Bioactive compounds as an alternative for drug co-therapy: overcoming challenges in cardiovascular disease prevention

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ABSTRACT

Different pharmacological interventions have been applied with success to reduce the progression of atherosclerosis. However, many patients are not good responders or must interrupt treatment due to adverse effects. Bioactive compounds such as omega-3 fatty acids (n-3 FA), plant sterol esters (PSE) and phenolic compounds (PHC) are natural molecules with great potential to reduce the atherosclerosis burden by reducing inflammation, LDL cholesterol (LDL-C) and oxidative stress, respectively. Although their physiological effects on biomarkers are much lower than those expected by drugs used for the same purpose, bioactive compounds can easily be incorporated into the daily diet and present no adverse effects. However, little is known about the combination of n-3 FA, PSE, PHC and drugs in atherosclerosis progression. This

review article summarizes potential effects of co-therapies involving n-3 FA, PSE and PHC combined with major hypolipidemic drugs on atherosclerosis biomarkers and clinical outcomes. Evidence of additive and/or complementary effects regarding drugs action reveals possible roles for bioactive compounds in disease management. Pharmaceutical companies, physicians and food scientists should be prepared to better understand this type of interaction and its consequences in terms of efficacy and life quality.

Keywords

Atherosclerosis, statin, omega 3, plant sterol, phenolic compounds

INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of mortality in many developed and developing countries (Weber and Noels, 2011; Reiner, 2013; Wong, 2014). Although significant progress has been achieved through new drugs and surgical procedures (Fuster, 2014), the number of deaths caused by CVD is still high. It has been estimated that 23.6 million will die as a consequence of CVD in 2030 (Cannon, 2013). On the basis of 2010 death rate data, one American dies of CVD every 40 seconds. The estimated total cost of this disease is US $315.4 billion, and is projected to rise to US $918 billion in 2030 (Go et al., 2014). For this reason, more efforts have been made in terms of primary prevention, including changes in lifestyle, diet and prescription drugs. However, the effectiveness of these measures is still low due to many factors. For example, it is well-known that the adherence of the patients to chronic drug prescriptions is universally poor, with less than half of those patients who are prescribed antihypertensive or lipid-lowering drugs continuing treatment beyond one year (Chapman et al., 2005). Among the factors that contribute to low patient adherence to medication are the economic burden, intolerance, complexity of treatment, side effects and the number of pills that the patient must take daily (Fuster, 2014; Scicchitano et al., 2014). In a previous study carried out by our group, patients took about eight pills per day, on average (Bertolami et al., 2014). In addition to the adherence limitation, atherosclerosis, the process that underlies CVD, actually starts early in life (Lusis, 2000; Mendis et al., 2005; Rader and Daugherty, 2008) when diet rather than drugs could be used as a preventive intervention. Thus, one alternative that could effectively contribute to early prevention, reduce drug doses or improve the patient response to treatment could be the inclusion of functional foods in the individual’s diet (Eussen et al., 2010).

About two decades ago, food companies started to add bioactive compounds to some food formulations, rendering them "functional." These bioactive compounds provide specific health benefits when consumed as part of the daily diet. Today, the number of functional foods has increased in different countries, offering several options for consumers. The United States is the world's largest functional food market with sales up to $43.9 billion in 2012, where six out of ten adults consume functional foods/beverages at least occasionally. Cholesterol-lowering foods/drinks were the most purchased condition-specific food or drink, sought by 29% of consumers (Sloan, 2014).

Despite weaker effects on CVD biomarker improvement when compared with drugs, functional foods do not present any side effects and can be included in the diet from childhood. Patients who are poorly responsive to pharmacological treatments could have a better response if functional foods are viable as a co-therapy, since bioactive compounds can act by different physiological pathways than drugs. In addition, functional foods may contribute to reduce high cholesterol prevalence (Scicchitano et al., 2014). About 5.6% of US adults have undiagnosed hypercholesterolemia and more than half of individuals are at borderline high risk, yet remain unaware of their condition (Go et al., 2014). These individuals could especially benefit from functional foods, since these over-the-counter compounds do not demand a physician's prescription to be bought in local markets.

Functional foods that are potentially beneficial for CVD prevention include different classes such as fruits, vegetables, legumes, nuts, chocolate, olive oil, fish or fish oil, tea, wine and soy protein. Dietary patterns and entire diets may also be cardioprotective, particularly the Mediterranean Diet (Alissa and Ferns, 2012). As there is a great number functional foods and bioactive

compounds with large variation of biological activity, this study will focus on main dietary components with anti-inflammatory, cholesterol lowering and antioxidant activity that are readily available for consumption. Among these, omega-3 fatty acids (n-3 FA) from fish and fish oil, plant sterol esters (PSE) found particularly in fortified foods and phenolic compounds (PHC) from green tea and red wine, will be addressed (**Figure 1**). The Food and Drug Administration (FDA) in the US recently approved the use of PSE health claim in food labeling, while more evidence is still necessary for n-3 FA and PHC health claims (FDA, 2004; FDA, 2010). Considering the increase in the functional food market, the combination of these bioactive compounds with drugs is already a reality and will become more evident along with a further reduction in functional foods prices. Thus, it is necessary to understand which biochemical mechanisms are involved when the major hypolipidemic drugs are taken with bioactive compounds. For this reason, the objective of this narrative review was to discuss potential effects of co-therapies involving n-3 FA, PSE and PHC combined with major hypolipidemic drugs on atherosclerosis biomarkers and clinical outcomes. A literature overview was conducted using the following databases: PubMed, Scopus and Web of Science. Articles were identified using the terms: atherosclerosis, statin, niacin, ezetimibe, fibrates, PCSK9 inhibitor, omega-3 fatty acids, plant sterols, phenolic compounds, green tea, wine, resveratrol. Clinical studies on combination therapy, presenting none or positive results as well as adverse effects, were included if they provided useful and clinically relevant information about the efficacy of the treatment and management of dyslipidemia.

CARDIOVASCULAR DISEASES

Cardiovascular or heart diseases (CVD) include different pathologies that directly affect the heart or vascular system, with high rates of morbidity and mortality (Wong, 2014). The predominant manifestation of CVD is caused by the ischemic heart disease associated with a restriction of the blood flux in arteries, capillaries or veins. In this condition, there is an interruption of the blood supply with severe consequences, such as fatal myocardial infarction (38-46%) or stroke (34-37%) (Wong, 2014).

Atherosclerosis is the process that underlies ischemic diseases and consists of chronic inflammation of the arteries caused by various factors. This condition is often associated with high consumption of lipids rich in saturated and trans fatty acids, cholesterol, simple sugars and salt, sedentarism, overweight or obesity, exposure to an oxidant environment and it is also strongly influenced by hereditary factors (Lusis, 2000). The mechanism proposed to explain atherosclerosis progression in humans involves the infiltration of low-density lipoprotein cholesterol particles (LDL) in the endothelium monolayer intima, where they contribute to monocyte recruitment and to foam cells formation (fatty streaks), as summarized in **Figure 2**. Briefly, excess LDL infiltrates the first external layer of the endothelium (tunica intima) at sites in the arterial tree where laminar flow is disrupted (Libby, 2002; Rader and Daugherty, 2008). Once retained in the intima, in part binding to proteoglycan, LDL particles are susceptible to oxidative modification by reactive oxygen species (ROS), or by enzymes such as myeloperoxidase (MPO) or lipoxygenase (LOX) released from inflammatory cells (Esterbauer et al., 1992; Weber and Noels, 2011). As the endothelium represents a site of chronic inflammation,

ROS within the vessel wall, such as superoxide anions produced by macrophages through the action of membrane-associated NADPH oxidase (NOX) (Heinecke, 1999) will promote LDL oxidation. In addition, LDL tyrosine residues can also be directly oxidized by hypochlorous acid (HOCl) generated by MPO (Levitan et al., 2010). Oxidized LDL (oxLDL) further induces the release of pro-inflammatory cytokines and monocyte chemotactic protein 1 (MCP-1) (Sanchez-Quesada et al., 2004) and can also inhibit the production of nitric oxide (NO), a chemical mediator with vasorelaxation properties (Lusis, 2000).

