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Progress and prospective of plant sterol and plant stanol research: Report of the Maastricht meeting

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A B S T R A C T

Abundant evidence over past decades shows that foods with added plant sterols and plant stanols lower serum LDL cholesterol concentrations. However, despite the overwhelming data, numerous scientific questions still remain. The objective of this paper is to summarize the considerations of 60 academic and industrial experts who participated in the scientific meeting in Maastricht, the Netherlands, on issues related to the health effects of plant sterols and plant stanols.

The meeting participants discussed issues including efficacy profiling, heterogeneity in responsiveness, effects beyond LDL-C lowering, and food formulation aspects of plant sterol and stanol consumption. Furthermore, aspects related to the potential atherogenicity of elevated circulatory plant sterol concentrations were discussed. Until the potential atherogenicity of plant sterols is resolved, based on the results >200 clinical trials, the risk to benefit of plant sterol use is favorable. Evidence on these topics in plant sterol and plant stanol research was presented and used to reach consensus where possible. It was concluded that endpoint studies looking at plant sterol and plant stanol efficacy are needed, however, there was no clear opinion on the best marker and best design for such a study. Based on the current scientific evidence, plant sterols and plant stanols are recommended for use as dietary options to lower serum cholesterol.

1. Introduction

The first expert meeting regarding the efficacy and safety of plant sterols and plant stanols was held in Stresa, Italy in 2001. As a result, a meta-analysis was published which included 41 placebo-controlled trials conducted with free plant sterols and plant stanols and plant sterols and plant stanols in ester form [1]. Consensus was reached that an intake of 2 g/d of plant sterols or plant stanols lowers serum low-density lipoprotein cholesterol (LDL-C) concentrations up to 10%, with little additional benefit at higher intakes. In the following years, several authoritative and scientific organizations recommended or approved health claims for the use of plant sterol-and plant stanol added products as part of a healthy diet to lower serum LDL–C and consequently cardiovascular disease (CVD) risk [2–8].

CVD remains the leading cause of morbidity and mortality worldwide, and the major prevention target for CVD is the reduction of LDL-C concentrations [9]. In this perspective results from all currently available intervention strategies suggest that lowering serum LDL-C concentration by diet is as valuable as lowering serum LDL-C concentration by lipid-lowering medication, e.g. HMG-CoA reductase inhibitors, to decrease the risk for a non-fatal myocardial infarct and coronary heart disease (CHD) [10,11]. The abundance of evidence that foods with added plant sterols or plant stanols lower serum LDL-C concentrations has resulted in recommendations for the inclusion of plant sterol and plant stanol added products into current dietary guidelines [2,12,13]. Plant sterols and plant stanols are common dietary components, sharing structural similarities with cholesterol. The average intake of plant sterols from habitual diets is approximately 250 mg/day, with the intake of plant stanols considerably lower, both are mainly derived from vegetable oils, grain products, nuts, seeds, fruits and vegetables. The most abundant plant sterols in the human diet are β-sitosterol, campesterol and stigmasterol, while plant stanols (the saturated derivatives) are less abundant and consist mainly of sitostanol and campestanol [14]. Humans are unable to synthesize plant sterols and plant stanols and serum concentrations are the result of intestinal absorption and biliary secretion. On average, plasma sitosterol concentrations range from 3.8 to 16.0 μM and campesterol concentrations from 6.9 to 27.9 μM [15], while plasma plant stanol concentrations vary between 0.05 and 0.3 μM in the normal population [16]. Plant sterols or plant stanol consumption reduces LDL-C concentrations by decreasing intestinal cholesterol absorption, which subsequently increases endogenous cholesterol synthesis and up-regulates LDL receptor expression [17,18]. However, recent insights also suggest plant sterols and plant stanols may also lower serum cholesterol by increased transintestinal cholesterol excretion [19].

Despite the amount of data generated over the past decades, several scientific questions still remain. Accordingly, the purpose of this review is to summarize the considerations of 60 academic and industrial experts on topics related to the health effects of plant sterols and plant stanols. These experts met in Maastricht, the Netherlands from October 30th to November 1st 2011, to discuss several specific topics related to the past, present and future of plant sterols and plant stanols in the management of cardiovascular

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health. Doses of plant sterols and plant stanols are expressed as the equivalent weights of free sterols and stanols.

2. Cholesterol lowering efficacy of plant sterols and plant stanols

Since the first Stresa meeting in 2001, numerous studies with plant sterols and plant stanols and their esters have been performed. Abumweis et al. published an updated meta-analysis of plant sterol and stanol studies in 2008 [20]. This meta-analysis identified 59 studies and demonstrated a dose–response effect with LDL-C reductions of 0.29, 0.32 and 0.42 mmol/L for plant sterol or stanol intakes of 1.5–2.0, 2.1–2.5 and >2.5 g/d, respectively. These results were in line with an updated meta-analysis by Demonty et al. published in 2009 [21]. The meta-analysis by Demonty et al. included 84 trials with 141 trial-arms and reported a mean pooled LDL-C lowering effect of −0.34 mmol/L (95%CI: −0.36 to −0.31) or −8.8% for an average plant sterol or stanol dose of 2.15 g/day. In this last meta-analysis no significant differences between the dose-response effects of plant sterols and plant stanols in the range of 0.45–9.00 g/d for a duration ranging from 21 to 182 d, were found. A non-significant 6.7% difference in the calculated maximal LDL-C lowering effect existed between plant sterols and plant stanols with a pooled calculated maximal LDL-C lowering effect for plant sterols and plant stanols of 12.3% [21]. No statistical significant difference was seen in the dose-response curves of free vs. esterified plant sterol and stanol, fat-based vs. non fat-based food formats or dairy vs. nondairy foods.

