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Review Article

Food Ingredients That Inhibit Cholesterol Absorption

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ABSTRACT: Cholesterol is a vital component of the human body. It stabilizes cell membranes and is the precursor of bile acids, vitamin D and steroid hormones. However, cholesterol accumulation in the bloodstream (hypercholesterolemia) can cause atherosclerotic plaques within artery walls, leading to heart attacks and strokes. The efficiency of cholesterol absorption in the small intestine is of great interest because human and animal studies have linked cholesterol absorption with plasma concentration of total and low density lipoprotein cholesterol. Cholesterol absorption is highly regulated and influenced by particular compounds in the food supply. Therefore, it is desirable to learn more about natural food components that inhibit cholesterol absorption so that food ingredients and dietary supplements can be developed for consumers who wish to manage their plasma cholesterol levels by non-pharmacological means. Food components thus far identified as inhibitors of cholesterol absorption include phytosterols, soluble fibers, phospholipids, and stearic acid.

Keywords: cholesterol absorption, functional food, nutraceutical, dietary supplement

INTRODUCTION

Cholesterol is an essential component of the human body. Cholesterol functions in a variety of capacities including, but not limited to, stabilizing cell membranes and serving as precursor for bile acids, vitamin D, and steroid hormones. Every cell of the human body can synthesize cholesterol when needed, but cells cannot catabolize cholesterol by oxidative processes. Therefore, any excess cholesterol must be transported to the liver, secreted into bile (as cholesterol or bile acids), and eliminated from the body by the intestinal route. Whole body cholesterol metabolism is in a delicate balance. When imbalances occur, cholesterol can accumulate in the gallbladder promoting gallstone formation. Cholesterol accumulation in the bloodstream (hypercholesterolemia) can cause atherosclerotic plaques to form within artery walls.

The discovery of transporters, receptors, and enzymes specific to cholesterol metabolism has changed our understanding of how sterols are absorbed into the body. The transport of cholesterol and phytosterols into the enterocyte is a tightly regulated process (Fig. 1). From a health standpoint, the efficiency of cholesterol absorption and the crosstalk with the processes of cholesterol and lipoprotein synthesis is of great interest, as many studies have linked cholesterol absorption with plasma total and low density lipoprotein (LDL) cholesterol concentration (1-5). This link is so important that a class of drugs was developed that blocks the intestinal absorption of cholesterol and, consequently, lowers plasma LDL cholesterol concentration. This class of drugs has also helped researchers elucidate some of the mechanisms of cholesterol transport at the cellular level (6,7). One of the drugs, ezetimibe, has become an important therapy in managing LDL cholesterol levels in individuals who can tolerate drug therapy. However, because drugs can produce severe side effects, it is desirable to learn more about natural food components that inhibit cholesterol absorption so that food ingredients and dietary supplements can be developed for consumers who wish to manage their plasma cholesterol levels by non-pharmacological means. Mc Auley et al. (8) developed a mathematical model of whole body cholesterol metabolism and the dysfunction that can follow with aging. The authors hypothesized that their model could be used to evaluate the effects of lifestyle modifications, such as phytosterol and fiber intake, to inhibit cholesterol absorption and ultimately the potential for lowering plasma LDL cholesterol.

When foods are consumed, cholesterol arrives in the small intestine from both the diet and bile (Fig. 1). Die-

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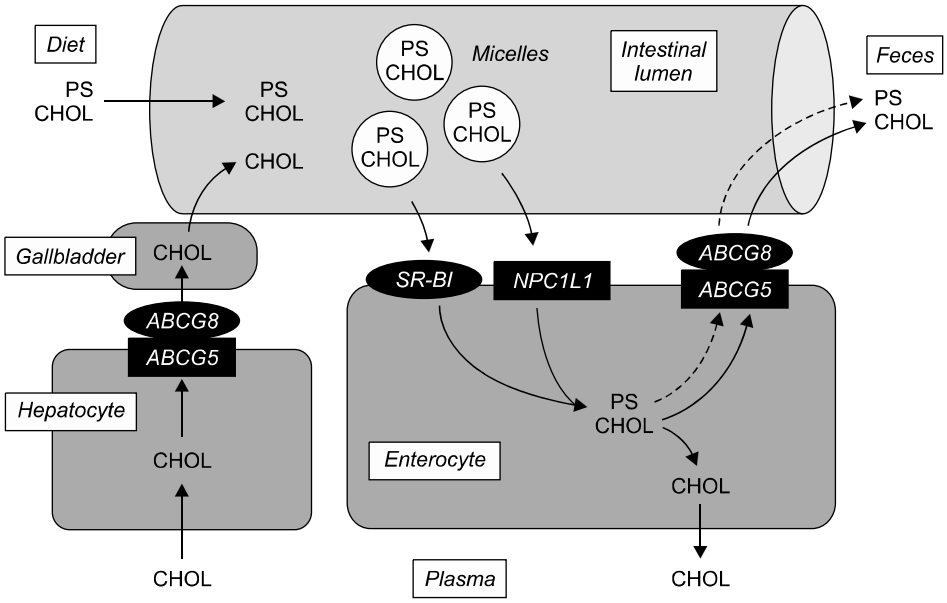