In the injured endothelium, monocytes transmigrate to the intima, proliferate and differentiate into macrophages (Lusis, 2000). These chemical modifications to LDL induce macrophages to phagocytize the oxLDL recognized by scavenger receptors, such as CD36 and SR-A, leading to foam cell formation (Libby, 2002). In addition, foam cells produce large amounts of MPO, thus driving a vicious cycle (Rose and Afanasyeva, 2003). The process also induces vascular smooth muscle cell proliferation in the intima or their migration from the media layer, causing intimal thickening along with fatty streaks, altering endothelial morphology and narrowing the lumen of the artery (Spagnoli et al., 2007; Rader and Daugherty, 2008). At this point, these cells secrete extracellular matrix proteins such as collagen, increase the aggregation of atherogenic lipoproteins and perpetuate a state of chronic inflammation. The plaque can continue to grow, resulting in clinically obstructive disease known as *angina pectoris* (Libby, 2002; Rader and Daugherty, 2008). On the other hand, the plaque can undergo abrupt rupture, caused by dissolution of the collagenous matrix of the fibrous cap, exposing the lipid core containing the pro-coagulant protein tissue factor to the vascular lumen, forming a thrombus that suddenly interrupts blood flow, often causing an acute fatal myocardial infarction or stroke

(Lusis, 2000; Libby, 2002; Rader and Daugherty, 2008). The inflammatory process not only promotes the initiation and evolution of atheromas, but also contributes to the production of proteolytic enzymes capable of degrading collagen, making the plaque more susceptible to rupture (Libby, 2002). Vulnerable plaques generally have thin fibrous caps and high number of inflammatory cells (Lusis, 2000). Interesting, the atherosclerotic process begins early in life, but generally manifests in older adults (Rader and Daugherty, 2008), when it is too late for preventive interventions, thus limiting the treatment to drugs and surgical procedures, including in this case all negative aspects such as side effects and high costs.

PHARMACOLOGICAL THERAPY FOR CARDIOVASCULAR DISEASE PREVENTION

Current strategies for prevention of cardiovascular events are mainly focused on attenuating hyperlipidemia, while the inflammatory and oxidative mechanisms of atheroprogression are not completely addressed (Weber and Noels, 2011). Although lowering LDL-C is the primary clinical approach to control hyperlipidemia and atherosclerosis risk, the pharmacological management of disease also covers different pathways of lipid metabolism (Figure 2). Strengths and drawbacks of the main drug therapies used to treat and control dyslipidemia are summarized in Table 1.

BIOACTIVE COMPOUNDS AND UNDERLYING MECHANISMS FOR CARDIOVASCULAR DISEASE PREVENTION

OMEGA-3 FATTY ACIDS

Polyunsaturated fatty acids (PUFAs) with especially important health effects include those in the omega-6 (n--6) and omega-3 (n--3) fatty acid families. Mammals cannot synthesize α-linolenic acid (ALA, C18:3 n-3) or linoleic acid (LNA, C18:2 n-6), the parent fatty acids of the n-3 and n-6 families, respectively (Adkins and Kelley, 2010; Poudyal et al., 2011). However, humans express enzymes necessary for the conversion of dietary ALA into longer chain PUFAs (LCPUFAs) as eicosapentaenoic acid (EPA, 20:5 n--3) and docosahexaenoic acid (DHA, 22:6 n--3), although these conversions are very limited (De Caterina, 2011).

ALA (n-3) and LNA (n-6) share a common metabolic pathway and therefore compete for the first enzyme (Δ6-desaturase) in metabolism, which consequently, represents a limiting step for n-3 LCPUFAs production (Poudyal et al., 2011). By sharing metabolic pathways, n-3 FA also compete with n-6 FA as substrates for the formation of pro-inflammatory mediators, such as leukotrienes, prostaglandins and cytokines, through complex pathways involving the cycloxigenase (COX) and LOX enzymes. Altogether, n-6 derived eicosanoids (as PGE2 and LTB4) are pro-inflammatory, while n-3 FA can be enzymatically converted to less active leukotrienes (LTB5) and prostaglandins (PGE3). Thus, displacing the pro-inflammatory n-6 FA pathway reduces the production of pro-inflammatory mediators by substrate competition; this mechanism is thought to be one of the main actions of n-3 FA in reducing inflammation (De Caterina, 2011; Calder, 2012). Furthermore, n-3 FA exert anti-inflammatory and inflammation-

resolving roles through other lipid mediators like resolvins and protectins. EPA and DHA give rise to mediators such as resolvin E1 and resolvin D1, respectively, while DHA leads to protectin D1. These mediators participate in the resolution of inflammation by limiting its progression and associated damage (Calder, 2012).

Besides reducing inflammation, n-3 FA also reduce the risk of cardiovascular disease associated with dyslipidemia. The hypotriglyceridemic effect of EPA and DHA is well established, and is the result of both increased lipolysis and decreased lipogenesis. These n-3 FA enhance fatty acid β-oxidation via the activation of PPARα. In addition, n-3 FA suppresses transcription of sterol regulatory element-binding protein-1c gene (SREBP-1c), inhibiting de novo lipogenesis by decreasing the expression of some genes like fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) (Figure 2). The reduced fatty acid availability for triacylglycerol (TAG) synthesis indirectly reduces hepatic VLDL synthesis, contributing to the hypotriglyceridemic effect (Nagao and Yanagita, 2008; Tai and Ding, 2010; Mozaffarian and Wu, 2011).

The health effects of EPA and DHA have been highlighted by results from epidemiologic and case-control studies that showed an inverse association between the consumption of fish or fish oil and cardiovascular events or mortality (Daviglus et al., 1997; Albert et al., 1998; Erkkilä et al., 2003; Kromhout et al., 2012). After 17 years of follow up, the Physician's study showed an inverse association between plasma n-3 FA and sudden death from cardiac causes, even among men without a history of cardiovascular disease (Albert et al., 2002). Consistent data were also observed by the Nurse's Health study, which after 16 years of follow-up showed a lower risk of overall mortality and lower risk of CVD associated with fish and n-3 FA intake, even after results were adjusted for confounding dietary variables (Hu et al., 2002). In the large-scale GISSI

Prevenzione trial, treatment with n-3 FA (1 g/day) resulted in significant reductions in all-cause mortality and cardiovascular mortality in patients surviving recent myocardial infarction (GISSI-Prevenzione Investigators, 1999), without affecting the risk of non-fatal coronary events. The authors suggested that the benefit of n-3 FA might not be mediated via antiatherosclerotic and antithrombotic effects, but rather antiarrhythmic, with these effects becoming apparent within only a few months after starting treatment (Marchioli et al., 2002). Thies et al. (2003) showed that treatment with fish oil (1.4 g/day n-3 FA) in patients awaiting carotid endarterectomy promoted the incorporation of n-3 FA in atherosclerotic plaque lipids, and enhanced atherosclerotic plaque stability, as observed by fibrous cap formation and decreased macrophage infiltration. Years later, the same group conducted another study with similar patients but using n-3 FA as ethyl acids (Omacor®), at 1.8 g EPA+DHA/day, rather than the TAG form. The authors once again observed the association between a higher EPA content in the plaque and lower plaque inflammation and instability (Cawood et al., 2010), which could contribute to lower cardiovascular mortality.