In 2010, two high- dose studies were published which evaluated the effect of daily intakes of plant stanol esters of up to 9 g/d on serum lipoprotein concentrations. Mensink et al. showed a linear dose-response relationship between plant stanol intake and LDL-C lowering, with a 17% reduction versus control in LDL-C concentration at an intake of 9 g/d [22]. A similar effect size was seen by Gylling et al., who reported a reduction of 17.4% versus control at an intake of 8.8 g/d of plant stanols [23]. In contrast, high dose studies performed with plant sterols have shown less consistent results. Clifton et al. found a reduction in LDL-C concentration of 12.4% from baseline at an intake of 6.6 g/d of plant sterol provided as esters [24], while Davidson et al. failed to find an additional LDL-C lowering effect at an intake of 9 g/d when compared to an intake of 3 g/d of plant sterol given as esters [25]. However, it must be noted that Gylling et al., Clifton et al. and Davidson et al. used differing food matrices so comparisons between the studies may have limited validity. A meta-analysis by Musa-Veloso et al. [26] included the above-mentioned high dose studies with plant stanol esters. This meta-analysis by Musa-Veloso et al. is the largest and most up-to-date meta-analysis on plant sterol or stanol consumption, including 114 trials representing 182 trial-arms and evaluating dose-response effects in LDL-C lowering separately for plant sterols and plant stanols [26]. The range of plant sterol and plant stanol intake was 0.2–9.0 g/d, while there were a limited number of studies with a plant sterol and plant stanol intake >4.0 g/d (i.e. 4 studies, 2 strata with plant sterols and 4 strata with plant stanols). These authors observed (I) that the maximal LDL-C lowering effect for plant stanols (16.4%) and plant stanol esters (17.1%) were significantly larger than the maximal LDL-C lowering effects for plant sterols (8.3%) and plant sterol esters (8.4%), and (II) that intakes of plant stanols in excess of the recommended 2 g/d dose are associated with additional and dose-dependent reductions in LDL-C concentration. In response, Demonty et al. expressed their concerns regarding the validity of drawing firm conclusions on maximal effects achievable at high doses [27]. This concern was based on (I) the fact that there are limited numbers of high dose studies available, and (II) the potential influence of the disproportionate number of low-dose plant sterol strata when compared to low-dose plant stanol strata. This concern was shared by Maki et al. in an editorial [28]. As a rebuttal, Musa-Veloso et al. reanalyzed their dose-response assessment by including the most recently published placebo-controlled plant sterol ester intervention study by Sialvera et al. [29] at that time, in which a decrease of 19.7% in LDL-C concentrations upon consumption of 4 g/d plant sterol esters was seen, and excluded 15 low-dose plant sterol strata (intake <0.8 g/d, which was the lowest dose shared amongst the plant sterol and plant stanol data sets) [30]. This re-assessment did not influence their findings, namely a significantly greater relative maximal LDL-C lowering effect for plant stanols relative to plant sterols as well as for plant stanol esters relative to plant sterol esters. Besides the meta-analysis by Musa-Veloso et al., Talati et al. [31] also aimed to evaluate the comparative efficacy of plant sterols versus plant stanols. The meta-analysis included 14 blinded (13 double, 1 single) randomized controlled trials that evaluated the effect of plant sterols versus plant stanols side-by-side in healthy or hypercholesterolemic patients ($n = 531$). The range of plant sterol and plant stanol intake was 0.6–2.5 g/d and within this range, no statistical or clinical difference in LDL-C lowering effect was identified between plant sterols and plant stanols [31]. This latter finding is in line with the meta-analysis by Muso-Veloso who also showed that only at higher intakes could a difference between the effect of plant sterols and plant stanols be identified, whereas at lower intakes effects are more comparable.

Conclusions cannot be drawn regarding the relative efficacy of plant sterols and plant stanols at higher doses before trials specifically designed to answer this question are conducted. In these trials the effects of high dose plant sterol versus plant stanol intakes should be compared head-to-head. The question as to whether a clinical role exists for plant sterols and plant stanols at intakes higher than currently advised (>2 g/d) was actively discussed. From clinical point of view, if indeed a larger LDL-C lowering effect can be reached with higher intakes of plant sterols and plant stanols this would be highly attractive as every additional degree of decrease in LDL-C is beneficial in terms of reducing CVD risk [10,11]. Besides the clinical benefit of high dose plant sterol and plant stanol added products, the practical implications such as cost-benefit ratio and compliance of the consumers must be considered. Nevertheless, the currently advised intake of plant sterols and plant stanols is 2 g/d, however EFSA recently approved a health claim for plant sterols and plant stanols added products at a dose of 3 g/d [7] and slightly increasing this dose up to 4 g/d would be feasible.

2.1. Conclusion

At doses up to 3 g/d plant sterols and plant stanols have been shown to have equal cholesterol lowering efficacy [7,31]. Recent studies indicate a potential additional LDL-C lowering effect of plant sterols and stanols at higher dosages (>2 g/d) however, more studies are needed to confirm the additional effect before recommendations for higher intakes can be made.

2.2. Recommendation

Clinical trials comparing the cholesterol lowering efficacy of plant sterols and plant stanols at doses above the 3 g/d are needed to support their equal and additional efficacy at higher intakes.

3. Do the effects of plant sterols and plant stanols differ between populations?

Intervention studies evaluating effects of plant sterols and plant stanols on serum cholesterol concentrations have been carried out

not only in healthy volunteers but also in different diseased populations, such as type 2 diabetic (T2DM) patients, metabolic syndrome (MetS) patients and familial hypercholesterolemia (FH) patients. The observed effects in these populations are of interest, not only to optimize clinical utility, but also to investigate mechanisms underlying phenotypic responses. At least 10 studies have evaluated the effects of plant sterols and plant stanols in T2DM and MetS patients. In 2009, Baker et al. performed a meta-analysis of five randomized, placebo-controlled trials to evaluate the effect of plant sterol and stanol consumption on serum lipoprotein concentrations in patients ($n = 138$) diagnosed with T2DM [32]. Plant sterol or stanol consumption (1.6–3.0 g/day) significantly reduced total and LDL-C concentrations by 0.26 and 0.31 mmol/L respectively, which is comparable with reductions seen in healthy subjects. Studies conducted with plant sterols and plant stanols in MetS patients show less consistent results. While some studies have provided substantial evidence that both plant sterols and plant stanols are able to lower serum total cholesterol and LDL-C concentrations in MetS patients [29,33,34], studies have also failed to find reduced LDL-C concentrations upon plant sterol consumption [35,36]. Taken together, studies performed in T2DM and MetS patients show large variability in extent of LDL-C lowering (see Table 1), with reductions ranging from 0% to 20% [37]. At an average intake of 1.85 g/d of plant sterols and plant stanols, the average LDL-C reduction in T2DM patients is 0.23 mmol/L (6.3%) and an intake of 3.24 g/d of plant sterols and plant stanols results in an average LDL-C reduction of 0.64 mmol/L (15.6%) in MetS patients. In comparison, in healthy subjects, the mean LDL-C reduction after consuming 1.85 g/d of plant sterols and plant stanols is 0.34 mmol/L (8.6%) and after consuming 3.24 g/d of plant sterols and plant stanols, LDL-C concentration is reduced by 0.40 mmol/L (11.0%) [21]. This shows a somewhat smaller response in T2DM patients and a larger response in MetS patients as compared to effects observed in healthy controls. In addition, reductions in TAG concentration are larger in MetS patients than in T2DM patients. The less consistent results in trials involving MetS patients might be ascribed to the broad range in criteria that define the metabolic syndrome [2,38]. Due to this broad range in definition, patients can be diagnosed with the MetS while having differing metabolic characteristics, which could modify LDL-C lowering efficacy. It is a challenge to identify which characteristics within the diversity in MetS patients might determine responsiveness.

MetS and T2DM patients are typically classified as having low absorption and high synthesis of cholesterol, based mainly on non-cholesterol sterol measurements. Lathosterol concentrations reflect absolute cholesterol synthesis, while sitosterol, campesterol and cholestanol concentrations are surrogate markers of dietary cholesterol absorption. Only a few studies have actually measured absolute cholesterol synthesis and absorption using direct methods, i.e. sterol balance and dual isotope techniques [39]. According to a review by Miettinen et al., surrogate markers do not adequately reflect cholesterol metabolism in all circumstances and conditions, so caution is warranted when assuming non-cholesterol surrogate markers are self-evident [40]. In addition, reciprocity appears to exist between cholesterol synthesis and cholesterol absorption in healthy subjects, but this relationship is less clear in diseased populations where the connection between cholesterol synthesis and absorption may become dysfunctional [40]. This raises the question of the value of surrogate markers in diseased populations. Based on surrogate markers alone, MetS and T2DM patients may be falsely classified as low-absorbers, whereas the observed high responsiveness towards plant sterols and plant stanols suggests otherwise. In addition, the measurement of serum non-cholesterol sterols and stanols is not standardized or quality-controlled in general, which impairs the reliability of the measurements and further the interpretation of the results.

