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Beyond blood lipids: phytosterols, statins and omega-3 polyunsaturated fatty acid therapy for hyperlipidemia

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Abstract

Phytosterols and omega-3 fatty acids are natural compounds with potential cardiovascular benefits. Phytosterols inhibit cholesterol absorption, thereby reducing total- and LDL cholesterol. A number of clinical trials have established that the consumption of 1.5–2.0 g/day of phytosterols can result in a 10–15% reduction in LDL cholesterol in as short as a 3-week period in hyperlipidemic populations. Added benefits of phytosterol consumption have been demonstrated in people who are already on lipid-lowering medications (statin drugs). On the other hand, omega-3 fatty acid supplementation has been associated with significant hypotriglyceridemic effects with concurrent modifications of other risk factors associated with cardiovascular disease, including platelet function and pro-inflammatory mediators. Recent studies have provided evidence that the combination of phytosterols and omega-3 fatty acids may reduce cardiovascular risk in a complementary and synergistic way. This article reviews the health benefits of phytosterols and omega-3 fatty acids, alone or in combination with statins, for the treatment/management of hyperlipidemia, with particular emphasis on the mechanisms involved.

Keywords: Hyperlipidemia; Phytosterols; Omega-3 fatty acids; Statins; Lipids

1. Introduction

Hyperlipidemia is a heterogeneous disorder involving multiple aetiologies. It is commonly characterised by an increased flux of free fatty acids (FFA), raised triglycerides, low-density lipoprotein (LDL)-cholesterol and apolipoprotein B (apoB) levels, and reduced plasma high-density lipoprotein (HDL)-cholesterol concentration, as a consequence of metabolic effects, or dietary and lifestyle habits [1].

The primary lipid abnormality involved in hyperlipidemia is an increase in circulating free (nonesterified) fatty acids originating from adipose tissue, caused by a down-regulation of signalling pathways, as well as inadequate esterification and FFA metabolism [2]. As a consequence, reduced retention of fatty acids by adipose tissue leads to an increased flux of FFA returning to the liver [1]. In turn, this stimulates hepatic triglyceride synthesis, promoting the production of apoB and the assembly and secretion of very low density lipoprotein (VLDL) [3]. In the presence of increased plasma triglyceride concentration, triglyceride-rich HDL particles are formed. These particles are more likely to be catabolised, and hence HDL cholesterol is reduced in the presence of elevated FFA. Elevated VLDL particles are lipolysed and hence fail to bind efficiently to LDL receptors, while the exchange of cholesterol esters with triglycerides forms triglyceride-rich lipoproteins, resulting in small dense (highly atherogenic) LDL-cholesterol particles (Fig. 1).

A strong independent association between elevated levels of LDL cholesterol and increased incidence of coronary artery disease has been established [4,5]. Elevated levels of LDL-cholesterol particles have been implicated in the development of the atherosclerotic plaque, characterised by reduced receptor-mediated clearance, increased arterial wall retention and an increased susceptibility to peroxidation [6].

In light of the increasing prevalence and health consequences associated with cardiovascular disease (CVD), there is an emerging need to identify treatments which alleviate risk factors associated with its development and

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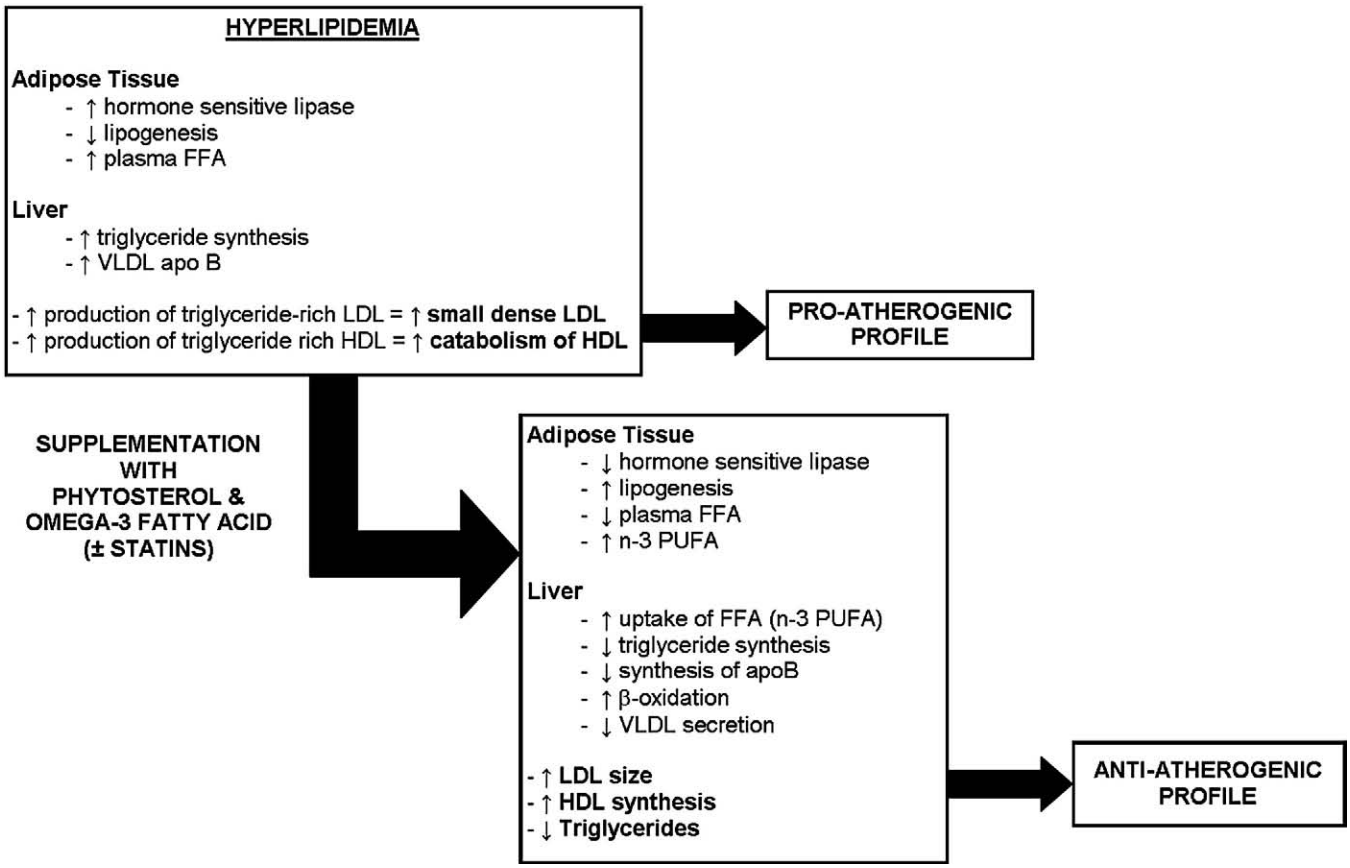