A systematic review that gathered data available until 2012 from randomized controlled trials and clinical trials concluded that n-3 FA intake for at least 6 months reduces cardiovascular events by 10%, cardiac death by 9% and coronary events by 18%, mainly in persons with high cardiovascular risk (Delgado-Lista et al., 2012). Controversially, other large trials have found that n-3 FA supplementation was not associated with major cardiovascular events reduction (Rizos et al., 2012; Roncaglioni et al., 2013). In the Origin trial, for example, consumption of 1 g of n-3 FA acids for six years did not reduce the rate of death from cardiovascular causes or other outcomes in patients at high risk for cardiovascular events (The Origin Trial Investigators, 2012).

In the Alpha Omega Trial (Kromhout et al., 2010), low dose of EPA-DHA (400 mg/d) for 40 months did not reduce the rate of cardiovascular events in patients surviving myocardial infarction.

PLANT STEROL ESTERS

Plant sterols or phytosterols are steroid alcohols synthesized *de novo* by the isoprenoid pathway, and are responsible for several functions in plant metabolism (Piironen et al., 2000). Plant sterol biosynthesis in higher plants occurs via cycloartenol and also via lanosterol, which is different from yeasts that use only lanosterol (Ohyama et al., 2009). They are derived from squalene and present a molecular structure similar to that of cholesterol (Figure 1). Present as free (FPS) or in an esterified form (PSE), they can contain a double bond in the ring (sterols) or be saturated (stanols) (De Smet et al., 2012). The main plant sterols present in the human diet are β-sistoterol, stigmasterol, campesterol and brassicasterol, found in vegetable oils, nuts, fruits and cereals (Piironen et al., 2000; Ostlund, 2007).

Due to their relative hydrophobicity, plant sterol absorption involves the cleavage of PSE into FPS in the lumen, solubilization into the emulsified fat phase and formation of a micelle that drives FPS to the brush border membrane (Figure 2), where they are absorbed by the same mechanism as cholesterol, via transporters proteins such as NPC1L1. Once inside the enterocyte cytoplasm, the most part of plant sterols will undergo efflux back to the lumen, mediated by a class of proteins known as ABC transporters, specifically ABCG5 and ABCG8. These two proteins are also expressed on the apical surface of hepatocytes, and are required to export plant sterols into the bile (Davis et al., 2004). The presence of plant sterols in the enterocyte reduces

the action of ACAT-2, the enzyme responsible for esterifying sterols, which is a necessary step for their incorporation into chylomicrons by microsomal triglycerides transfer protein (MTP) and further release into the lymph. However, it has been suggested that ACAT-2 reduction may be only a consequence of a reduction in cholesterol flux through the enterocyte (Davis et al., 2004). In addition, during digestion, plant sterols can displace cholesterol in micelle formation, reducing cholesterol absorption. All conjoint mechanisms contribute to a reduced cholesterol (40-60%) and plant sterol (< 15%) absorption rate (De Smet et al., 2012).

Several clinical studies and meta-analyses have shown that consumption of 2-2.5 g/day of PSE promotes a consistent LDL-C reduction of 10%, in average (Miettinen et al., 1995; Ostlund, 2007; Demonty et al., 2009; Ras et al., 2014). Based on epidemiological data and clinical trials with cholesterol-lowering drugs, long-term use of PSE could lower CVD risk up to 20% over a lifetime (Katan et al., 2003). However, as far as we know, no epidemiological study has evaluated the effects of PSE supplementation over CVD outcomes up to now. Available data also suggest a moderate reduction of TAG after PSE intake, with little or no effect over HDL-C and CRP levels (Gylling et al., 2014; Rocha et al., 2016).

In 2010, the FDA approved the health claim for functional foods that provide 1.3 g of plant sterols/day (FDA, 2010). Based on this approval, food companies started to commercialize food products such as margarine, milk, yogurts, biscuits and others containing PSE. In fact, the consumption of these functional foods has been recommended by physicians to patients with hypercholesterolemia, and is nowadays incorporated into National Cholesterol Education Program (NCEP) guidelines (De Smet et al., 2012).

A recent study carried out by our group showed that the response in terms of LDL-C reduction varies according to individual differences (Bertolami et al., 2014). This variation depends on an individual's ability to absorb and synthesize sterols. For example, it has been suggested that individuals with high basal cholesterol synthesis are less responsive to PSE interventions than those who present low endogenous synthesis and, consequently, are better sterol absorbers (Rideout et al., 2010; Mackay et al., 2015). For this reason, measurements of lathosterol and plant sterols in plasma could provide important information about an individual's capacity for cholesterol synthesis and absorption, respectively, improving the prescription's efficiency.

PHENOLIC COMPOUNDS

Phenolic compounds (PHC) are secondary metabolites in plants and comprise a large class of phytochemicals with different chemical structures (Cheynier, 2012). The phenolic content of plants varies according to the plant and its condition of development (Soto-Vaca et al., 2012), which also influences the type of compound and its corresponding role in the organism. Some examples include the formation of pigments, protection against insects and UV radiation, and antioxidant protection against reactive species (Cheynier, 2012). Evidence from clinical trials and epidemiological studies have suggested that the consumption of fruits and vegetables provides health benefits beyond basic nutrition and also protection against chronic diseases (Dauchet et al., 2009; Boeing et al., 2012), which triggered interest in investigating PHC and their possible role in CVD prevention (Soto-Vaca et al., 2012). Although controversy remains whether PHC-rich food/beverage consumption decreases CVD risk, protective effects of green

tea and red wine have been highlighted by numerous studies (Del Rio et al., 2013; Pang et al., 2016).

Green Tea

The consumption of green tea predominantly occurs in Asia, especially in countries such as China and Japan (Yang and Wang, 2011; Ozen et al., 2012). Green tea is obtained by processing the leaves of *Camellia sinensis*, which affects its composition and the amount of phenolic compounds. Processing promotes the inactivation of enzymes and the stabilization of tea components (Yang and Wang, 2011) resulting in 80-90% catechins and 10% other flavanols (Deka and Vita, 2011). Catechins, epigallocatechin-3-gallate (EGCG) (Figure 1), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate(EGC) and (-)-epicatechin (EC) are the main phenolic compounds present in green tea (Yuan et al., 2011).

Epidemiological studies carried out especially in Asia have suggested an inverse association between the risk of cardiovascular disease and elevated consumption of green tea, which could be linked to flavonoids (Moore et al., 2009; Deka and Vita, 2011). Regarding dyslipidemia, an LDL-C lowering effect has been observed after the consumption of flavin-enriched green tea for 12 weeks (Deka and Vita, 2011). Moreover, some studies have suggested beneficial effects of green tea consumption (or extract) in reducing blood pressure, decreasing the risk of diabetes mellitus and diminishing body weight. Even though most of these studies demonstrated a beneficial effect on CVD and metabolic syndrome, some other observational studies did not obtain the same outcomes, mostly justified by differences of in study characteristics, such as population, dosage, follow-up time and selected biomarkers (Deka and

Vita, 2011). Some experimental and human interventional trials have suggested possible mechanisms of action of catechins in the context of reducing CVD events, including anti-inflammatory, anti-proliferative and anti-thrombotic effects (Moore et al., 2009; Deka and Vita, 2011).

Inflammation and endothelial dysfunction play important roles during atherosclerosis development, and catechins have been demonstrated to target some important elements in these process (Naito and Yoshikawa, 2009; Moore et al., 2009; Deka and Vita, 2011). *In vitro* studies have revealed that EGCG inhibits the migration of neutrophils and macrophages and promotes a reduction in ROS production by inflammatory cells. Regarding endothelial function, EGCG may improve the availability of NO by stimulating eNOS phosphorylation and consequently increasing the production of NO and improving endothelial-dependent vasodilation (Deka and Vita, 2011). Additionally, it has been reported that catechins may reduce cellular adhesion molecule expression through the inhibition of ICAM-1/VCAM-1 expression (Naito and Yoshikawa, 2009). Considering the antioxidant effects of green tea, studies have been controversial. However, increased capacity to scavenge ROS has been mentioned as a possible beneficial effect, taking into account the susceptibility of LDL to oxidation (Basu and Lucas, 2007; Deka and Vita, 2011). Despite the fact that there is not yet a clear association between green tea consumption and clinical outcomes, green tea is currently considered a safe drink and a possible healthy choice for CVD risk reduction (Deka and Vita, 2011; Pang et al., 2016).