Few studies have evaluated the LDL-C lowering capacity of plant sterols and plant stanols in FH patients. FH is characterized by very high serum LDL-C concentrations and a high incidence of premature CHD, therefore, treatment modalities at least in adolescents and adults are based on cholesterol lowering drugs. However, FH patients might also benefit from foods with added plant sterols or plant stanols as adjunctive therapy to their current treatments. In 2006, a systematic review was conducted of four randomized controlled trials in which the primary objective was to evaluate the effects of plant sterols and plant stanols on serum lipid concentrations in heterozygous FH patients ($n = 124$) [41]. The duration of

Table 1
Characteristics of studies conducted with plant sterols/stanols in T2DM and MetS patients.

Reference	Patients (n)	Design	Duration	Plant sterol/stanol dose[a] g/d (matrix)	Change in TCH (mmol/L)[b]	Change in LDL-C (mmol/L)[b]	Change in HDL-C (mmol/L)[b]	Change in TAG (mmol/L)[b]
Gylling and Miettinen, 1994	T2DM (11)	RC	6 weeks	Stanols: 3.0 (spread)	−0.36, −5.8%	−0.37, −9.3%	0.11, +10.7%	−0.06, −1.3%
Gylling and Miettinen, 1996	T2DM (8)	PA	7 weeks	Stanols: 3.0 (spread)	−0.7, −10.5%	−0.6, −14.3%	−0.05, −2.1%	No change
Lee et al., 2003	T2DM (81)	PA	12 weeks	Sterols: 1.6 (spread)	−0.12, −1.9%	−0.10, −2.3%	0.03, +2.4%	−0.17, −8.5%
Lau et al., 2005	T2DM (14)	RC	3 weeks	Sterols: 1.8 (spread)	−0.36, −6.2%	−0.84, −25.9%	0.10, +7.3%	0.53, +19.6%
Yoshida et al., 2006	T2DM (13)	RC	3 weeks	Sterols: 1.8 (bar)	−0.07, −1.3%	−0.08, −2.2%	No change	−0.06, −2.8%
Plat et al., 2009	MetS (18)	PA	9 weeks	Stanols: 2.0 (yoghurt drink)	−0.81, −10.9%	−0.66, −11.7%	0.05, +4.3%	−0.46, −31.7%
Gagliardi et al., 2010	MetS (35)	PA	5 weeks	Sterols: 2.4 (spread)	−0.29, −5.4%	−0.49, −15.1%	−0.18, −15.9%	No change
Hernández-Mijares et al., 2011	MetS (24)	PA	12 weeks	Sterols: 2.0 (low-fat milk)	0.12, +2.0%	−0.02, −0.5%	0.03, +2.7%	0.25, +16.0%
Sialvera et al., 2011	MetS (108)	PA	8 weeks	Sterols: 4.0 (yoghurt drink)	−1.01, −16.3%	−0.83, −19.8%	No change	−1.11, −22.1%

	Average plant sterol/stanol dose (g/d)	Average response			
		Change in TCH (mmol/L)	Change in LDL-C (mmol/L)	Change in HDL-C (mmol/L)	Change in TAG (mmol/L)
T2DM patients					
Non-weighted	2.24	−0.32, 5.1%	−0.40, −10.8%	0.04, +3.7%	0.05, +1.4%
Weighted by number of subjects	1.85	−0.20, 3.2%	−0.23, −6.3%	0.04, −3.1%	−0.06, −3.7%
MetS patients					
Non-weighted	2.60	−0.50, 7.7%	−0.50, −11.8%	−0.03, −2.2%	−0.33, −9.5%
Weighted by number of subjects	3.24	−0.71, 11.3%	−0.64, −15.6%	−0.03, −2.2%	−0.66, −13.9%

RC, Randomized crossover design.
PA, Parallel arm controlled design.
[a] Dose is expressed as the equivalent weights of free sterols and stanols.
[b] Changes are expressed vs. placebo.

the studies included ranged from 4 to 12 wk and spreads with added plant sterols and plant stanols were administrated with an average dose of 2.3 g/day. Compared to placebo, serum LDL-C concentrations were decreased by 10–15% with a mean decrease of 0.64 mmol/L (95% CI −0.86, −0.43 mmol/L). Heterozygous FH patients need to reduce their LDL-C by at least 40% to reach target concentrations [41]; indicating that plant sterol or stanol intake alone is not sufficient to reach cholesterol targets. Plant sterol or stanol intake does offer an additional effect to the cholesterol lowering strategies in the treatment of FH patients, which is particularly interesting in FH children since lipid-lowering medication is not advised at a young age.

3.1. Conclusion

Plant sterol and plant stanol added products have been shown to be effective in many different disease populations such as individuals with T2DM, MetS or FH adults and children. However, the evidence for equivalent efficacy in these disease populations compared to the general population is still limited by the relatively small number of studies, with variable doses and treatment durations, conducted in each of these populations.

3.2. Recommendation

Further research into the use of plant sterol and plant stanol products in T2DM, MetS and FH is recommended.

4. Triglyceride lowering effect of plant sterols and plant stanols

Elevated serum LDL-C, although the most important, is not the only blood lipid related risk factor for CVD. A meta-analysis of 29 Western prospective studies including 10,158 CVD cases in 262,525 participants indicated that elevated fasting TAG concentrations are an independent risk factor for CVD [42]. High TAG concentrations are common with approximately one-third of the US population having increased fasting TAG concentrations (>1.7 mmol/L) [43]. Therefore a need exists for TAG-lowering strategies. Most intervention studies have reported that plant sterols and plant stanols do not affect HDL-C or TAG concentrations. Some studies, however, have found slight changes in HDL-C or TAG, which have generally been attributed to other (dietary) factors and not due to plant sterol or stanol intake [44]. In 2008, Naumann et al. performed a meta-analysis examining the effects of subject baseline characteristics on serum lipoprotein response to plant stanol consumption [45]. This analysis revealed a relationship between plant stanol intake and TAG-lowering, with larger TAG-lowering seen in subjects with higher baseline TAG concentrations. Two studies specifically designed to investigate TAG-lowering by plant stanols were performed in metabolic syndrome and hypertriglyceridemic patients with elevated TAG concentrations at baseline and reductions of 27% and 11% were found, respectively [46,47]. Demonty et al. performed a meta-analysis of 12 randomized controlled trials evaluating the effects of plant sterols on TAG concentrations and showed that plant sterol intake in the 1.6–2.5 g/d range significantly lowered TAG by 6.0% [48]. Larger reductions in absolute TAG reductions achieved with plant sterol consumption were observed with higher TAG concentrations at baseline [48]. The postulated mechanism by which plant sterols and plant stanols might exert their TAG-lowering effect is through lowered hepatic production of large TAG-rich VLDL-1 particles [33]. Some suggestion exists from animal studies that the reduced TAG concentration induced by plant sterols and plant stanols coincides with increased fecal fat excretion [49], but the exact mechanism producing the TAG-lowering is yet unknown. The potential TAG-lowering effect of plant sterols and plant stanols would be of great benefit for populations with elevated fasting serum TAG concentrations, especially MetS patients, who often suffer not only from elevated LDL-C but also elevated TAG concentrations.