Fig. 1. Cardiovascular risk factors associated with hyperlipidemia. The consumption of statins and fibrates, omega-3 fatty acids or phytosterols can reduce cardiovascular risk via different mechanisms. The combination of omega-3 fatty acids and phytosterols may provide a complementary and synergistic reduction in CVD, with the possibility of reducing the dose of pharmacotherapy. LDL, Low-density lipoprotein; HDL, high-density lipoprotein; CVD, cardiovascular disease.

progression. Cardiovascular risk factors such as hyperlipidemia, hypertension and thrombosis contribute to the underlying mechanisms of atherosclerotic disease, promoting endothelial dysfunction, oxidative stress and pro-inflammatory pathways.

Lipid management guidelines have immense potential for significant health gain; however, conventional lipid-modifying treatments such as statins and fibrates are significantly underused in Australia [7]. Lipid guidelines from the National Heart Foundation of Australia place great emphasis on LDL- and HDL cholesterol as atherogenic and anti-atherogenic components, respectively. Conversely, the Adult Treatment Panel III (ATP III) guidelines of the US National Cholesterol Education Program (NCEP) place greater emphasis on triglyceride levels [7–10]. Statin monotherapy may not be sufficient to achieve the recommended non-HDL goals, especially given their modest effects on triglyceride concentration. The tendency of cardiovascular risk factors to cluster in individuals suggests the benefit of treatments targeted towards multiple risk factors [11–13]. Inevitably, a polypill which improves hypertension, lipid profile and insulin resistance is likely to target multiple risk factors with improved compliance.

Pharmacological therapies including bile acid sequestrant resins, statins, fibrates, niacin and cholesterol absorption inhibitors are common treatment options for hyperlipidemia; however, the use of alternative therapies is also becoming increasingly popular. Controlled trials using a range of functional foods (*i.e.*, policosanols, flaxseed, red yeast rice, guggulipid, garlic, viscous fibre, almonds and macadamia nuts and soy proteins) have been examined as potential complementary alternatives in the management of hyperlipidemia [14]. These functional foods are effective in reducing both total cholesterol and LDL cholesterol; however, they have no effect on triglycerides or HDL-cholesterol concentration. Long-chain omega-3 fatty acids and phytosterols have emerged as efficacious dietary supplements for the management of hyperlipidemia as well as other risk factors such as inflammation and thrombosis.

This article is focused to review the data from human clinical trials that have studied the efficacy of phytosterols and omega-3 fatty acids to treat cardiovascular risk factors such as lipid aberrations, inflammation, aggregation and hypertension. We also explore evidence supporting the use of combining pharmacotherapy with phytosterols or omega-3 fatty acids in patients with hyperlipidemia. Evidence for

the complementary and synergistic efficacy of the combined phytosterols and omega-3 fatty acids, with particular reference to the mechanisms involved, will be presented.

2. Phytosterols

2.1. Structure and derivatives

Plant sterols and stanols (referred to collectively as phytosterols) are nonnutritive compounds, structurally analogous to cholesterol, with the same basic functions in plants as cholesterol in animals; that is, to regulate membrane fluidity and other physiological functions associated with plant biology [15]. Phytostanols are characterised by a reduction at the double bond and are consequently saturated versions of phytosterols and are therefore less abundant [16]. Although structurally similar to cholesterol (steroid nucleus and a hydroxyl group at C3), phytosterols are differentiated by their degree of saturation and by their side-chain configuration at the C24 position [17,18]. There are more than 250 different phytosterols, with the most common belonging to the 4-desmethyl sterol family, made up of three compounds which account for most of the total phytosterol mass. These include β-sitosterol (includes an extra ethyl group at the C24 position, *e.g.*, 24-ethylcholesterol), campesterol (includes an extra methyl groups at the C24 position, *e.g.*, 24-methylcholesterol) and stigmasterol (includes an additional ethyl group at C24 and a double bond at the C22 position, *e.g.*, Δ^{22}-24-ethylcholesterol), accounting for 65%, 30% and 3%, respectively, of total dietary phytosterol intake [18–20].

2.2. Dietary sources and intakes

Phytosterols are found in all plant-based foods, such as fruit, vegetables, nuts, seeds, legumes and cereals. The most concentrated source can be found in vegetable oils such as corn oil (800–1500 mg/100 g) and palm oil (70–100 mg/100 g) [21]. The average intake of phytosterols in Western societies is approximately 100–300 mg daily [22]. Appreciably, the quantity of phytosterols consumed will vary between populations such as the Japanese or vegetarians, who consume an estimated 300–500 mg daily [23].