Red wine

After studies based on the “French Paradox”, which highlighted the association between dietary intake of wine and risk of cardiovascular death, wine has been a target of interest, especially by lowering the prevalence of coronary heart diseases after regular moderate intake (Gresele et al., 2011; Fernández-Mar et al., 2012). The cardiovascular protection observed after the consumption of red wine and grapes is attributed to its phenolic compound content (Fernández-Mar et al., 2012). Among them, resveratrol may be the main phenolic related to cardiovascular outcomes (Figure 1) (Szkudelska and Szkudelski, 2010; Gresele et al., 2011). Resveratrol is a stilbene derivate that has been shown to exert beneficial effects as modulation of lipoprotein metabolism, antioxidant activity through the inhibition of ROS (quenching), modulation of platelet function and activation of eNOS (Gresele et al., 2011; Fernández-Mar et al., 2012). Anti-inflammatory effects have also been reported, including modulation of COX-2 activity (Das and Das, 2010; Tang et al., 2014) and inhibition of phospholipase-D, pro-inflammatory cytokine (IL-1, TNF-alpha, IL-6) and TNF-induced NF-kB activation (Tang et al., 2014).

During initial atherosclerotic lesion formation, resveratrol decreases the expression of adhesion molecules such as ICAM-1/VCAM-1. Moreover, it has been shown to reduce MCP-1 expression through the PI3k/Akt pathway, consequently diminishing monocyte recruitment. Considering foam cells, resveratrol may reduce their formation by modulating cholesterol transport and removal. These effects are related to increased cholesterol efflux, free cholesterol removal, downregulation of oxLDL uptake and stimulation of mature HDL particles (Tang et al., 2014).

A systematic review that gathered results from seven controlled trials concluded that resveratrol supplementation has little effect on lipoprotein metabolism and that cardioprotection may rather be associated with its anti-inflammatory and antioxidant effects (Sahebkar, 2013). Although clinical data about resveratrol is still very limited, many resveratrol supplements are commercially available and widely consumed.

Besides resveratrol, other bioactive compounds, as hydroxytyrosol (Fernández-Mar et al., 2012) catechins and quercetin (Bertelli and Das, 2009) are present in wine. For this reason, different polyphenols in wine may act together, providing cardiovascular benefits related to wine consumption (Gresele et al., 2011; Calabriso et al., 2016). However, it has been suggested that to attain cardiovascular protection, moderate red wine consumption should focus on wines with high *in vitro* antioxidant activity (Macedo et al., 2012). Although this kind of information is not available on bottle labels, and health claims for alcoholic beverages are not recommended, the product price may provide an initial indication of its functionality, although this is still under investigation. Llobodanin et al. (2014) evaluated the *in vitro* antioxidant activity of 666 samples of red wine and concluded that there was an increase in antioxidant activity from US $27.00 to US $37.00/bottle, while above this value no additional benefit in terms of antioxidant activity could be achieved.

More studies are needed to be certain of the beneficial effects of wine in humans, although it is certain that moderate consumption of red wine has an important influence on cardioprotection.

BIOACTIVE COMPOUNDS AS CO-THERAPY WITH DRUGS

Some studies have suggested that n-3 FA combined with statin therapy improves lipid biomarkers in hyperlipidemic patients (Barter and Ginsberg, 2008) and may be preferable to other drug combinations (Micallef and Garg, 2009a; Eussen et al., 2010). The Japan EPA Lipid Intervention Study (JELIS) was a pioneering study that evaluated the combination of n-3 FA and statin treatment. The authors observed that EPA supplementation reduced major coronary events by 19% in statin treated patients for secondary prevention, while a non-significant 18% reduction was observed in patients with no history of coronary artery disease (Yokoyama et al., 2007). In the same line of study, the GISSI-HF trial enrolled approximately 7000 patients with heart failure treated with multiagent therapy. The results showed a significant benefit of n-3 FA co-therapy (850--882 mg of EPA plus DHA), which was effective at reducing both all-cause mortality and admissions to hospital for cardiovascular reasons (Tavazzi et al., 2008).

Additionally, the COMBOS trial (Davidson et al., 2007) evaluated the lipid lowering efficacy of n-3 FA (4 g/d) combined to simvastatin (40 mg/d) in 254 subjects with persistent hypertriglyceridemia. After eight weeks, the combined treatment significantly reduced non-HDL-C by 9.0%, while the reduction after placebo plus simvastatin treatment was only 2.2%. The combination of n-3 FA with simvastatin also reduced triglycerides by 29.5%, VLDL-C by 27.5%, significantly increased HDL-C by 3.4% and significantly reduced the total cholesterol:HDL-C ratio (9.6%). Similar results were also observed in the ESPRIT trial (Maki et al., 2013).

Besides plasma lipid lowering effects, n-3 FA in combination to statins may also alter the lipoprotein profile toward less atherogenic particles (Nordoy et al., 2001; Micallef and Garg, 2009a). It was shown that n-3 FA combined with atorvastatin therapy increased the average LDL particle size without increasing the particle concentration (Maki et al., 2011).

Since the combination of n-3 FA with statins seems to be effective, especially on the TAG concentration, adding a third compound to this combination, such as PSE, may provide complementary action on circulating lipids, besides providing more comprehensive health benefits via anti-inflammatory effects and improved vascular function (Micallef and Garg, 2009a). Adding PSE to statin treatment promotes an additive reduction of 10%-15% on total cholesterol and LDL-C, which is similar or even more effective than doubling the statin dose (Katan et al., 2003; Scholle et al., 2009). This mean reduction in LDL-C does not seem to increase significantly further with PSE doses above 2.5 g/day or in long-term consumption. For example, the intake of 3.0 g/day of PSE by type 1 diabetic patients on statin treatment also reduced mean total and LDL-C by 10--16% compared with the baseline values, and by 8--15% compared with the control group (Hallikainen et al., 2011). A mean LDL-C reduction of 10% was also observed after adding even higher doses (5.1 g/d of PSE) to the daily diet of statin treated patients, for eight weeks, in a placebo-controlled clinical trial that enrolled 141 hypercholesterolemic patients (Blair et al., 2000). The authors also observed that this effect was similar for all four types of statins included in the study (atorvastatin, pravastatin, simvastatin and lovastatin). Furthermore, similar results were observed after long-term consumption of margarine enriched with PSE by statin treated patients (De Jong et al., 2008). Consumption of

2.5 g of plant sterol or stanol esters lowered LDL-C concentrations by 8.7 and 13.1%, respectively, over a period of 1.5 years (De Jong et al., 2008).

There are still few available data about combined therapy with statins and PHC. Naruszewicz et al. (2007) first observed the effects of combined treatment of statins and flavonoids in patients with coronary disease. Supplementation with 255 mg/day of a chokeberry flavonoid extract (about 25% anthocyans, 50% polymeric procyanidines and 9% phenolic acids), for six weeks, significantly reduced oxidative markers as serum 8-isoprostans and the oxLDL concentration (by 38 and 29%, respectively), and also reduced inflammation, as observed by a 23% reduction in hs-CRP (Naruszewicz et al., 2007).