4.1. Conclusion

As many plant sterol and plant stanol studies exclude subjects with high TAG concentrations, the TAG-lowering potential of plant sterols and plant stanols may have been overlooked. TAG-lowering by plant sterols and plant stanols appears to be greater in individuals with higher baseline TAG concentrations. The exact mechanism by which plant sterols and plant stanols lower is yet unknown.

4.2. Recommendation

The potential TAG-lowering effect of plant sterols and plant stanols deserves further investigation, especially potential TAG-lowering effects in the postprandial condition, since postprandial TAG concentrations are emerging as an important CVD risk factor [50].

5. Heterogeneity in LDL-C responsiveness to plant sterols and plant stanols

Evidence from available meta-analyses consistently show that plant sterols and plant stanols yield a mean reduction in LDL-C concentrations of approximately 10% at doses around 2 g/day [1,20,21,26]. However, considerable variability in mean LDL-C lowering can be seen at the same dosage across trials. This variability has, among others, been attributed to differences in consumption frequency [51,52], product matrix [53], and consumption timing [54]. The range of variability seen in LDL-C lowering between participants in the same study population following the same study regimen is also very large [55–57]. Rideout et al. reported an LDL-C lowering response range from −40 to +20% after providing 1.95 g of plant sterols per day to 56 mildly hypercholesterolemic participants for 4 weeks [55]. This considerable variability in individual responsiveness to plant sterol consumption could in part be due to variable compliance; however compliance is less of a factor in trials where plant sterol or stanol consumption is supervised.

Response to plant sterols and plant stanols has shown to be affected by specific characteristics of cholesterol metabolism that differ between individuals. Fuentes et al. showed that plasma sitosterol to cholesterol ratio, a marker of cholesterol absorption, at baseline in 30 participants with familial hypercholesterolemia (FH) predicted cholesterol lowering potential of plant sterol esters [58]. Individuals with higher sitosterol to cholesterol ratios had increased LDL-C lowering in response to plant sterol consumption. Casas-Agustench et al. also showed the same relationship between high cholesterol absorption and enhanced LDL-C lowering in response to plant sterol intervention in hypercholesterolemic individuals [59]. Low serum lathosterol, a precursor of cholesterol and a surrogate marker of cholesterol synthesis, at baseline was associated with enhanced LDL-C lowering [60]. Moreover, individuals with higher serum lathosterol concentrations, indicative of higher cholesterol synthesis, demonstrated reduced LDL-C lowering than those with low baseline lathosterol concentrations. Thuluva et al. demonstrated that the plasma ratio of lathosterol to campesterol, which would reflect overall cholesterol metabolism, predicts response to plant stanol consumption [61]. Individuals with higher lathosterol to campesterol, suggesting greater cholesterol synthesis

and lower absorption, showed a diminished degree of cholesterol lowering following plant stanol consumption. These findings using non-cholesterol sterol surrogates of cholesterol metabolism lend support to results using direct stable isotope measurements. Rideout et al. showed that responders to plant sterol intervention, those with a >5% LDL-C reduction, had significantly lower fractional cholesterol synthesis (FSR) than individuals with a <5% reduction in LDL-C in a retrospective clinical analysis of 3 trials that used deuterium incorporation as a direct measure of cholesterol synthesis [62].

Unfortunately, as yet the impact of dose has not been evaluated with respect to responsiveness to plant sterol or stanol consumption. Individuals with lesser response in trials using the typical 2–3 g dose range may respond to a higher dose. The plant sterol and stanol response phenotype could represent a threshold of plant sterols and plant stanols required for optimal cholesterol lowering effect. This potential variability in threshold dose for cholesterol lowering could be responsible for much of the variability in dose-response seen across meta-analyses [21,26] where more individuals respond, yielding a greater mean cholesterol lowering, in trials with higher doses of plant sterols and plant stanols.

Rudkowska et al. demonstrated that the extent of response to plant sterol consumption is reproducible within participants in a crossover trial with multiple plant sterol treatment phases where plant sterol or placebo products were consumed under supervision [56]. This reproducibility of plant sterol response has also been seen in trials without supervised dietary consumption [59]. The correlation of LDL-C lowering between repeated plant sterol intake regimens within the same individual demonstrates that response may be genetically influenced. Variations in genes such as ABCG5 and G8, NPC1L1, APOA4, SR-BI, HMG-CoAR, CETP, APOE and CYP7A1 have all been investigated in relation to extent of cholesterol lowering response to plant sterols and plant stanols [56,63–66]. As yet very few trials have demonstrated significant associations between a single nucleotide polymorphism (SNP) in a gene and serum LDL-C lowering response to plant sterol or stanol consumption. Zhao et al. showed that the ABCG8 T400K polymorphism associated with plant sterol response in individuals with high basal plant sterol concentrations in a 4 week randomized crossover trial in 82 participants [63]. However, the same T400K polymorphism in ABCG8 was found not to be associated with plant sterol or stanol response in studies by Plat et al. [64] in an 8 week parallel arm trial with 112 participants, by Rudkowska et al. [56] in a 4 week randomized crossover in 26 participants and by Gylling et al. [66] in a 1 year parallel arm trial with 282 participants. De Castro-Oros et al. [65] showed that a variation (−204A > C) in the promoter region of the CYP7A1 gene, which encodes for the rate limiting enzyme in the synthesis of bile acid from cholesterol, was associated with response to plant sterol consumption. Compared to AA carriers of the variant, C-carriers showed significantly higher reductions in LDL-C (0.13 vs. 0.43 mmol/L) in response to plant sterol consumption. The C-variant was associated with a 78% increase in CYP7A1 expression in HepG2 cells. It was hypothesized that the C-variant increases the size of the bile acid pool which enhances cholesterol absorption by improving the capacity to form micelles in the intestinal lumen [65].

If genetics dictate response it is unlikely that a single SNP will have an unequivocal impact on the dietary responsiveness to plant sterol or stanol consumption. Polymorphisms in several genes considered together may be needed to explain the variance.

5.1. Conclusion

There is evidence demonstrating that plant sterol and plant stanols have a higher efficacy in individuals with a high absorption, low synthesis of cholesterol phenotype.

Cholesterol lowering response to plant sterol and plant stanol consumption has been related to genetic variants in only a few studies. The degree to which genetics influence observed heterogeneity in LDL-C lowering between participants has not yet been fully accessed.

5.2. Recommendation

Clinical trials assessing the impact of dose on responsiveness to plant sterols and plant stanols should be undertaken, specifically looking at higher plant sterol or stanol doses in individuals who have previously not responded to lower doses.

Collaborative multicenter retrospective clinical analyses looking at genetics in combined study populations where plant sterol or stanol response phenotypes have already been established should be conducted to increase the potential of identifying SNPs associated with response. In this respect, trials with prospective recruitment for genotypes or phenotypes of interest should also be undertaken, to assess gene associations *a priori* rather than *post-hoc*. The use of emerging technologies including gene arrays and eventually exome or whole genome sequencing will be the key to elucidating the true impact of genetics on response to plant sterols and plant stanols. Outcomes of genetic studies largely depend on the definition of the clinical phenotype; therefore, care must be taken to accurately define response. Crossover trials with repeated plant sterol or stanol consumption phases and/or trials where consumption is supervised are critical in minimizing the potential of non-compliance contributing to the non-response phenotype.