Fig. 1. Transport of cholesterol (CHOL) and phytosterols (PS) in the intestinal lumen, enterocytes, hepatocytes, and plasma. Dietary CHOL and PS mix with biliary CHOL from the gallbladder to form micelles within the intestinal lumen. The micelles also contain other digested lipids that are transported to the brush border where the lipids are delivered to the enterocyte. Specific transporters, scavenger receptor class B type I (SR-BI) and Neiman-Pick C1 Like 1 (NPC1L1), transport the majority of CHOL and PS into the enterocyte. However, nearly all of the PS is redirected back to the intestinal lumen by the transporters ATP binding cassette (ABC) transporter G5 and G8 (ABCG5 and ABCG8, respectively) for excretion from the body. Some CHOL may also be transported back into the intestinal lumen by ABCG5 and ABCG8, although evidence for this is less conclusive. About half of the CHOL is packaged into lipoproteins and transported into the lymphatic system and eventually the bloodstream for delivery to the liver. Consequently, cholesterol absorption efficiency is generally estimated to be about 50~60%.

tary cholesterol accounts for approximately 300 mg/d (9-11), whereas biliary cholesterol is estimated to contribute 800~1,400 mg/d (12,13). The liver—not the diet—is therefore the primary source of cholesterol available for intestinal absorption, a point that is often underappreciated. Consequently, therapies that block cholesterol absorption are effective at lowering LDL cholesterol mainly because they prevent the reabsorption of endogenous cholesterol back to the liver. This explains why individuals who consume no animal products (i.e., no cholesterol) will also experience reductions in LDL cholesterol when given absorption-blocking therapies.

Biliary cholesterol enters the small intestine unesterified, along with the other major components of bile (phosphatidylcholine and bile acids). As the components of bile mix with dietary lipids, micelles form spontaneously. Micelles are lipid aggregates that form when a critical concentration of lipid from bile mixes with lipids entering the small intestine from the diet. Bile acids act as detergents allowing the lipids to "dissolve" in an aqueous environment, facilitating their delivery to the brush border. The proportion of bile acids, phospholipid, and cholesterol must be maintained within a specific range to optimize the formation of micelles in the lumen of the intestine (14).

Furthermore, bile acids are required for the micellar solubilization of cholesterol, but are also synthesized from cholesterol within the hepatocyte. After micelles have facilitated the solubilization, digestion, and absorption of lipids in the proximal small intestine, bile acids are reabsorbed in the distal small intestine (ileum) by the apical sodium-dependent bile acid transporter specific bile acid transporter, ASBT (15). Pharmaceutical compounds known as bile acid sequestrants (cholestyramine and colestipol) are effective at lowering plasma LDL cholesterol because they promote intestinal bile acid excretion, which limits their reabsorption, and results in the liver using more cholesterol for bile acid synthesis. This increased demand for cholesterol in the liver causes an upregulation of LDL uptake from the plasma, thus resulting in lower plasma LDL cholesterol concentration. In this pathway, micelle formation and cholesterol absorption is not affected by treatment with bile acid sequestrants or inhibition of ASBT, because the liver compensates for the reduced bile acid reabsorption by increasing the synthesis of bile acids (16,17). This allows the total bile acid pool size to remain unchanged, even though the turnover of bile acids due to pharmaceutical or dietary factors is much greater.

The discovery of intestinal cholesterol transporters has been a key component to the continued understanding of cholesterol absorption. Transport into enterocytes is mediated by a specific transporter, Neiman-Pick C1 Like 1 (NPC1L1) protein in the proximal small intestine and has been shown to be inhibited by the pharmaceutical ezetimibe (6). NPC1L1 also transports dietary phytos-

terols into the enterocyte. Davis et al. (18) first demonstrated NPC1L1 knockout mice have significantly reduced uptake of cholesterol and phytosterols, compared to wild type mice. Cholesterol and phytosterols are utilized quite differently once taken up into the enterocyte. Despite the ability of NPC1L1 to transport both cholesterol and phytosterols, less than 1% of dietary phytosterols enter the circulation, whereas 50～60% of intestinal cholesterol enters the circulation. This alternate processing of phytosterols is due to the ATP binding cassette (ABC) transporter G5 and G8 (ABCG5 and ABCG8, respectively) effectively secreting phytosterols back into the lumen of the intestine (19-21). Though the role of intestinal ABCG5 and ABCG8 in cholesterol transport is not well studied, there is some support to the hypothesis that upregulating intestinal ABCG5 and ABCG8 transporters will divert more cholesterol from the enterocyte back into the intestinal lumen, effectively creating an anti-hyperlipidemic effect (22). Yet the potential inability (or diminished ability) of cholesterol to interact with ABCG5 and ABCG8 is one possible explanation for the differential absorption rates between cholesterol and phytosterols.