The interaction of moderate consumption of wine and statin treatment has not been studied so far. However, after six months of follow-up, a randomized, placebo-controlled trial evaluated the effects of a resveratrol-enriched grape supplement (Stilvid®) in statin-treated patients (Tomé-Carneiro et al., 2012). The authors observed that consumption of one capsule of Stilvid® daily (containing 350 mg grape polyphenols, including 8 mg resveratrol) significantly decreased apoB (9.8%) and oxLDL (20%) levels, whereas LDL-C was only slightly reduced (4.5%). On the contrary, the consumption of yerba mate (1.7 g of total phenols per day) promoted additional 10.0% and 13.1% reductions in LDL-C after consumption for 20 and 40 days, respectively ($p<0.001$), in hypercholesterolemic individuals on statin therapy. Polyphenol rich beverage intake also increased HDL-C by 6.2% after 40 days (De Morais et al., 2009). Further improvement in lipoprotein status was also observed after one year of combined treatment with statins and flavonoids (Curtis et al., 2012). Consumption of 27 g/day of a

flavonoid-enriched chocolate (containing 850 mg flavan-3-ols [90 mg epicatechin] and 100 mg isoflavones [aglycone equivalents)]/day) by type 2 diabetic patients significantly reduced LDL-C and the total cholesterol:HDL-C ratio, and attenuated the estimated 10-year risk of CVD (Curtis et al., 2012).

Combination of bioactive compounds and fibrate has also been investigated. The intake of n-3 FA by hypertriglyceridemic patients on fibrate treatment was shown to reduce monocyte secretion of TNF-α, IL-1β, IL-6 and MCP-1, in addition to a significant reduction in TAG (Krysiak et al., 2012). Even patients with severe hypertriglyceridemia stably treated with fenofibrate (130 mg) benefitted from n-3 FA supplementation, showing an additive and significant TAG reduction of 17% (Roth et al., 2009).

There is little or no information regarding treatment with PCSK9 inhibitors and ezetimibe combined with bioactive compounds in humans. Although ezetimibe is a strong pharmacologic inhibitor of intestinal cholesterol absorption, its combination with PSE increases cholesterol fecal excretion and reduces plasma LDL-C, indicating that this compound may act by different pathways on cholesterol metabolism (Lin et al., 2011) (Figure 2).

Regarding to niacin, a pilot study evaluated the effects of n-3 FA and niacin therapy, either alone or in combination. Despite including only a small number of dyslipidemic subjects (n = 29), the results showed that 3.4 g of n-3 FA combined with 3 g of niacin reduced TAG by 50% and increased HDL-C by 30%. The concentration of LDL-C was not altered in either treatment group. Moreover, the addition of n-3 FA did not affect niacin flushing (Isley et al., 2007). A larger study including 60 participants evaluated the effects of extended-release niacin

and n-3 FA on metabolic syndrome. The results corroborated previous findings, showing that niacin increased HDL-C, while n-3 FA improved hypertriglyceridemia. Although the LDL-C concentration was not altered by this combination, LDL particle characteristics were changed, leading to less atherogenic forms (Shearer et al., 2012).

Some of the findings on the use of single bioactive compounds as co-therapy with drugs are summarized in **Table 2.**

CONSIDERATIONS AND FUTURE DIRECTIONS

Altogether, clinical studies regarding bioactive compounds as co-therapy with drugs were mainly designed to investigate the effect of combining statins with n-3 FA or PSE. Among them, trials with n-3 FA enrolled a higher number of patients and for longer periods. The large number of positive results from previous cellular and animal models, added to the wide availability of n-3 FA capsules in the drugstores, was especially responsible for boosting n-3 FA research. On the contrary, combination of statins and PSE has been given through functional foods rather than capsules. Whereas PSE can be easily added to different kinds of products, the food industry still struggles with technological issues in adding n-3 FA to food formulations, especially at high doses. Oxidative stability and sensorial characteristics of n-3 FA enriched products are still obstacles to be overcome in order to provide adequate amounts of these bioactive compounds through diet.

Dietetic approach to cardiovascular disease undergoes a change of concept as focus shifts from prevention towards disease treatment. The studies performed with combination of bioactive compounds and drugs suggest potential additive effect after intervention, although differences

among biomarkers and clinical outcomes were observed. While some compounds act on the same biochemical parameter as drugs, complementary effects by reduction of a secondary target can also be observed after co-therapy. Lack of effect has also been reported, and can be attributed to the fact that statin treatment may dilute the preventive cardiovascular effects of bioactive compounds, as it has been previous suggested for n-3 FA (Eussen et al., 2012). Nevertheless, studies so far have not associated any risk to the different combinations that have been tested.

Diet therapy may not only contribute to increase responsiveness to drugs but also to increase treatment compliance. One of the proposed strategies to increase treatment adherence, and that has been gaining strength in the last years, is the use of "Polypill"- a pill that combines four to five drugs (Wiley and Fuster, 2014). However, the Polypill concept of fixed-dose combination goes against the current trend of personalized medicine. Rather than multidrug combination, we believe that future efforts should focus on multi-bioactive combination.

A few studies already observed the effects of combining n-3 FA and PSE (**Table 3**). In a recent study carried out by our group, hyperlipidemic patients were supplemented with a combination of n-3 FA, PSE and green tea (data not shown). We observed a reduction on lipid parameters (LDL-C and TAG), as well as inflammatory and oxidative stress markers. However, the results were strongly related to patient's individual characteristics. In general, the outcomes observed from these studies suggest both synergistic and complementary effects through multi-bioactive combination.

Considering the results observed for drug combination with bioactive compounds and for multi-bioactive combination, we believe that all mechanistic pathways attributed to each individual compound/drug (that are summarized in Table 1 and in Figure 2) can be simultaneously activated during treatments and do not interfere with each other. This is especially relevant in terms of dietary co-therapy and highlights the potential for new food products development where different compounds may be present in the same food matrix.

Current strengths and drawbacks for the use n-3 FA, PSE and PHC in CVD prevention are summarized in Table 4.

CONCLUSION

The combination of bioactive compounds with drugs appears to be a safe and effective therapy to reduce CVD progression. However, many drug interactions with bioactives, especially phenolic compounds, are still poorly understood and documented. Although data so far indicate a potential additive and/or complementary effect, large clinical trials are still necessary to evaluate changes in biomarkers and clinical outcomes. Based on this information, physicians may recommend specific combinations to improve patient's treatment. It is worthy to highlight that patients respond individually to bioactive compounds, as well as to drugs. Thus, the effect of bioactive compounds and drug combination will be particular to each patient. The number of bioactive compounds sold in the drugstores for individuals who aim to prevent or treat CVD shows that the combination is already a reality. Pharmaceutical companies, physicians and food scientists should be prepared to better understand this type of interaction and its consequences in terms of efficacy and life quality.

List of abbreviations

ALA	α-linolenic acid
CE	cholesteryl ester
CETP	cholesteryl ester transfer protein
COX	cycloxigenase
CVD	Cardiovascular diseases
DGAT2	diacylglycerol O-acyltransferase 2
DHA	docosahexaenoic acid
EGCG	epigallocatechin-3-gallate
eNOS	endothelial nitric oxide synthase
EPA	eicosapentaenoic acid
FC	free cholesterol
FDA	Food and Drug Administration
FFA	free fatty acids
FPS	free plant sterol
GSH-Px	glutathione peroxidase
HDL-C	high-density lipoprotein cholesterol
HMG CoA	3-hydroxy-3-methylglutaryl coenzyme A
LA	linoleic acid

LDL low-density lipoprotein

LDL-C low-density lipoprotein cholesterol

LDLr LDL receptor

LOX lipoxigenase

LPL lipoprotein lipase

MCP-1 monocyte chemotactic protein 1

MM LDL minimally modified very low density lipoprotein

MPO myeloperoxidase

n-3 FA omega-3 fatty acids

NCEP National Cholesterol Education Program

NF-kB nuclear factor kappa B

NO nitric oxide

NOX NADPH oxidase

NPC1L1 Niemann-Pick C1 Like 1

oxLDL oxidized low density lipoprotein

PAF platelet-activating factor

PCSK9 Proprotein convertase subtilisin/kesin type 9

PHC phenolic compounds

PPAR-α Peroxisome proliferator-activated receptor alfa

PSE plant sterol/stanol esters

ROS reactive oxygen species

SOD superoxide dismutase

SREBP-1c sterol regulatory element-binding protein-1c gene

SREBP-2 sterol regulatory element binding protein-2

TAG triacylglycerol

VLDL-C very low density lipoprotein cholesterol.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

BS designed the manuscript; BS, IAC and HSJK wrote the paper; IAC had primary responsibility for final content. All authors read and approved the final manuscript.