6. Formulation and matrixing issues in relation to efficacy

Although it was originally felt that the presence of fat was required for maximum efficacy of esterified plant sterol and stanol products, subsequent studies with low fat and no fat foods, liquid or solid foods, and free or esterified plant sterols or plant stanols have shown similar effects on LDL-C. The Demonty et al. meta-analysis [21], also confirmed this observation. In addition, a number of studies even demonstrated that plant sterol and plant stanol tablets and capsules exert a similar LDL-C lowering effect as can be reached with plant sterol or plant stanol added foods [67,68].

Many protocols exist for the preparation of plant sterols or stanols with a microcrystalline structure, however there are no data in the literature describing the relationship between crystal size and cholesterol lowering efficacy. Studies have been published in which no LDL-C lowering effect was found [69,70], and it is speculated that this lack of effect may have been caused by the formation of crystalline sterols, which were unable to compete for incorporation into mixed micelles, in the preparations used. If true, this indicates that it is essential that the sterols and stanol are available for incorporation into mixed micelles starting in the stomach and continuing in the very upper part of the GI tract [68].

Besides matrices, also timing and frequency of intake are important when evaluating the LDL-C lowering efficacy of plant sterols and plant stanols. Some but, not all evidence points towards enhanced efficacy of plant sterols and plant stanols consumed at multiple time points [21], as compared to once per day [51,52]. When consumed once a day, the timing of plant sterol or stanol consumption may be essential in determining efficacy. It seems that plant sterols and plant stanols should be consumed during or after a meal, preferably a larger meal such as lunch or dinner in which bile flow is triggered [20]. Consumption of plant sterols and plant stanols with a meal is more relevant for some matrices, e.g. spreads vs. beverages, as for instance consumption of spreads is more likely to coincide with meals than beverages.

Plant sterols and stanols are typically added to foods or beverages as either sterol esterified to fatty acids or free sterols. In the above-mentioned meta-analysis by Demonty et al. [21] it was concluded that no difference exists in LDL-C lowering efficacy between free plant sterols and plant stanols and their esterified forms. Little attention has been paid to the actual fatty acid used for esterification, at least one study in hamsters shows that stearate is superior to the more commonly used linoleate or oleate [71], and sterol stearates have been also shown to be effective in humans with 11% LDL-C lowering with 1 g three times/day [60], but not to any greater extent than seen in other studies with unsaturated fatty acid esters of sterols. Although most sterol and stanol esters are effectively hydrolysed in the small intestine [72] there may be a difference in rates of hydrolysis between different sterols [73,74] and between different esters [73]. However, whether these observed differences are clinically significant remains to be seen. Whether non-hydrolysed sterol esters are active is not clear but Rasmussen has suggested this as a possibility [71]. Free plant sterols and plant stanols may have greater ability to incorporate into mixed micelles and therefore have greater cholesterol lowering efficacy when particle size is reduced, for example by sub-crystalline formulations or by an emulsified formulation, such as incorporation into lecithin [68,75].

Over 200 different plant sterol species with 4 types of conjugates exist: the 3β-OH group is esterified to a fatty acid or a hydroxycinnamic acid (e.g. ferulic acid-gamma oryzanol from rice), or glycosylated with a hexose (usually glucose) or a 6-fatty-acyl hexose. Rice bran oil possesses a complex mixture of plant sterols, with most studies performed using rice bran oil showing reductions in LDL-C concentrations [76,77] when the oil is rich in desmethyl sterols (e.g. sitosterol and campesterol ferulate) with no effect of dimethylsterols (cyloartenol) [78].

Moreau et al. assessed the extent of hydrolysis of common plant sterol conjugates, including hydroxycinnamate (ferulic acid) sterol esters in corn fiber oil and rice bran oil, and concluded that these compounds are cleaved by cholesterol esterase [79]. However, additional information concerning the cleavage of plant sterol conjugates deserves further investigation. Regarding matrices and formulations, the FDA has stated that plant sterol and plant stanol added foods are allowed to possess health claims for esterified and free forms, while plant sterol and plant stanol supplements are only allowed to use health claims if they are in esterified form [80].

6.1. Conclusion

Although potential differences exist between types of free plant sterol as well as ester forms, there are no obvious clinical differences in LDL-C lowering except for the 4,4 dimethylsterols which are ineffective [78], as are the triterpene alcohols from shea nuts [81]. Plant sterol and plant stanol formulations have been shown to be effective in higher fat, low fat and fat free foods, as well as in both liquid and solid food formats. However, there are few head-to-head studies comparing differing formats as they relate to relative cholesterol lowering efficacy.

6.2. Recommendation

More head-to-head comparisons of plant sterol or stanol food formats and matrices are needed to elucidate the full impact these on LDL-C lowering ability of plant sterol and stanol added foods and supplements. Particle size of free plant sterol or stanol preparations is one aspect which has not yet been fully explored.

Food formulation and matrixing must be done in such a way as to maximize the ability of the product to deliver the plant sterol or plant stanol into the upper part of the GI track in a format where it can be incorporated into mixed micelles and bring about cholesterol lowering.

Therefore, in all future scientific publications of studies it is important that detailed information (source and type of sterol, use of emulsifiers, and particle size) concerning formulation be provided in order to identify how differences in formulation may alter the potency of the plant sterol or stanol preparation under investigation.

7. Effects of plant sterols and plant stanols beyond improving lipid metabolism

7.1. Alzheimer's disease

Because of the clear role of ApoE4 in Alzheimer's disease and cognitive decline disturbances in cholesterol metabolism (including familial hypercholesterolemia) have been suggested to be related to the pathogenesis of the disease [82,83]. However, people with phytosterolemia, a rare genetic disorder that is characterized by extremely elevated plasma plant sterol concentrations, do not appear to have an increased rate of cognitive decline, though no formal study looking at cognitive function and phytosterolemia has been done. In the ABCG5−/− phytosterolemic mouse model fed a normal chow diet, it was shown that serum and brain plant sterol concentrations increased 35–70-fold and 5–12-fold, respectively [84]. However, despite this increase in plant sterols and decreases in desmosterol and 24(S)–OH–cholesterol in the hippocampus, no differences were found between the ABCG5−/− and wild type mice in terms of memory function, anxiety or mood-related behavior [84]. In humans, the effect of long-term plant sterol and plant stanol consumption on neurocognitive functioning has also been investigated [85]. Participants consuming 2.5 g/d of plant sterols or plant stanols for 85 weeks underwent cognitive assessment before and after the intervention. Despite a significant increase in serum concentrations, neither plant sterol nor plant stanol intake affected cognitive performance, which included memory function similar to the data in ABCG5−/− mice. Another human study compared cerebrospinal fluid (CSF) and plasma plant sterol concentrations between 67 AD patients and 29 age- and gender-matched controls [86]. This study showed that patients with AD had decreased CSF brassicasterol concentrations compared to control participants. No differences were found in other plant sterols.