Dietary components reduce cholesterol absorption through one or more mechanism and a primary mode of inhibiting cholesterol absorption is through the binding of bile acids and disrupting the formation of micelles. When dietary lipids are consumed, all of the components of the micelle are transported from the lumen of the intestine to the brush border. While absorption of dietary triacylglycerol is absorbed very efficiently (90～100%); cholesterol absorption is generally found to be less well absorbed at a rate of 50～60% (23). Although the impact on whole body cholesterol balance may be slight, there is evidence to support the hypothesis that biliary cholesterol is absorbed more efficiently than dietary cholesterol, due to its association with the bile acids and phospholipid in bile (24).

The following sections of this review focus on specific dietary components that inhibit cholesterol absorption through known or hypothesized mechanisms.

PHYTOSTEROLS

Phytosterols are structural components of plant cell membranes and function similarly to cholesterol in animal cells. The most abundant phytosterols are sitosterol, campesterol, and stigmasterol, with the structural differences occurring in the side chain attached to the steroid ring. Plant stanols are saturated sterols (i.e., no double bond in the steroid ring) and are much less abundant in nature compared to their corresponding sterols. In the human diet, phytostanols comprise about 5～10% of the total sterol/stanol present (10,25,26). Due to their low abundance in the diet, phytostanols are often reported as a part of the total "phytosterol" content of a particular food or dietary intake. Western societies consume about 200～300 mg phytosterols per day (10,25-30), whereas Asian and vegetarian diets provide higher amount of phytosterols in the diet (31,32).

The cholesterol lowering properties of dietary phytosterols have been known for decades. Moderate levels of phytosterols consumed in usual diets probably exert some minimal effect on cholesterol absorption, although higher amounts (1～3 g/d) are needed to produce significant reductions in plasma LDL cholesterol. As a therapeutic food ingredient, phytosterols/stanols are often esterified with long-chain fatty acids to increase their solubility for incorporation into food products. Meta-analyses have consistently shown that intake of 1～3 g/d of phytosterol (or stanol) esters lowers plasma LDL cholesterol concentration up to 15% compared to placebo (33-36). An inverse dose response relationship exists between phytosterol/stanol ester intake and LDL cholesterol concentration, but tapers off at intakes above 3 g/d with little added benefit at higher intakes (37).

The mechanisms whereby phytosterols inhibit cholesterol absorption are not entirely clear and likely includes multiple pathways to disrupt cholesterol absorption. Proposed mechanisms include the inhibition of cholesterol ester hydrolysis; competition with cholesterol for solubilization into mixed micelles; co-crystallization of cholesterol and phytosterols; competition for transport across the apical membrane of enterocytes; and impaired intracellular re-esterification of cholesterol by acyl-coenzyme A:cholesterol acyltransferase-2 (ACAT-2) for incorporation into chylomicrons and secretion into the lymphatic system.

First, the interaction of phytosterol esters with digestive enzymes is a possible point of regulation, although the extent of interaction is still uncertain. On one hand, Nissinen et al. (38) demonstrated that high levels of sitostanol esters infused into healthy subjects were rapidly hydrolyzed and incorporated into micelles, causing cholesterol and its esters to accumulate in the oil phase. Preferential interaction of phytosterol esters with digestive enzymes would also preclude dietary cholesterol esters from hydrolysis, further limiting cholesterol absorption. In contrast, it has been suggested that phytosterol esters are poorly hydrolyzed by digestive enzymes (39). If the phytosterol esters remain intact within the intestinal lumen, they could attract other lipophilic compounds, including cholesterol and cholesterol esters, and carry them to distal parts of the intestinal where cholesterol absorption is much less efficient. Absorption of dietary cholesterol esters is dependent upon hydrolysis by pancreatic cholesterol esterase (PCE) to yield free cholesterol and a free fatty acid. The free cholesterol is then solu-

bilized by bile to form mixed micelles in the lumen of the intestine (40). PCE is responsible for the hydrolysis of both cholesterol and phytosterol esters. One study has found that both sterol and fatty acid components of a sterol ester influence the rate of hydrolysis by PCE, with cholesterol esters having the highest rate of hydrolysis by PCE, followed by sitosterol, stigmastanol, and stigmasterol esters (41). The authors hypothesized that PCE may play a discriminatory role in the hydrolysis of sterol esters, specifically phytosterols that can compete with cholesterol for incorporation into mixed micelles. In a similar study, phytosterol esters fed to hamsters were effective at inhibiting cholesterol absorption if the phytosterol esters were hydrolyzed and the free sterol was allowed to compete with cholesterol for incorporation into micelles (42). Further research is clearly needed to resolve this issue.