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Table 1: Strengths and drawbacks of current pharmacological therapies for cardiovascular disease prevention

Drug	Mechanism of action	Strengths	Drawbacks
Statins	Cholesterol synthesis inhibition, primarily in the liver, by blocking 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the principal rate-limiting enzyme of cholesterol biosynthesis (Brautbar and Ballantyne, 2011; Lamon-Fava, 2013)	Statins are ubiquitously the first-line drug therapy for LDL-C lowering.	Even patients treated with statins have a considerable residual burden of cardiovascular risk (Libby et al., 2011).
		However, they also provide cardiovascular protection beyond their cholesterol-lowering action, called pleiotropic effects, which include anti-inflammatory and antioxidant effects at the vascular wall, thus improving endothelial function and enhancing atherosclerotic plaque stability (Takemoto and Liao, 2001; Ridker et al., 2008; Babelova et al., 2013; Antoniades and Channon, 2014).	Additionally, many patients are unable to tolerate the maximal dose of statin therapy, and others do not tolerate any dose at all (Brautbar and Ballantyne, 2011). Adverse effects range from myalgia to serious muscle damage, which is often accompanied by evidence of renal dysfunction. Cognitive impairment, memory problems and elevation of liver enzymes have also been described (Fernandez et al., 2011; Psaty and Rivara, 2012).
Ezetimibe	Cholesterol absorption inhibition. Ezetimibe	Ezetimibe is frequently administered in	Combined therapy with ezetimibe and statins appears

	binds to Niemann-Pick C1 Like 1 (NPC1L1), the principal cholesterol transporter in the brush border of the enterocyte (Reiner et al., 2011).	association with statins, particularly in the treatment of homozygous familial hypercholesterolemia or statin intolerant patients. Combined ezetimibe and statin therapy provides an additional 15--20% LDL-C reduction (Reiner et al., 2011).	to have little effect on the progression of atherosclerosis (Kastelein et al., 2008) and only a modest reduction on the occurrence of cardiovascular events (Cannon et al., 2015) in clinical practice.
Niacin	Decreases triacylglycerol (TAG) synthesis by the inhibition of diacylglycerol O-acyltransferase 2 (DGAT2) in the liver, and decreases TAG hydrolysis in adipose tissues and free fatty acid (FFA) flux to the liver. Niacin also increases high-density lipoprotein cholesterol (HDL-C) through the induction of the	Following nicotinic acid therapy, HDL-C increases in a dose-dependent manner up to 25%, and typically reduces LDL-C by 15--18% and TAG by 20--40% (Remaley et al., 2014). Option treatment for statin-intolerant patients with contraindications for other drugs (like ezetimibe) and who have high LDL-C (Lloyd-Jones, 2014).	Main drawback of niacin administration is the occurrence of side effects, especially cutaneous flushing (Olsson, 2010). Even niacin combined with laropiprant (formulation that minimizes facial flushing effects of niacin) was shown to provoke serious adverse events such as an increased occurrence of diabetic complications, serious bleeding, gastrointestinal events, serious infection, myopathy and skin-related events (HPS2--THRIVE

	hepatic production of apoAI (Chapman et al., 2010; Remaley et al., 2014).		study) (Landray et al., 2014).
Fibrates	Fibrate acts as an agonist of peroxisome proliferator-activated receptor alpha (PPAR-α), which is thought to lower plasma TAG concentrations by activating lipoprotein lipase (LPL) and by decreasing hepatic synthesis of fatty acids (Chapman et al., 2010; Katsiki et al., 2013).	A modest increase in HDL-C (up to 5--15%) can be observed after fibrate administration, as a consequence of stimulated production of apoAI and apoAII by PPAR-α (Ewang-Emukowhate and Wierzbicki, 2013).	Fibrates have been observed to raise plasma creatinine concentration, even though its effects on the kidney are still unknown (Wilkinson et al., 2014). Additionally, fibrates can interact with other drugs. One example is the increased risk of myopathy and rhabdomyolysis resulting from the interaction of gemfibrozil with statins (Chapman et al., 2010; Wilkinson et al., 2014).
PCSK9 Inhibitors	PCSK9 Inhibitors are a new and emerging class of therapeutic options for lowering LDL-C, and are still on study. Proprotein convertase subtilisin/kesin type 9 (PCSK9) is secreted mainly in the liver and binds LDL receptor	Phase II trials showed that PCSK9 inhibitors, particularly monoclonal antibodies, reduce LDL-C by between 60 and 70%, even in the presence of background statin therapy (Norata et al., 2014; Stein and Raal, 2014). Recently, the FDA approved	The lack of conclusive data about possible adverse events and undesirable consequences for the patient, especially that could be related to a very low concentration of LDL-C (Dadu and Ballantyne, 2014), have been some of the obstacles to fully understand these new emerging therapies. For this reason, it is necessary

	(LDLr) on the hepatocyte surface. This complex is internalized and undergoes hepatic degradation, leading to a decreased number of available LDLr, and thereby increasing circulating LDL (Dadu and Ballantyne, 2014). Therefore, PCSK9 inhibition increases LDL-C clearance.	Praluent® (alirocumab) for use in adult patients with heterozygous familial hypercholesterolemia in addition to maximally tolerated statin therapy or patients with clinical atherosclerotic cardiovascular disease such as heart attacks or strokes, who require additional lowering of LDL-C (FDA, 2015; Perkel, 2015).	to observe and look forward for more results and data about this new strategy of targeting PCSK9.

Table 2: Human intervention studies of drug therapy combined to bioactive compounds

Combination therapy	Study population	Follow-up	Major findings	Ref.
n-3 FA and statin (1800 mg EPA)	All subjects	4-6 years	Relative risk reduction of 19% in major coronary events	JELIS Yokoyama et al. (2007)
	9326 EPA			
	9319 control			
	Secondary prevention	4-6 years	19% reduction in major coronary events	
	6897 EPA			
	6900 control			
n-3 FA and multiagent therapy (850--882 mg of EPA plus DHA)	6975 subjects with heart failure	3-9 years	Reduction of all-cause mortality and admissions to hospital for cardiovascular reasons	GISSI-HF Tavazzi et al. (2008)
n-3 FA and statin (4g/d FA)	254 subjects on stable statin treatment	8 weeks	9% non-HDL cholesterol reduction; 29.5% TAG reduction; 27.5% VLDL reduction and 3.4% HDL increase	COMBOS Davidson et al. (2007)
n-3 FA and statin (4g/d FA)	432 patients with persistent hypertrygliceridemia	6 weeks	6.9% non-HDL cholesterol reduction; 20.6% TAG reduction	ESPRIT Maki et al. (2013)

n-3 FA and statin (4g/d FA)	237 subjects with mixed dyslipidemia.	8 weeks	Increase in average LDL particle size without particle concentration increase	Maki et al. (2011)
n-3 FA and statin (400 mg of EPA plus DHA)	4153 patients with history of myocardial infarction	41 months	n-3 FA co-therapy had no effect on major cardiovascular events, despite a significant reduction in triglycerides	Eussen et al. (2012)
PSE and statin (3g/d PSE)	24 type 1 diabetic patients	4 weeks	LDL reduction of 10-16% compared to baseline, and 8-15% compared to control group	Hallikainen et al. (2011)
PSE and statin (5.1g/d PSE)	141 hypercholesterolemic patients	8 weeks	LDL incremental reduction of 10%, regardless statin type	Blair et al. (2000)
PSE and statin (2.5 g/d PSE)	54 subjects on stable statin treatment	1.5 years	LDL reduction of 8.7-13.1%	De Jong et al. (2008)
Flavonoids and statin (255 mg/d chokeberry extract)	44 patients with history of myocardial infarction	6 weeks	38% reduction of 8-isoprostans; 29% reduction of oxLDL and 23% reduction of hs-CRP	Naruszewicz et al. (2007)
Grape supplement and statin (8 mg/d resveratrol)	75 subjects on stable statin treatment	6 months	Reduction of 9.8% in ApoB and 20% in oxLDL	Tomé-Carneiro et al. (2012)