7.2. Cancer

The potential effects of plant sterols and plant stanols on cancer, in particular the potential of plant sterols to decrease oxidative stress and/or increase apoptosis, are being investigated [87]. A study carried out in 463 newly diagnosed primary lung cancer patients and 465 hospitalized controls showed that plant sterol consumption, from foods without added plant sterols, was associated with a reduced risk of lung cancer [88]. Plant sterol intake was shown to be negatively associated with stomach cancer by the same group [89], while a reduced risk of developing ovarian cancer was reported at higher intakes of stigmasterol, compared to lower intakes [90]. On the other hand, Normen et al. failed to show any association between a high dietary intake of plant sterols and plant stanols and the risk of either colon or rectal cancer in a Dutch cohort study [91].

The possible anti-carcinogenic effect of plant sterols could be explained via multiple mechanisms of action, including inhibition

of tumor growth and tumor metastasis, as well as promoting cellular apoptosis [87]. *In vitro,* plant sterols inhibit growth of colon cancer cells [92], breast cancer cells [93] and prostate cancer cells [94] at physiological concentrations. In addition, plant sterol-treated breast cancer cells showed reduced invasiveness, suggesting a reduction of metastatic ability [95]. Moreover, Awad et al. replicated these findings *in vivo* by showing that plant sterols inhibit tumor growth and reduce tumor metastasis in human breast and prostate cancer cell lines grown in a severe combined immunodeficiency (SCID)-mouse model [96,97]. Besides these effects on cell growth and metastasis, plant sterols have been shown to induce apoptosis in cancer cell lines, which is an important mechanism in the inhibition of carcinogenesis [98].

7.3. Conclusion

Thus both animal and human data are consistent in showing no relationship between brain plant sterol levels and cognitive function. At present clear evidence confirming the benefit of plant sterols on cancer prevention is lacking.

7.4. Recommendation

The potential benefit of plant sterol containing foods on cancer could be elucidated by examining the use of these foods in ongoing cancer cohorts such as European Prospective Investigation into Cancer and Nutrition (EPIC) but these data have not yet been gathered or published.

8. Plant sterols and atherosclerosis

Plant sterols naturally occur in diet and are consumed in the range of 0.2–0.4 g/d in typical Western diets [14]. Some vegetarian diets possess upwards of 0.7 g/d [99] and analysis of ancestral diets suggest that humans may have consumed as much as 1 g/d of plant sterols [100]. In healthy adults without consumption of foods with added plant sterols or plant stanols serum plant sterol range from 3.8 to 27.9 μM [15], while plasma plant stanol concentrations vary between 0.05 and 0.3 μM in the normal population [16]. The typical daily intake of plant sterols and plant stanols recommended for cholesterol lowering is 2 g/d, an amount unattainable without consumption of foods with added plant sterols or plant stanols. Concerns have been raised that plant sterol intake exceeding what is possible to consume with normal diets is approaching a pharmacological supplementation rather than a nutritional intervention. As such, the safety of increased plant sterol intakes and subsequent increased serum plant sterol concentrations is still hotly debated [101–103]. It has been demonstrated that during customary plant sterol added margarine consumption, the serum plant sterol concentrations increased from 19 to 30 μM with no change in serum plant stanol values, whereas during customarily used plant stanol added margarine, the serum plant stanol concentrations increased from 0.2 to 0.7 μM, but serum plant sterols decreased by 16–23% [104].

Safety concerns regarding elevated plant sterol concentrations in blood stem from characteristics observed in patients suffering from phytosterolaemia, a rare autosomal recessive genetic disorder that results in an inability to effectively clear absorbed plant sterols and plant stanols from blood and tissues [105]. The inability causes circulating and tissue plant sterol concentrations to increase 10–25 times above normal up to 0.2–1.6 mM [105]. Serum plant stanol levels are also elevated in phytosterolemics [106]. However, during consumption of plant sterol added foods the circulating plant sterol concentrations are in the 19–30 μM range [104], much lower than in phytosterolemia. Phytosterolemia has been linked to mutations in the ABGC5 or ABGC8 genes which are responsible for the export of sterols from the intestines and liver into the lumen and bile, respectively [107]. Phytosterolemics can often develop xanthelasma and/or tendon xanthomas, and experience early malignant atherosclerosis [108]. Mildly elevated cholesterol concentrations can also be seen in individuals with phytosterolemia [14]. CVD risk is severe in phytosterolemia since myocardial infarctions and death have been attributed to phytosterolemia in individuals as young as 3 years of age [67].

Serum plant sterol concentrations in normolipidemic individuals vary considerably and are highly heritable [109,110]. The high circulating plant sterol concentrations and premature CHD and death seen in phytosterolemia has led to the investigation of the association between plant sterol concentrations in the normal range and CHD risk. These studies have recently been summarized by Baumgartner et al and divided into different categories of study designs, i.e. prospective cohort studies and case–control studies [111]. Briefly, Glueck et al. [112] were the first to suggest that elevated plant sterol concentrations in the normal range may be associated with increased CHD risk. This suggestion was based on campesterol and total plant sterol concentrations correlating positively with cholesterol concentrations, and high campesterol in the top quintile of the 3472 hypercholesterolemic patients being associated with increased personal or familial CHD. This link between elevated plant sterol concentrations and atherogenesis has since been supported by Sutherland et al. [113], Rajaratman et al. [114], Sudhop et al. [115], Assmann et al. [116], Matthan et al. [117], Silbernagel et al. [118,119] and Weingartner et al. [120] in various studies using a range of methods to access CHD risk or mortality. However, not all studies have shown an association between circulating plant sterol levels and the risk of CHD. Wilund et al. [121], Pinedo et al. [122], Windler et al. [123], and Miettinen et al. [124] failed to identify an association between plant sterol concentrations and CVD or CVD risk. Fassbender et al. [125], Strandberg et al. [126], Escurriol et al. [127], and Weingartner et al. [128], reported data which suggested atheroprotective associations with higher plant sterol concentrations. The mixed data available to date do not provide a clear link between circulating serum plant sterol concentrations in the normal range and the development of cardiovascular disease. Moreover, Genser et al. [129] conducted a meta-analysis looking at the relationship between serum plant sterols on cardiovascular risk. For this purpose, 17 studies were identified involving 11,182 participants reporting either risk ratios (RR) of CVD in relation to plant sterol concentrations (8 studies) or standardized mean differences in plant sterol concentrations between CVD cases and controls (15 studies). The meta-analysis provides no evidence of an association between serum concentrations of plant sterols and risk of CVD [129]. Interestingly, if the difference in mean serum plant sterol concentrations in each of the tertiles is compared, it can be concluded that there is no relation with CVD risk over at least a three-fold range difference in serum plant sterol concentrations. This is an important observation given the fact that serum plant sterol concentrations at the utmost double during consumption of plant sterol added foods.

The potential relationship between serum plant sterol concentrations and the risk of CVD has also been investigated using Mendelian randomization. A genome wide association study identified 2 SNPs in ABCG8 and 1 SNP in ABO which were significantly associated with serum campesterol, sitosterol and brassicasterol concentrations and irrespective of serum cholesterol level [130].