The intestinal absorption of individual phytosterols varies markedly to that of cholesterol (<5% *vs*. 20–60%). Absorption of phytosterols depends very much on the nature of the C24 side chain, where increasing complexity of the side chain increases hydrophobicity, which reduces absorption [24–26]. The overall net absorption of phytosterols is <5% of that consumed, most of which is rapidly excreted by the liver (retaining <1%) [25,27].

2.3. Absorption and metabolism

Phytosterols or cholesterol must be incorporated into micelles for absorption. The sterol-laden micelles interact with the intestinal brush border membrane, thereby facilitating their uptake by enterocytes. The exact mechanism by which phytosterol absorption occurs is still yet to be well defined; however, it is believed to require the Nieman–Pick C1 Like 1 Protein [28]. Once inside, the enterocyte, cholesterol and a small percentage of phytosterols are esterified by acyl cholesterol acyl transferase (ACAT), packaged into chylomicrons and secreted into the lymphatic system. Unesterified cholesterol and phytosterols are transported back into the intestinal lumen by the ATP-binding cassette (ABC) proteins (ABCG5 and ABCG8) [29]. Once taken up by the liver, phytosterols are incorporated into very low density lipoproteins or secreted via the bile. Due to the low affinity of ACAT for β-sitosterol in the liver, it has a greater secretion rate and higher hepatic clearance than campesterol [30]. In humans, β-sitosterol and campesterol concentrations are approximately 0.30 and 0.42 mg/dl, respectively, and when supplemented at 2–3 g/day, levels increase by 34% and 73%, respectively [31,32].

2.4. Mechanism of action

Phytosterols are effective in reducing the absorption of both dietary and biliary cholesterol from the intestinal tract, by displacing cholesterol from micelles, hence limiting intestinal solubility of cholesterol and decreasing the hydrolysis of cholesterol esters in the small intestine [33–35]. Phytosterols have the potential to reduce cholesterol absorption by 30% to 50% [36].

Competitive solubilization experiments of cholesterol microemulsion droplets, which mimic the functionality of micelles, show that the presence of phytosterols within the micelle significantly lowers the relative solubility of cholesterol [37]. This is supported by further studies suggesting that β-sitosterol has an increased affinity for biliary micelles, compared with cholesterol, demonstrated at high sterol concentrations [35]. Another potential mechanism for action is thought be that phytosterols induce the expression of the ABCA1 transporters, which are unable to differentiate between cholesterol and β-sitosterol, thus increasing the efflux of cholesterol [38,39]. *In vitro* studies have also shown that ACAT-mediated esterification is less efficient for phytosterols than it is for cholesterol [40]. ACAT is responsible for reducing intracellular free cholesterol concentration and it has been suggested that phytosterols may suppress ACAT activity, consequently reducing intestinal cholesterol uptake [41].

The inhibition of cholesterol absorption by the above mechanisms produces a state of relative cholesterol deficiency, which is followed by an up-regulation of cholesterol biosynthesis and LDL-receptor activity [35]. Chronic phytosterol-feeding studies show whole-body cholesterol biosynthesis increases by 38–53%, LDL-receptor expression increases by 25–45%, VLDL-cholesterol production by the liver was reduced by 20% and plasma concentration of dense LDL cholesterol was reduced by 22% [38,42]. Phytosterols encompass a wide variety of

biological interactions. Above all, they are known for their efficacious cholesterol-lowering properties [20,43–45]. The mechanisms by which phytosterols reduce cholesterol absorption and consequently reduce LDL cholesterol will be further explored throughout this review.

The dose–response effect of phytosterols and omega-3 fatty acids is also an important issue to address when considering the efficacy of such nutritional therapies. A large majority of studies provide evidence of both phytosterols and omega-3 fatty acids to be effective lipid-lowering agents over the short-term; however, long-term studies are needed to establish sustained, continued efficacy of such functional foods. In a 6-week dose–response study by Clifton *et al*. [46], responses from soybean oil, tall oil and a mix of tall oil and rapeseed oil were tested in free-living subjects with hypercholesterolemia. LDL Cholesterol was modestly reduced in a dose–response manner, and after phytosterol withdrawal, plasma sterols decreased by 50% within 2 weeks, dependent upon baseline concentrations. In a long-term, open-label, cross-over study by Amundsen *et al*. [47], children and their parents with familial hypercholesterolemia were asked to consume 20 g/day of a phytosterol-enriched spread (1.76 g phytosterols). Significant reductions in total lipid profile were achieved with a mean consumption of 1.2 and 1.5 g/day of phytosterols in children and their parents, respectively. Sustained efficacy of cholesterol reduction and long-term compliance of a phytosterol-enriched spread were demonstrated in this study, with a 26-week follow-up. This study demonstrates sustained efficacy of cholesterol reduction and long-term compliance of a phytosterol- enriched spread. Whether lipid lowering or health benefits of phytosterols can be achieved in free-living populations needs to be established.

2.5. Phytosterol-enriched functional foods

In response to the growing body of evidence supporting significant cholesterol lowering from phytosterol-enriched foods, the ATP III [9] recommends the inclusion of 2 g/day of phytosterols in the diet of individuals with elevated LDL cholesterol, which is also endorsed by the International Atherosclerosis Society and the National Heart Foundation of Australia [9,48,49]. Recent advancements in food technologies have seen the emergence of foods such as margarines, yoghurts, salad dressings, milk and snack bars enriched with an esterified form of phytosterols and promoted as 'nutraceuticals' or foods with therapeutic value.

Several studies have shown that the consumption of phytosterol esters (around 2 g/day) can achieve reductions in LDL cholesterol in the order of 10–15% in about 90% of individuals [50–52]. The comparable LDL cholesterol-lowering capabilities of phytosterols reflect their strong ability to reduce cholesterol absorption [53].

