firm conclusions from these meta-analyses. It is important, however, to point out that the participants in the studies included in these meta-analyses did not consume plant-sterol-enriched foods and that their plasma plant-sterol concentrations were below levels observed in those who do consume such foods.

In summary, human studies that have analysed the relationship between plasma plant-sterols and CVD risk have shown
2. Plant sterols and atheroma

2.1. In vitro data

It has been shown in vitro that sitosterol promotes the production of proinflammatory cytokines (tumour necrosis factor [TNF]-α, interleukin [IL]-6, IL-1β) by macrophages [36]. In addition, campesterol reduces cholesterol efflux from macrophages [36].

2.2. Animal studies

In mice, a slight increase in plasma plant-sterols induced by a plant-sterol-enriched diet (2%) significantly reduced endothelium-dependent vasodilation [24,37]. In addition, in stroke-prone spontaneously hypertensive rats, it has been shown that a diet enriched with plant-sterol rendered erythrocyte cell membranes more rigid, which could be a factor contributing to the shortened lifespan of these rats [38]. Indeed, in the same animal model, it was shown that a diet enriched with plant-sterol induced a significant reduction in lifespan [39,40]. One study also reported that the rats’ lifespan was significantly and inversely associated with concentrations of plant sterols in the diet \( r = -0.72 \), in the liver \( r = -0.72 \) and in the brain \( r = -0.84 \) [39].

In contrast, in apoE-deficient mice, a plant-sterol-enriched diet was shown to be associated with a significant reduction in the size of aortic atherosclerotic lesions [4,41].

2.3. Studies in humans

Several studies have investigated the consequences of consuming plant-sterol-enriched margarine on endothelial function. All showed a lack of any improvement in endothelium-dependent vasodilation, although plasma LDL-C was significantly reduced. In a study of 200 hypercholesterolaemic patients receiving no lipid-lowering drugs, no improvement in endothelium-dependent vasodilation was observed after 3 months of daily intakes of plant stanol-enriched margarine, although plasma LDL-C was significantly reduced by 9% [42]. Several other studies involving adults [43,44] and children [45,46] have also reported the absence of any improvement in endothelium-dependent vasodilation after 3 months of daily intakes of plant-sterol- or stanol-enriched margarine, despite significant reductions in plasma LDL-C levels. In addition, it has been reported that daily intakes of plant-sterol-enriched margarine for 12 weeks can induce a significant reduction in brachial artery diameter [43].

Plant sterols are prone to oxidation in plasma. In fact, a significant increase in oxidized plant sterol (7α-OH-sitosterol) was found in patients with sitosterolaemia and in plant-sterol-enriched lipid emulsions [47]. Oxidized plant sterols are known to be cytotoxic to macrophages in culture [48].

In a study of 45 patients taking statins, 16 weeks of plant sterol or stanol consumption had no effect on markers of antioxidant

discrepancies. However, it should be noted that the majority of the studies, the largest prospective studies and all the GWASs indicated a positive association between plasma plant-sterol levels and CVD risk (Fig. 1).

Fig. 1. Graphic representation of human studies investigating the association between plasma plant-sterol levels and cardiovascular risk.
status, oxidative stress, endothelial dysfunction or low-grade inflammation, despite significant reductions in LDL-C [49].

3. Conclusion

Data on the association between plasma plant-sterol levels and CV risk are ambiguous. Human studies have produced conflicting results, with some indicating a positive association and others showing no association or even an inverse relationship. These discordant findings make it impossible to draw any firm conclusions, even though the largest prospective trials have suggested that high plasma levels of plant sterols increase CV risk. The available data are still not sufficient to confirm whether plant sterol consumption increases CV risk, although they do suggest the presence of such a risk and so cannot be ignored. It is for this reason that all recent reviews of the topic [5,4], and the recent Maastricht meeting on plant sterols, have concluded that only a prospective interventional trial to analyse the effects of plant-sterol-enriched food on the occurrence of CV events will be able to determine whether or not the consumption of these foods increases CV risk.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References