The SNP rs41360247 in ABCG8 was associated with decreased plant sterol concentrations and ratios to cholesterol and SNPs rs4245791 in ABCG8 and rs657152 in ABO were associated with increased plant sterol concentrations and ratios to cholesterol. These SNPs were then associated with coronary artery disease (CAD) in 13,764 cases and 13,630 controls. The SNPs showed a similar relationship with CAD as they did with plant sterol levels, with rs41360247 in ABCG8 being associated with decreased CAD odds, and SNPs rs4245791 in ABCG8 and rs657152 in ABO being associated with increased CAD odds. This Mendelian randomization study suggests a potential association between campesterol levels and CAD risk. However, it must be realized that the potential association between serum plant sterol concentrations and CVD risk may also be epiphenomenal. Plant sterol concentrations reflect cholesterol absorption levels [131], thus it may be increased absorption of cholesterol, and not an atherogenic role of plant sterols themselves, which associates with CAD risk [119]. This notion is supported by the fact that associations seen between CVD and plant sterols are also seen with cholestanol [117,118], a cholesterol derivative not of plant origin, which reflects cholesterol absorption [117,119]. Unfortunately, in the Teupser et al. paper serum cholestanol concentrations were not investigated in relation to the above mentioned SNPs in ABCG5, ABCG8 and ABO regarding CVD risk as done for campesterol. This information could have helped in evaluating whether the hypothesized atherogenic plant sterol effect may actually be related to differences in cholesterol absorption. Additionally, it must be acknowledged that data relating natural circulating levels of plant sterols and CVD in epidemiological and Mendelian randomization studies may not truly reflect the effect of plant sterol supplementation because plant sterol levels in these trials are elevated due to physiological and genetic factors rather than increased plant sterol consumption [15,109,130].

Animal models have been used to investigate effects of plant sterol supplementation on atherogenesis with mixed results [132–138]. Weingartner et al. [132] examined the effects of plant sterol (PS) and/or ezetimibe (EZE) supplementation on top of a western diet (WD) or normal chow (NC), in ApoE (−/−) mice. Compared to the WD, WD + PS, WD + EZE and WD + PS + EZE all reduced atherosclerotic lesion formation, although the effect was larger with ezetimibe than with PS. Similar results were seen on top of the NC. However, within the 3 groups with similar cholesterol concentrations (NC, WTD + PS, and WTD + EZE) sitosterol and campesterol concentrations strongly correlated with atherosclerotic lesion formation. The level of plant sterol supplemented in this study was 2% (w/w) of the diet, which translates to a human equivalent of approximately 200 g of plant sterols per day, or 100 times the typically consumed supplemental dose in humans. It should, however, be realized that all animal studies so far have used dosages in this higher range since it reflects a ratio compared to dietary cholesterol intake seen in humans. Even at these extremely high concentrations of supplementation, plant sterols have been found to reduce serum cholesterol concentrations and suppress atherosclerotic plaque development. Plat et al. [133], investigated the addition of plant stanol or plant sterols to (LDL-R +/−) mice already on atorvastatin therapy. The addition of plant sterols or plant stanols to atorvastatin therapy decreased plasma cholesterol concentrations by 39% and 41%, respectively. Plant sterol to cholesterol ratios increased 4- to 11-fold during sterol treatment versus stanol treatment. However, lesion size decreased similarly in the sterol (−99% vs. control) and the stanol (−98% vs. control) groups, at comparable cholesterol reductions and maximally differing serum plant sterol changes, suggesting that increased serum plant sterol concentrations are not atherogenic. In this study plant sterols and plant stanols were supplemented at 1% (w/w) of the diet, or roughly 50 times higher than the typical human supplementation dose.

No studies as yet have looked at effects of plant sterol and plant stanol consumption on clinically relevant hard endpoints for the primary and secondary prevention of CVD in humans. Therefore, the actual characterizations of the anti-atherogenic properties of plant sterols and plant stanols or pro-atherogenic properties of plant sterols or plant stanols are still restricted to risk factor reduction such as changes in serum cholesterol concentration or

A

Reference	Patients (n)[1]	Design	Plant sterol/stanol dose g/d	Average absolute change in FMD vs. placebo
Gylling et al., 2009	Moderately hypercholesterolemics (282)	Parallel	Sterol: 2.15 Stanol: 2.13	Sterol: +0.37% Stanol: +0.58%
Hallikainen et al., 2006	Hypercholesterolemics (76)	Parallel, crossover	Sterol: 1.93 Stanol: 1.98	Sterol: +0.90% Stanol: +0.12%
De Jongh et al., 2003	FH children (41)	Crossover	Sterol: 2.3	Sterol: +0.50%
Hallikainen et al., 2008	Type 1 diabetics (19)	Parallel	Stanol: 2.15	Stanol: -2.47%
Raitakari et al., 2008	Hypercholesterolemics (190)	Parallel	Stanol: 2.0	Stanol: +0.91%
Jakulj et al., 2005	FH children (41)	Crossover	Stanol: 2.0	Stanol: +0.05%

[1] study arms were weighted by number of subjects included

B

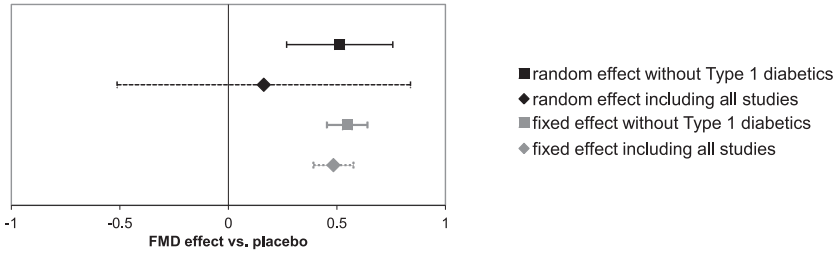

Fig. 1. Characteristics of studies evaluating the effect of plant sterols/stanols on endothelial function in humans.

evaluating changes in surrogate markers of atherosclerosis. In seven studies the effect of plant sterols or plant stanols on surrogate markers were studied such as intima-media thickness, flow-mediated dilatation (FMD), and/or arterial stiffness [67,139–143]. In most of the studies no significant improvement in arterial health could be demonstrated, but in one study plant stanol ester consumption for two years was associated with beneficial changes in carotid artery compliance [143], and in another study carotid artery compliance and flow-mediated dilatation was improved in subjects with initially reduced respective values [142]. Results of the 6 studies using FMD are combined in Fig. 1, panel A. In general, plant sterol and stanol consumption did not significantly change FMD, despite significant reductions in LDL-C concentrations. Nevertheless, the combined analysis as shown in Fig. 1, panel B suggests that if anything there is an improvement both for studies with plant sterols as well as plant stanols. It is very likely that each of the individual studies was underpowered to show significant effects on FMD and these potential effects need further investigation in a larger study population. In a study by Kelly et al. [144] which investigated plant sterol and plant stanol margarines compared to control margarine in statin users, an association between serum campesterol concentrations and changes in the arteriolar and venular diameters in the microcirculation of the fundus during a 1.5 year intervention period was seen when all treatment groups were combined. It should be acknowledged, however, that the intervention itself failed to show any significant change in the diameter of the arterioles or venules between baseline and endpoint, or between margarine treatments.

8.2. Recommendation

The present evidence supports plant sterols and plant stanols as viable option for lowering LDL-C in individuals at increased risk of CVD.