Recent studies have also investigated the consumption of regularly consumed foods enriched with phytosterol esters such as cream cheese, salad dressing, yoghurt, milk, cereal bars and margarine. These products have shown to be an effective means of reducing total cholesterol and LDL cholesterol in adults and children with hyperlipidemia [54–56].

A convenient means of delivering phytosterol esters in the diet has been their dispersion into fat spreads. As fats are needed to solubilise sterols, margarines are an ideal vehicle to increase the lipid solubility and facilitate the incorporation of phytosterols into the micelles. The estimated effects apply to an average serving of 20–25 g/day providing 1.6–2 g of sterol esters per serving. In a study by Nestel *et al*. [57], the consumption of 20 g/day of a sterol ester-fortified margarine (2.4 g/day) for 4 weeks provided a median reduction in total cholesterol (12.2%) and LDL cholesterol (13.6%) in line with those reported by Weststrate and Meijer [58] and Gylling and Miettinen [59]. A recent systemic review of the efficacy of phytosterols in lowering total cholesterol and LDL-cholesterol concentrations in familial hyperlipidemic subjects shows differences between treatment and control groups for total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides [60]. Fat spreads enriched with phytosterols (2.3±0.5 g/day) significantly reduced total cholesterol (7–11%), with a mean decrease of 0.65 mmol/L (95% CI −0.88 to −0.42 mmol/L; $P<.00001$), and LDL cholesterol (10–15%), with a mean of 0.64 mmol/L (95% CI −0.86 to −0.43 mmol/L; $P<.00001$), in 6.5±1.9 weeks compared to the control group, without any adverse effects. Triglycerides and HDL-cholesterol concentrations were not affected. These studies suggest that the cholesterol-raising effects of margarine and butter products are counteracted by the inclusion of phytosterol esters. In a long-term (12 months) study by Hendriks *et al*. [61], mildly hyperlipidemic participants consuming 20 g/day of a phytosterol-enriched spread (providing 1.6 g phytosterols) showed a consistent reduction in total- and LDL cholesterol, 4% and 6%, respectively, on average over 1 year. Phytosterol supplementation did not affect red blood cell deformability, hormone levels, clinical and haematological parameters or fat-soluble vitamin concentration. However, lipid adjusted α- and β-carotene concentration was reduced by 15–25%, relative to control. A simple method of preventing [62] changes in carotenoid concentration is to increase fruit and vegetable consumption during phytosterol supplementation [62–64]. It has also been suggested that carotenoid responses to phytosterols may vary according to apolipoprotein E (ApoE) genotype; more specifically, carriers of ApoE4 may have a tapered response to phytosterols consumption [65]. Although there are a limited number of studies investigating the long-term effects of phytosterol-enriched foods, there is no evidence to suggest their consumption to be unsafe, making them an effective functional food for the management of hyperlipidemia.

In a community intervention program based in Maastricht, Netherlands, observations on the voluntary use and effectiveness of phytosterol/stanol-enriched fat spreads were made during the time of their introduction to the Dutch

market [66]. Blood samples taken in 1999 and again in 2003 show significant changes in total-cholesterol concentration in nonusers (+2%), enriched-spread users (−4%), cholesterol-lowering drug users (−17%) and combination users (spread+statin) (−29%). Although the recommended dose was not consumed (14±9 g/day), a modest reduction in cholesterol is apparent in this free-living community setting.

When comparing phytosterol-enriched margarine products with other enriched foods, it is clear that margarine is an effective food vehicle. In a study by Noakes *et al*. [67] either a phytosterol-enriched milk (300 ml/day; 2.0 g phytosterols/day) or yoghurt (300 g/day; 1.8 g phytosterols/day) product was supplemented into the diets of modestly hypocholesterolemic (total cholesterol 5–7.5 mmol/L) participants for 3 weeks. The milk and yoghurt products yielded changes in LDL cholesterol of 6–8% and 6%, respectively. In another study [68], a breakfast cereal and bread product enriched with phytosterols (2.4 g/day) showed a reduction in total cholesterol (8.5%) and LDL cholesterol (13.6%) after 4 weeks of supplementation. This was comparable with results of a study by Clifton *et al*. [69] which supplemented milk, yoghurt, bread and cereal products (1.6 g/day) for 3 weeks in mildly hypocholesterolemic subjects.

Phytosterols are recognised as an important facet of healthy diets designed to reduce hypercholesterolemia, given the extensive scientific consensus supporting their role in improving LDL-cholesterol concentration.

2.6. Phytosterol and statin combinations

Since phytosterols and statins reduce plasma cholesterol via two different mechanisms, *i.e.*, inhibition of cholesterol absorption and inhibition of cholesterol synthesis, respectively, the added benefit of concurrent therapy with the two agents has been demonstrated in recent clinical trials [54,70].

This additive nature of phytosterols is convincingly demonstrated by Simons *et al*. [71] in a study with four parallel treatment arms. Hypocholesterolemic participants were asked to consume 25 g/day of a sterol ester-enriched margarine (providing 2 g/day) in addition to a lipid-lowering drug cerivastatin (40 mg/day) for 4 weeks. This combination of phytosterols and statins produced a further 8% reduction in LDL cholesterol. The additive effect of this combination is equivalent to doubling the dose of a statin [72]. Similarly, Neil *et al*. [73] showed an 11% reduction in LDL cholesterol with a statin plus sterol ester margarine (2.5 g/day) *vs*. placebo, for 8 weeks.

These studies show that the combination of phytosterols as an adjunct to statin therapy is additive rather than synergistic, resulting in incremental decreases in LDL cholesterol ranging from 10–20%, as reviewed by Thompson *et al*. [56]. A series of randomised, controlled trials [74–76] combining phytosterols with statin medication show a 4.5±2.4% additive effect, which translates to an additional 9–14% reduction in cardiovascular events, using risk modelling equations [77,78]. Accordingly, statin-induced inhibition of cholesterol synthesis may insufficiently reduce LDL cholesterol, produce adverse effects and may be uneconomical, such that suboptimal doses may be required [79]. The addition of phytosterol-enriched foods as an adjuvant to statin therapy may provide sufficient improvement to total and LDL cholesterol to counteract the reduction in lipid-lowering medication.