Yerba mate and statin (1.7g of total phenols)	102 subjects	40 days	Additional 13.1% reductions in LDL and 6.2% increase in HDL	De Morais et al. (2009)
Flavonoid enriched chocolate and statin (850 mg flavan-3-ols and 100 mg isoflavones)	93 type 2 diabetic patients	1 year	Significant reduction on LDL and attenuated the estimated 10-year risk of CVD	Curtis et al. (2012)
n-3 FA and fibrates (4 g/d FA)	46 subjects with isolated hypertriglyceridemia	90 days	35% reduction in TAG and reduction on monocyte secretion of TNF-alfa, IL-1beta, IL-6 and MCP-1	Krysiak et al. (2012)
n-3 FA and fibrates (4 g/d FA)	58 patients with severe hypertriglyceridemia stably treated with fenofibrate	8 weeks	Additive and significant TAG reduction of 17%	Roth et al. (2009)
n-3 FA and niacin (3.4 g/d FA)	29 dyslipidemic subjects	12 weeks	TAG reduction of 50% and HDL increase of 30%.	Isley et al. (2007)
n-3 FA and niacin (4 g/d FA)	60 metabolic syndrome subjects	16 weeks	TAG reduction of 34% and HDL increase by 7.8md/dL; LDL particles changed to the less atherogenic, large, buoyant	Shearer et al. (2012)

			particles	

Abbreviations. n-3 FA, omega 3 fatty acids; TAG, triacylglycerol; PSE, plant sterol esters.

Table 3: Human intervention studies with combination between n-3 fatty acids and plant sterols.

Combination dosage	Study population	Follow-up	Main findings	Ref.
phytosterol-enriched spread (2 g/d) and n-3 FA capsules (1.4 g/d)	60 hiperlipidemic subjects	3 weeks	LDL reduction of 12.5%, HDL-C increase of 8.6% and TAG reduction of 25.9% hs-CRP reduction of 39%	Micallef and Garg (2008; 2009b)
spreads containing a fixed amount of PSE (2.5 g/day) and varying amounts of EPA+DHA (0.0, 0.9, 1.3 and 1.8 g/day)	314 hypercholesterolemic subjects	4 weeks	LDL-C reduction of 13% and TAG reduction of 9--16% in dependence on the EPA+DHA dose.	Ras et al. (2014);
spreads containing a fixed amount of PSE (2.5 g/day) and varying amounts of EPA+DHA (0.0, 0.9, 1.3 and 1.8 g/day)	282 hypercholesterolemic subjects	4 weeks	Shifts in the lipoprotein distribution: VLDL particles were reduced in cholesterol and TAG content; HDL particles were increased in cholesterol and TAG.	Jacobs et al. (2015)
2 g/d plant sterol (yogurt drink) and 2 g/d omega-3 fatty acids from fish oil	178 mildly hypercholester-olemic subjects (total cholesterol 5.0--8.0	4 weeks	LDL-C reduction 4.2% and non-HDL-C reduction of 3.9% (non-	Khandelwal et al. (2013)

(capsules)	mmol/l)		significant)	

Table 4: Strengths and drawbacks of n-3 FA, PSE and PHC non-pharmacological therapies for CVD prevention

Compound	Mechanism of action	Strengths	Drawbacks
n-3 FA	Anti-inflammatory activity through substrate competition with n-6 FA and production of inflammation-resolving mediators as resolvin E1, resolvin D1 and protectin D1 (Calder, 2012). Hypotriglyceridemic effect: decreases TAG as the result of both increased lipolysis and decreased lipogenesis (Mozaffarian and Wu, 2011).	The intake of 2-4 g n-3 FA reduces about 25-30% of plasmatic TAG in hypertriglyceridemic patients (TAG < 500 mg/dL) with reduced or no side effects (Jacobson, 2008). n-3 FA may also affect atherosclerotic plaque composition, contributing to lower inflammation and instability (Cawood et al., 2010).	Its effects over CVD outcomes are still controversial. Oxidative stability is still an obstacle for n-3 FA enrich food products.
PSE	Cholesterol absorption inhibition through competition for enzymes and transporters. PSE can also displace cholesterol in micelle formation during digestion (De Smet et al., 2012).	Consumption of 2-2.5 g of PSE/day promotes an average reduction of 10% in LDL cholesterol, even in a statin background. This additive reduction is similar or even more effective than doubling the statin dose (Katan et al., 2003; Scholle et al., 2009). PSE enriched food products are readily available at food markets and no adverse	There are no available data on the long-term effects of PSE over CVD outcomes.

		effect has been documented.	
PHC	Protective effects of PHC are not fully elucidated. Main described activities include anti-inflammatory and antioxidative effects through several mechanisms (Del Rio et al., 2013).	Regular moderate intake of red wine has been associated with lower risk of cardiovascular death. Also, green tea and different types of PHC-rich food/beverage are commercially available and are considered safe.	There is not yet a clear association between green tea consumption and clinical outcomes.

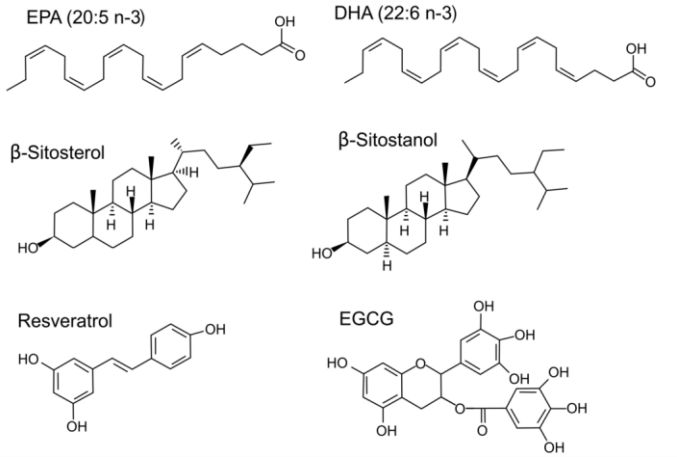

Fig. 1 Bioactive compounds with potential cardiovascular protection activity Molecule structure of representative omega-3 fatty acids (EPA and DHA), plant sterol/stanols (β-sitosterol and β-sitostanol) and phenolic compounds (resveratrol and EGCG). Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; EGCG, epigallocatechin-3-gallate.