9. General discussion and the need for endpoint studies

It has been identified that a need exists for endpoint studies using plant sterol or plant stanol intervention. The ideal endpoint trial would compare plant sterol to plant stanol consumption versus a placebo, as both plant sterols and plant stanols similarly lower cholesterol concentrations [1,20,21], while having opposite effects on plant sterol concentrations [145]. An endpoint trial would provide evidence for actual effect of elevated plant sterol or plant stanol concentrations versus lowered LDL-C concentrations seen in response to plant sterol or plant stanol consumption. While support was voiced that an endpoint trial is necessary to provide conclusive evidence, the group failed to reach agreement about the best trial design and best endpoint markers as well as the feasibility of such a trial. Whether to set-up a primary or secondary prevention trial, trial duration, trial population size, and the use of food products or capsules in relation to compliance were all discussed. A follow-up discussion around this issue is certainly warranted. Even without an endpoint trial, based on the wealth of current clinical trial evidence demonstrating LDL-C lowering with plant sterol and plant stanol consumption, the use of plant sterols and plant stanols can be encouraged in hypercholesterolemic individuals who are at increased risk of CVD.

Conclusions regarding the use of plant sterol and stanols where agreement was reached
At doses up to 3 g/d plant sterols and plant stanols have been shown to have equal LDL-C lowering efficacy in a wide range of populations including T2DM, MetS and FH individuals. An average ~ 10% reduction in LDL-C can be achieved with intakes of ~2 g/d plant sterols or plant stanols.
A role exists for plant sterol and plant stanol esters in modestly lowering fasting triglyceride concentrations, however, the effects on TAG metabolism in the postprandial state needs further study.
Although potential differences exist between types of free plant sterol or plant stanol, such as the use of emulsifiers or particle size, there are no obvious clinical differences in LDL-C lowering effects when comparing free plant sterols and plant stanols vs. esterified forms.
Plant sterol and plant stanol formulations have been shown to be effective in higher fat, low fat and fat free foods, as well as in both liquid and solid food formats.
In all cases, but especially when non fat-based food products are used, attention has to be paid to the formulation and matrixing of the added sterol/stanol ingredient.
The suitability of using surrogate markers for assessing cholesterol absorption efficiency in specific populations, like obese, T2DM or MetS individuals need to be evaluated.
Future plant sterol and plant stanol studies should provide detailed information concerning the formulation of the used plant sterol or stanol blend in order to assess its impact on efficacy.
Recommendations for future studies and research needs regarding plant sterols and plant stanols
Head-to-head comparison studies between one or more high dose (>3 g/d) plant sterol or plant stanol intakes are needed to assess a potential difference or similarity in reaching optimal LDL-C lowering efficacy.
Standardization and cross-comparability in relation to the various analytical methodologies used to measure plant sterol and stanol concentrations in serum and tissues needs to be achieved.
The role of plant sterols and plant stanols in postprandial TAG metabolism is unclear and should be investigated.
The effects of plant sterol and plant stanol dose should be assessed in individuals who have already been characterized as non-responders to typical (2–3 g/d) doses of plant sterols or plant stanols to investigate if increased plant sterols and plant stanols can overcome poor responsiveness.
A collaborative multicenter retrospective clinical analysis investigating the potential genetic basis for the heterogeneity in plant sterol and plant stanol response should be undertaken where genetic data from hyper- and non-responsive individuals from already completed clinical trials are pooled to increase the potential of identifying SNPs associated with response to plant sterol and stanol consumption.
More research regarding the potential role for plant sterols and plant stanols in other diseases including Alzheimer's disease and cancer is warranted.
An endpoint trial using defined clinical outcomes was deemed to be needed to assess the functionality of the achieved LDL-C reduction through plant sterol or plant stanol consumption. The best design of such a trial, i.e. whether focusing on primary or secondary prevention, evaluating a hard endpoint such as mortality rates, or a surrogate marker, such as FMD, and whether to use a food vs. a supplement, needs to be decided.

8.1. Conclusion

Until the discussion around potential atherogenicity of plant sterols is resolved, based on the results of >200 clinical trials demonstrating LDL-C lowering efficacy, the risk to benefit of plant sterol use is favorable.

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Finally we want to thank all participants of the symposium for their participation in the discussion during this symposium. (Appendix 1)

Appendix 1

Invited speakers	Affiliation
Atif Awad	University at Buffalo, NY, USA
John Chapman	INSERM, Pitié-Salpétrière Hospital Paris, Paris, France
Peter Clifton	Baker IDI Heart and Diabetes Institute, Adelaide, AU
Helena Gylling	Helsinki University, Helsinki, Finland
Peter Jones	University of Manitoba, Winnipeg, Canada
Dieter Lutjohann	Bonn University, Bonn, Germany
Winfried Marz	Synlab Center of Lab Diagnostics Heidelberg, Germany
Bob Moreau	USDA ARS ERRC, Wyndmoor, Pennsylvania, USA
Richard Ostlund Jr.	Washington University, St Louis, USA
Jogchum Plat	Maastricht University, Maastricht, The Netherlands
Todd Rideout	University at Buffalo, NY, USA
Emilio Ros	Lipid Clinic, End and Nutrition Service, Barcalona, Spain
Ernst Schaefer	Tufts University, Boston, USA
Daniel Teupser	University Hospital Leipzig, Leipzig, Germany
Erkki Vartiainen	National Public Health Institute, Helsinki, Finland
Oliver Weingartner	University of Homburg, Homburg/Saar, Germany
Antonis Zampelas	Agricultural University of Athens, Athens, Greece
Invited industry	
Alvin Berger	Cargill, USA
Asif Malik	Cargill, USA
Ingmar Wester	Raisio, Finland
Susanna Rosin	Raisio, Finland
Gert Meijer	Unilever, The Netherlands
Elke Trautwein	Unilever, The Netherlands
Rouyanne Ras	Unilever, The Netherlands
Jean-Michel Antoine	Danone, France
Stephanie Jeansen	Danone, France
Hana Koutnikova	Danone, France
Renny Ison	MCNeil, USA
Collette Short	MCNeil, USA
Horst Messinger	BASF, Germany
Peter Horlacher	BASF, Germany
Christina Ehrhardt	BASF, Germany
Cecilia Brañes	Arboris, USA
Thomas Harting	Arboris, USA
Invited discussants	
Gilbert Thompson	Hammersmith Hospital, London, UK
Matti Tikkanen	Helsinki University, Helsinki, Finland
Mandana Amir Shaghaghi	University of Manitoba, Winnipeg, Canada
David Baer	Beltsville Human Nutrition Research Center, USDA, USA
Diana Ansorena	University of Navarra, Pamplona, Spain
Sabine Baumgartner	Maastricht University, Maastricht, The Netherlands
Francisco Blanco-Vaca	IIB Sant Pau, Barcelona, Spain
Lars Ellegard	Gothenburg University, Gothenburg, Sweden
Joan Carles Escolà-Gil	IIB Sant Pau, Barcelona, Spain.
Kirsi Laitinen	University of Turku, Turku, Finland
Dylan Mackay	University of Manitoba, Winnipeg, Canada
Semone Myrie	University of Manitoba, Winnipeg, Canada
Tatu Miettinen	Helsinki University, Helsinki, Finland
David Mymin	Health Sciences Centre, Winnipeg, Canada
Matthew Robinson	exCLAIM International, Amsterdam, The Netherlands
Cathy Rompelberg	RIVM, The Netherlands
Essi Sarkkinen	Foodfiles, Kuopio, Finland
Guenther Silbernagel	Eberhard-Karls-University, Tübingen, Germany
Els de Smet	Maastricht University, Maastricht, The Netherlands
Stoffer Loman	NutriClaim, Utrecht, The Netherlands

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