3. Omega-3 polyunsaturated fatty acids

3.1. Structure and dietary sources

The two most important metabolically active polyunsaturated fatty acids are the parent fatty acids linoleic acid (LA) and α-linolenic acid (ALA) of the n-6 and the n-3 families, respectively [80]. LA and ALA are elongated and desaturated in animal cells, forming the metabolically active longer chain n-6 (arachidonic acid, AA) and n-3 (eicosapentaenoic, EPA; docosapentaenoic, DPA; and docosahexaenoic acid, DHA) polyunsaturated fatty acids and their derivatives (2- and 3-series eicosanoids, respectively) [81]. ALA is a key constituent of dark green leafy vegetables (*i.e.*, broccoli, cabbage and spinach), many seed oils, and cereal products. The long-chain omega-3 fatty acids (EPA, DPA and DHA) are predominantly found in oil-rich seafood including tuna, salmon, trout, herring and sardines [82]. Increasing demand for long-chain omega-3 fatty acids has led to the development of novel sources such as those derived from algae (commercially available) and genetically modified seed oils with limited success to date [83].

Also, there is a constant growth in the availability of foods fortified with omega-3 fatty acids in the consumer market. Fortified sources include eggs produced by chickens fed omega-3-rich diets, fish oils or algae-derived omega-3 fatty acids supplements, margarine spreads, milk and breads [84,85].

3.2. Mechanism of action

Omega-3 fatty acids are pleiotropic molecules with a broad variety of biological actions including hypotriglyceridaemic, anti-aggregatory, anti-inflammatory and anti-arrhythmic responses [84]. These fatty acids could play a key role in the management of hypertension and hyperlipidemia and in the prevention of several diseases such as coronary heart disease, type 2 diabetes and insulin resistance [86]. These effects are mediated by alterations in circulating plasma lipids, eicosanoids, cytokines and physicochemical properties in the phospholipid membrane. When added to the diet, both EPA and DHA present in fish oil can alter the membrane phospholipid composition of the cells, impact eicosanoid synthesis and regulate transcription factor activity [84].

Supplementation with omega-3 fatty acids favourably modifies serum and tissue lipid alterations; the most consistent finding is a drastic reduction in fasting and postprandial serum triglycerides and FFA [87]. This has been observed with EPA and DHA alone [88] and with their combination in fish oil. Reduced VLDL production by the liver [89] largely results from (i) decreased availability of FFA released from adipose stores, (ii) suppression of lipogenic activity, (iii) an increase in the activity of triglyceride-synthesising enzymes (DGAT and PAP), (iv) the induction of genes involved in fatty acid oxidation and (v) an increase in phospholipid synthesis [90]. This regulation of gene expression proceeds through the inhibition of SREBP-1 and the activation of PPAR, which omega-3 fatty acids can interact with [80]. Net production of apoB is also reduced [91]. An increased lipolytic activity of lipoprotein lipase (LPL) in extrahepatic tissues completes the hypotriglyceridemic effect witnessed with omega-3 supplementation [92].

As a consequence in the reduction of triglycerides and VLDL, HDL synthesis is indirectly affected. The cholesteryl ester transfer protein (CETP) plays a pivotal role in HDL metabolism, as evidenced by the CETP amino acid polymorphism [93]. This transfer protein enables cholesteryl esters to be transferred from HDL to VLDL and LDL, and the synthesis of triglyceride-rich HDL [94]. In the absence or reduction of circulating triglycerides, CETP is reduced thereby reducing the amount of triglycerides being transferred from VLDL to HDL, which results in a modest increase in triglyceride-poor HDL and possibly apoA-I concentration (Fig. 1).

3.3. Role in eicosanoids production

The essentiality of LA and ALA is that they are precursors for some of the more important highly unsaturated fatty acids (AA, DHA and EPA), which are necessary for the synthesis of the family of bioactive mediators known as the eicosanoids. The metabolism of AA via the cyclooxygenase (COX) pathway generates pro-inflammatory 2-series eicosanoids, including prostaglandins (PGE_2 and $PGF_{2\alpha}$) and thromboxanes (TXA_2). The metabolism of AA via the lipooxygenase (LOX) pathway generates the chemotactic 4-series leukotrienes (LTB_4, LTC_4 and LTE_4) [95]. Metabolism of EPA and DHA generates an anti-inflammatory response by competitively inhibiting the production of 2-series eicosanoids and producing the less biologically active 3-series prostaglandins (PGE_3 and $PGF_{3\alpha}$) and thromboxanes (TXA_3) via the COX pathway and the 5-series leukotrienes (LTB_5, LTC_5 and LTE_5) via the LOX pathway.

Inflammatory and immune cells isolated from blood collected from humans consuming a typical Western diet contain high amounts of AA and low proportions of EPA and DHA [96,97]. The exact amount of AA depends on the cell type and lipid fraction examined, although as an example, typical human mononuclear cells (70:20:10 mixture of T lymphocytes, B lymphocytes and monocytes) contain 15–25% AA and 6–10% LA [81].

3.4. Omega-3 fatty acid supplementation and human health

Secondary prevention trials [98–100] have shown that omega-3 fatty acid supplementation reduces premature mortality from coronary artery disease, reduces risk of impaired glucose tolerance and diabetes, and has beneficial effects on thrombosis and arterial compliance. It is well accepted that high doses of omega-3 fatty acids reduce triglycerides and improve HDL-cholesterol concentrations [101,102]. Epidemiological and experimental evidence suggests that the consumption of omega-3 fatty acids is associated with a reduced risk of CVD, certain types of cancer, inflammatory disease, diabetes mellitus, multiple sclerosis and clinical depression [85,103]. These effects are mediated by alterations in circulating plasma lipids, eicosanoids, cytokines and physicochemical properties in the membrane [104].