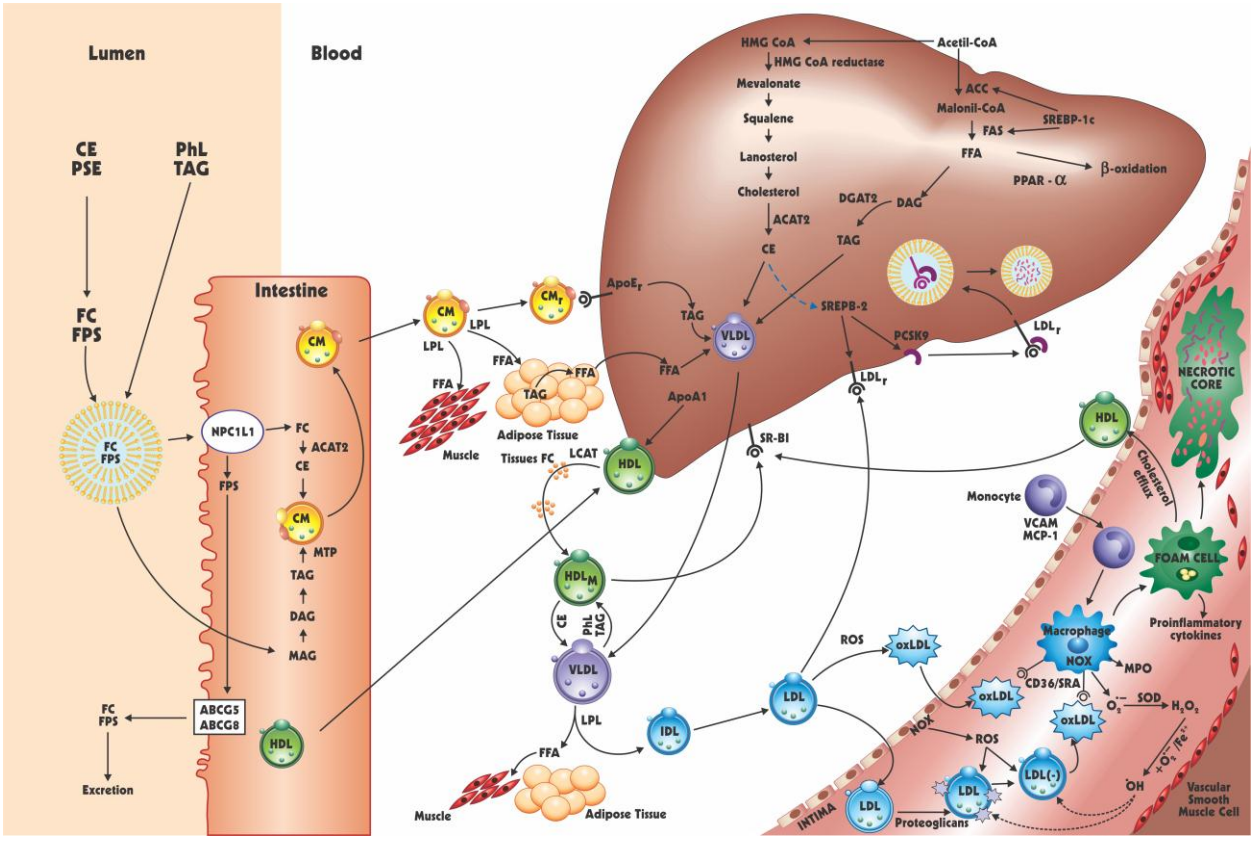

Fig. 2 Lipid metabolism and the atherosclerotic process Cholesterol, plant sterols, phospholipids and triglycerides obtained through diet are incorporated into mixed micelles in the intestinal lumen. Free cholesterol and free plant sterols are absorbed through the NPC1L1 transporter while monoacylglycerols and diacylglycerols enter into the enterocyte by facilitated diffusion at the brush border. Esterified cholesterol and triacylglycerols are further packed into chylomicrons, which are transported by the lymph to the circulation, delivering free fatty acids to peripheral tissues, through the activity of LPL. Chylomicron remnants undergo hepatic uptake, where contribute to the formation of VLDL along with esterified cholesterol (synthesized through the HMG CoA pathway) and TAG (synthesized through the malonyl-CoA pathway). VLDL particles are secreted into the bloodstream where they deliver FFA to tissues and

exchange TAG, CE and PhL with mature HDL. Further VLDL hydrolysis gives rise to IDL and LDL particles. LDL distributes CE to tissues and undergoes hepatic uptake through LDL receptors (LDLr). However, LDL particles may infiltrate the endothelial intima where they are retained via matrix proteoglycan binding. The retained LDL particles undergo modification, especially oxidation, resulting in electronegative LDL and oxidized LDL (oxLDL). This oxidative reaction can also occur in the bloodstream. Oxidized LDL (oxLDL) triggers an inflammatory process that includes monocyte migration, infiltration and differentiation into macrophages. Specific scavengers receptors in the macrophages, such as CD36 and SRA, recognize and internalize ox LDL, leading to the formation of foam cells and fatty streaks. In response to chemoattractants secreted by macrophages and foam cells, smooth muscle cells move into the intima and proliferate, forming a fibrous cap along with extracellular matrix molecules, such as elastin and collagen. With the progression of the atherosclerotic plaque, foam cells undergo apoptosis and give rise to a lipid-rich necrotic core. Proinflammatory mediators, smooth muscle cell death and protease degradation of the extracellular matrix weakens the fibrous cap of the mature plaque, making it susceptible to rupture and inducing the thrombus. The atherosclerotic process may be attenuated by HDL, as it promotes cholesterol efflux from other tissues by LCAT and also from the macrophages. HDL is recognized by SRB1 receptors in the liver, keeping the "cholesterol reserve transport" cycle. Drugs and bioactive compounds reduce atherosclerotic risk through the following mechanisms: statins reduce cholesterol synthesis through the inhibition of HMG CoA reductase; PCSK9 antibodies reduce LDL by inhibiting LDL_r degradation; ezetimibe and FPS inhibit cholesterol absorption through NPC1L1 competition; FPS also dislocates cholesterol from mixed micelles and reduces ACAT2 activity,

increasing cholesterol excretion; Niacin reduces TAG hydrolysis in adipose tissue, decreases TAG synthesis through DGTA2 and increases HDL through increased apoA1 synthesis; fibrates also increase HDL through apoA1, increase VLDL hydrolysis through LPL and increase TAG oxidation through PPARα; n-3 FA also activates PPARα, thus increasing β-oxidation, and reduces FA synthesis through SREBP-1c downregulation, reduces monocyte infiltration, may reduce endothelial NOX activity and activate eNOS; Phenolic compounds also activate eNOS, reduce the expression of adhesion molecules, reduce the expression of proinflammatory cytokines and diminishes oxidative stress. **Abbreviations:** ABCG5, ATP-binding cassette, sub-family G, member 5; ABCG8, ATP-binding cassette, sub-family G, member 8; ACAT2, acetyl-CoA acetyltransferase 2; ACC, Acetyl-CoA carboxylase; SRA, scavenger receptor class B/A; CE, cholesterol ester; CM, chylomicron; CM_r, chylomicron remnants; DAG, diacylglycerol; DGAT2, diacylglycerol O-acyltransferase 2; FAS, fatty acid synthase; FC, free cholesterol; FFA, free fatty acids; FPS, free plant sterols; HMG Coa-Reductase, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; HDL_M, mature high-density lipoprotein; HOCl, hypochlorous acid; IDL, Intermediate Density Lipoproteins; LCAT, lecithin-cholesterol acyltransferase; LPL, lipoprotein lipase; MAG, monoacylglycerol; MCP-1, monocyte chemotactic protein 1; MPO, myeloperoxidase; MTP, microsomal triglyceride transfer protein; NOX, NADPH oxidase; NPC1L1, Niemann-Pick C1 Like 1; oxLDL, oxidized LDL; PCSK9, proprotein convertase subtilisin/kesin type 9; PhL, phospholipids; PPAR-α, Peroxisome proliferator-activated receptor alpha; PSE, plant sterol ester; ROS, reactive oxygen species; SRA, scavenger receptor class B/A; SBR1, Scavenger receptor class B member 1; SOD, superoxide dismutase; SREBP-1c, sterol-regulatory-element-binding protein-1c; SREBP-2, sterol regulatory element-binding transcription

factor 2; TAG, triacylglycerol; VCAM, vascular cell adhesion molecule; VLDL, Very Low Density Lipoproteins.