It is largely agreed that omega-3 fatty acids reduce hepatic secretion of triglyceride-rich lipoproteins (VLDL and LDL) in the order of 32% [105,106]. These studies, in part, firmly establish that this mechanism involves the reduction of triglyceride concentrations via a reduction in VLDL secretion rates. Grimsgaard *et al*. [107] reported on the effects of supplementation with highly purified EPA (3.8 g/day) or DHA (3.6 g/day) for 7 weeks in healthy, nonsmoking male volunteers. They found a reduction in plasma triglycerides that was at least as marked in the DHA group (26%) as in the EPA group (21%). In addition, HDL cholesterol increased only in the DHA group [107]. These results provide convincing evidence that EPA and DHA are equally effective at reducing serum triglycerides, but that DHA may raise HDL-cholesterol as well as LDL-cholesterol particle size (*i.e.*, both anti-atherogenic outcomes).

Omega-3 fatty acids have contrasting effects on LDL cholesterol, with a general tendency toward slightly increased LDL-cholesterol concentrations; however, the size of the LDL molecule is also increased, which is thought to be less atherogenic [22]. The mechanism by which this occurs is largely unknown and many speculations have been made. It is thought that LDL synthesis rates are increased, rather than the receptors themselves; however, the potential associated cardiovascular risk is largely compensated for by the incorporation of omega-3 fatty acids into the surface phospholipids of small, dense lipoprotein particles, which change their structure to exhibit antioxidant properties [108]. Since omega-3 fatty acids are shown to have beneficial effects on other CVD risk factors such as HDL-, VLDL cholesterol, triglycerides, CETP, triglyceride-synthesising enzymes (DGAT and PAP), fatty acid oxidation, lipogenesis, blood pressure and inflammatory biomarkers, this may offset any potentially negative effects on changes in LDL cholesterol. Moreover, the LDL particle size is increased following dietary supplementation with omega-3 fatty acids to reduce the atherogenecity of these particles.

3.5. Omega-3 and statin combinations

Combination of statin–fibrate drugs is usually prescribed in patients with mixed hyperlipidemia to reduce circulating pools of triglycerides and cholesterol. While these combinations have been shown to reduce triglycerides by up to 50% [109,110], there are reports of adverse events, such as liver and muscle toxicity [111–113]. When omega-3 fatty acids are administered as an adjunct to a statin therapy in hypercholesterolemic patients with persistent hypertriglyceridemia, benefits in lipid parameters (total cholesterol and triglycerides) are enhanced [114,115].

In the Combination of Prescription Omega-3 with Simvastatin (COMBOS) study, the efficacy of a highly purified omega-3 fatty acid (465 mg EPA+375 mg DHA per 1-g capsule) was trialled in 254 patients on a stable statin therapy with persistent hypertriglyceridemia [116]. Non–HDL cholesterol was significantly lowered after treatment with omega-3 fatty acids and statins, compared to the placebo group (−9.9% *vs*. −2.2%). Also, reductions in triglycerides and VLDL cholesterol had a median decrease of 30% and 28%, respectively. These findings have been found in a number of other studies [106,117]. In another omega-3 (8 g/day DHA-rich supplement) and statin combination study, a 27% reduction in triglycerides after 3 months was found [118]. Also, a significant correlation between dosage of fish oil and the extent of cholesterol reduction (r=−0.344, P<05) was found; however, no significant changes in VLDL, IDL and LDL were present. There is a significant amount of research to show that statin therapy in combination with omega-3 fatty acids significantly reduces lipids compared to a placebo, particularly triglycerides and lipoprotein subfractions. This supports data showing that omega-3 fatty acid supplementation decreases coronary mortality in established coronary heart disease patients [119] and reduces CVD in the long-term. Given the strength of the evidence, omega-3 fatty acids in addition to a statin may be preferable to drug combinations for the treatment of combined hyperlipidemia.

4. Potential health benefits of combination therapies

The benefits of cholesterol-lowering treatments on the risk of coronary heart disease and mortality have been clearly established in large clinical trials involving the use of inhibitors of cholesterol synthesis (statins) [75,120–122]. However, a monotherapy of statins is frequently insufficient for reducing plasma cholesterol concentration to target levels in practice, especially in hypercholesterolemic patients with increased intestinal absorption [123]. Given that statin therapies are the most widely used method for lipid lowering in these patients, these drugs may only be used at suboptimal doses.

In 2005, the National Heart Foundation of Australia (NHFA) [7], along with the Cardiac Society of Australia and New Zealand (CSANZ) [8], released a position statement on lipid management in an aim to establish a cost-effective risk factor management strategy for those individuals at a high risk of a cardiovascular event. The interventions outlined by the NHFA and CSANZ to achieve a 25% relative risk reduction in high-risk groups place emphasis on LDL cholesterol, HDL cholesterol and total cholesterol [124]. The NCEP suggests that an aggressive LDL-cholesterol reduction could in large ameliorate the risk of diabetes, coronary artery disease and insulin sensitivity associated with combined hyperlipidemia [125].

In combination, phytosterols and omega-3 fatty acids may offer a more comprehensive strategy for not just optimising circulating lipid levels, but also for providing additional health benefits via anti-inflammatory, -hypertensive and -arrhythmic effects. There is considerable evidence to suggest that omega-3 fatty acid supplementation is involved in improved vascular function and lipoprotein profile, lower arterial pressure, diminished thrombogenicity and modification of atherogenic processes, all of which are important cardiovascular preventative actions. Additionally, inflammatory cytokines, adhesion molecules and vasoconstrictive eicosanoids have all shown to be beneficially reduced with the consumption of omega-3 fatty acids [126]. In the GISSI Prevenzione Trial, 11,324 patients were randomised to receive 1 g/day of EPA plus a DHA supplement or placebo [127]. After 3.5 years, there was a remarkable reduction in most cardiovascular end points and, ultimately, a 20% reduction in cardiovascular-related deaths, nonfatal infarctions and nonfatal strokes [127]. This clinical trial provides evidence-based medicine which underpins the validity of epidemiological findings and physiological plausibility of omega-3 fatty acids being protective against CVD. Comparably, very little work has been done to investigate the cardioprotective effects of phytosterols in human nutrition, apart from its hypocholesterolemic effect. The findings from a study by Madsen *et al*. [52] suggest that the consumption of 2 g/day of phytosterols had no effect on apo A-1, Lp(a) and hs-CRP, but significantly decreased apoB (4.6%), a strong predictor of coronary events; these findings are also supported by Ridker [128] and Yusuf *et al*. [129].

Since the 1970s, phytosterols have been esterified to improve their functionality, solubility and incorporation into food products [130,131]. The esterification of phytosterols to long-chain omega-3 fatty acids does not impair their hypolipidemic properties, yet enhances their solubility in oil by 10-fold [132]. The extent to which the fatty acid moiety of phytosterol esters influences cholesterol absorption has been examined in a limited number of animal and human trials. In a study by Rasmussen *et al*. [133], phytosterols were esterified with fatty acids from soybean oil, beef tallow or purified stearic acid, and tested in 35 male F_1B Syrian hamsters for 4 weeks (50 g/kg phytosterol esters esterified with fatty acids). This study showed that beef tallow and stearic acid are more effective than soybean oil in reducing cholesterol absorption, liver cholesterol and plasma

non–HDL-cholesterol concentration in hamsters. In a similar study by Ewart *et al*. [134], male hamsters were fed phytosterol esters esterified to fish oil, which showed a significant reduction in non–HDL-cholesterol concentration compared to the control. Furthermore, in insulin-resistant rats fed the same phytosterol-fish oil esters, serum triglyceride and total-cholesterol levels were significantly reduced compared to the control rats [135]. Unfortunately, in the studies by Ewart *et al*. [134], Russell *et al*. [135] and Demonty *et al*. [136], their study design did not allow for a phytosterol-only or fish oil-only group for comparison. Therefore, it is difficult to determine whether the combined phytosterol-fish oil esters were the most effective compared with phytosterol esters or fatty acid esters alone.

To date, phytosterols provided as food matrices, mostly as spreads or soft-gel capsules, have been shown to affect LDL-cholesterol concentrations in both normo- and hyperlipidemic individuals [137–140]. In a cross-over design study by Jones *et al*. [136,141], 21 moderately overweight hypercholesterolemic subjects consumed an isoenergetic diet for 28 days, each supplemented with foods containing one of three phytosterol ester preparations (fish oil, sunola oil and olive oil). Each treatment contained 1.7 g/day phytosterols, and the phytosterol-fish oil treatment contained 5.4 g/day fish oil (EPA+DHA). The changes in total-, LDL- and HDL cholesterol did not significantly differ between the three diets; however, the phytosterol-fish oil ester significantly reduced fasting and postprandial triglyceride concentration. Furthermore, plasma TNF-α, IL-6, CRP, prostate-specific antigen and fibrinogen concentrations were unaffected by the three phytosterol preparations.

These studies taken together suggest that the phytosterol carrier may indeed play a role in the exerted effects of various phytosterol preparations, and the interaction between phytosterols and various fatty acid esters needs to be further explored.

In our laboratory, we designed a study to directly compare the efficacy of a combination treatment with phytosterols and omega-3 fatty acids to treat hyperlipidemia. This clinical trial was a 3-week randomised, double-blind, placebo-controlled, 2×2 factorial study involving 60 adults with hyperlipidemia [142]. The study was designed so that participants were randomised to receive either sunola oil capsules alone or in combination with 25 g/day of a spread (containing 2 g/day phytosterols) or 1.4 g/day of omega-3 fatty acids (high DHA) capsules alone or in combination with 25 g/day of a phytosterol-enriched spread. Plasma lipid profile was significantly improved with a 13.3% reduction in total cholesterol, a 12.5% reduction in LDL cholesterol, a 25.9% reduction in triglycerides and an 8.6% increase in HDL cholesterol in the combined phytosterol and fish-oil group, and these changes were more prominent compared to other groups. In further analysis, we also showed significant reductions in a range of inflammatory markers, important in the development of atherosclerosis. For the first time, we showed a reduction in hs-CRP, IL-6, TNFα and LTB_4 with the consumption of phytosterols in combination with omega-3 fatty acids supplementation [143]. Using these findings to calculate overall cardiovascular risk (using the Framingham equation), we showed a 22.6% reduction in individuals consuming the combination treatment, compared to 15.1% and 15.3% CVD risk reduction in the phytosterol and fish-oil groups alone. Together, these findings suggest that reducing plasma lipids, along with systemic inflammation in hyperlipidemia, represents an important mechanism by which omega-3 fatty acids, combined with cholesterol-lowering phytosterols, confer their putative cardiovascular benefit. Given the lack of evidence, it is difficult to speculate the exact mechanism by which phytosterols are anti-inflammatory; however, it should be noted that phytosterol supplementation did have a significant main effect on C22:6n-3 concentration and so the authors speculate that the conversion of C18:3n-3 to C22:6n-3 may be a possible mechanism by which the combination of these two functional foods elicits an anti-inflammatory response. To a large extent, reducing the inflammatory milieu to provide benefit among cardiovascular risk factors is yet to be fully understood in the context of hyperlipidemia.

5. Conclusion and future direction

It is well established that dietary supplementation with phytosterols reduces blood levels of total- and LDL cholesterol with no effects on HDL cholesterol and triglycerides. Since phytosterols reduce plasma cholesterol by reducing cholesterol absorption, evidence for the added benefit of phytosterol supplementation in conjunction with statin drugs, which inhibit cholesterol biosynthesis, has been presented. Neither phytosterols nor statin drugs have any significant effect on plasma triglycerides or anti-inflammatory/anti-aggregatory properties. On the other hand, there is ample evidence in the literature to demonstrate the triglyceride-lowering as well as anti-aggregatory and anti-inflammatory potential of long-chain omega-3 fatty acids. It is therefore conceivable that a combination of omega-3 fatty acids with either statin drugs or phytosterols may offer not only complementary lipid-lowering effects but also anti-aggregatory and anti-inflammatory effects resulting in greater CVD risk reduction in high-risk individuals (Fig. 2). Moreover, phytosterol supplementation appears to counteract the LDL-raising effects of high-DHA fish oil. In fact, the combined therapy with phytosterols and omega-3 fatty acids has been shown to have not only complementary but also synergistic effects on circulating lipid levels, without adverse effects. Therefore the combination of phytosterols and omega-3 fatty acids may offer greater cardiovascular benefits than either of the supplements alone, with improved compliance but without any adverse effects seen with statin drugs.

The mechanisms by which the combination of phytosterols and omega-3 fatty acids provides synergistic effects are

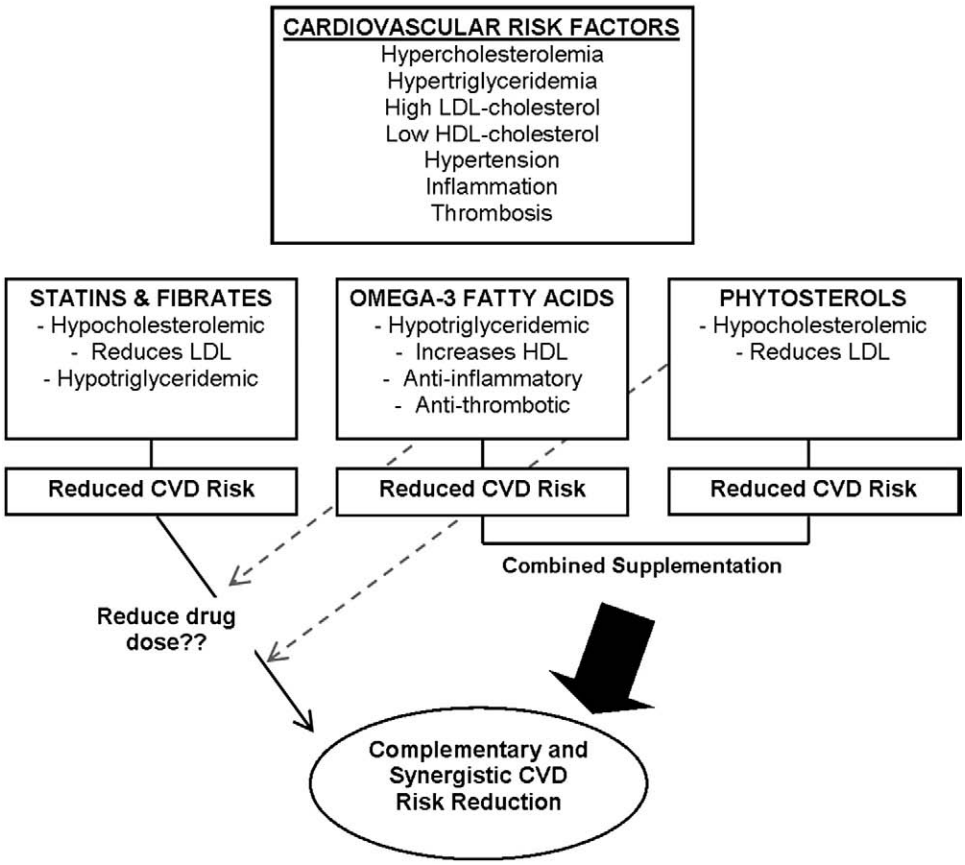

Fig. 2. The mechanism by which hyperlipidemia provides a pro-atherogenic environment. Concurrent dietary supplementation with phytosterols and omega-3 fatty acids optimises the lipid profile, thereby creating an anti-atherogenic potential. FFA, Free fatty acids; VLDL, very low density lipoprotein; apoB, apolipoproteins B; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

not known at present. It is likely that omega-3 fatty acids replace cholesterol from the micelles more efficiently compared to saturated, monounsaturated or omega-6 fatty acids, resulting in enhanced reduction in cholesterol absorption. Phytosterols are absorbed, albeit inefficiently, from the gastrointestinal tract; therefore, long-term supplementation may result in considerable increases in plasma phytosterol concentrations and therefore may influence metabolic pathways, other than simply reducing cholesterol absorption. However, to date, no such evidence has been presented in the literature. Any interactive influence of phytosterols and omega-3 fatty acids following absorption from the gut on haemostatic factors, inflammation mediators and eicosanoid metabolism remains to be examined. ApoE genotypes have been shown to be a determining factor for the plasma lipid lowering and carotenoid depletion following phytosterol supplementation [144].

It would be desirable to develop a single functional food incorporating phytosterols and omega-3 fatty acids for ease of consumption and improved compliance. However, there are technological difficulties in achieving this due to limited solubility of phytosterols and oxidising potential of omega-3 fatty acids, particularly at dose levels required to optimise blood lipids and influence anti-inflammatory and anti-aggregatory pathways. Further research is needed to successfully incorporate phytosterols and omega-3 fatty acids into food matrices and to develop industrial processes to optimise the retention of these ingredients in the final products.

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