Second, as mentioned previously, micelles play a key role in lipid absorption, acting as vehicles that transport both lipophilic and amphiphilic compounds towards the intestinal wall. Cholesterol not dissolved in micelles will form a separate oil phase within the intestinal lumen, making it generally unavailable for absorption (43). Using model bile solutions *in vitro*, Ikeda and coworkers reported that cholesterol solubility was significantly decreased in the presence of sitosterol (44-46). They further demonstrated that sitosterol, infused with cholesterol into rat intestinal tracts as an artificial "bile" mixture, significantly reduced cholesterol absorption *in vivo* (45). Armstrong and Carey (40) conducted a thermodynamic analysis of micellar solubilities and found that sitosterol, compared to cholesterol, had a higher binding affinity for micelles. *In vitro* studies in our laboratory suggest that the higher affinity of phytosterols causes cholesterol to be displaced from the micelle (47). Heinemann et al. (48) published an intestinal perfusion study in healthy volunteers and found that both sitosterol and sitostanol reduced cholesterol absorption by disrupting cholesterol solubility in micelles. In another infusion study, Nissinen et al. (38) observed that in subjects receiving either high or low amounts of plant stanol esters, cholesterol solubility in micelles was decreased due to displacement by plant stanols. Using a variety of *in vitro* techniques, Mel'nikov et al. (49) found the both sitosterol and sitostanol reduced the concentration of cholesterol in dietary mixed micelles via a dynamic competition mechanism. These investigators further concluded that cholesterol, sitosterol and sitostanol compete equally for solubilization in micelles. While there is general agreement that phytosterols compete with cholesterol during micelle formation, the degree of solubilization likely depends on the composition of other lipids—dietary and biliary—present in the intestinal lumen (14).

The third proposed mechanism whereby phytosterols reduce cholesterol absorption is co-crystallization of cholesterol with phytosterols. The concept that co-crystallization would render cholesterol unavailable for absorption has been considered for some time, but the data are quite limited. Christiansen et al. (50) investigated the solubility and phase behavior of sitosterol and cholesterol (and mixtures thereof) in the presence and absence of water. As expected, the solubility of both sitosterol and cholesterol were significantly reduced in water-acetone solutions compared to acetone alone, but the decrease in solubility was much greater with sitosterol. When mixtures of cholesterol/sitosterol in ratios of 3:1, 1:1, and 1:3 were co-precipitated from the water-acetone solution, the total sterol solubility decreased with increasing proportions of sitosterol, suggesting that the more hydrophobic sitosterol promotes co-crystallization with cholesterol. Under more realistic conditions, Mel'nikov et al. (51) examined the co-crystallization properties of cholesterol /sitosterol and cholesterol/sitostanol mixtures from triglyceride oil that was hydrolyzed to mimic the intestinal environment during digestion. However, during lipolysis of the model dietary emulsions, no crystal formation was detected. The researchers concluded that the solubility of sterols significantly increased in the products of lipid hydrolysis and that their solubility increased in parallel with solvent polarity (free fatty acids> diglyceride oil> triglyceride oil). These results suggest that co-crystallization of phytosterols and cholesterol may not occur to a great extent *in vivo* and would not be a major contributor in reducing cholesterol absorption (51).

The fourth proposed mechanism involves the regulation of the intestinal transporters NPC1L1, ABCG5, and ABCG8. As described earlier, NPC1L1 resides in the brush border membrane and transports both cholesterol and phytosterols into the enterocyte (6,18,52-54), whereas ABCG5 and ABCG8 transport phytosterols and possible cholesterol back to the intestinal lumen (21). In this way, phytosterols could compete with cholesterol for binding to NPC1L1, although direct evidence for this is lacking. It is also possible that phytosterols could inhibit gene expression of NPC1L1 (55) or, conversely, enhance expression of ABCG5 and ABCG8, which could promote cholesterol efflux if, indeed, cholesterol is transported by ABCG5 and ABCG8. However, Field et al. (56) recently reported that NPC1L1 mRNA was not changed in hamsters fed plant stanols. They also found that ABCG5 and ABCG8 mRNA was decreased by plant stanols rather than increased, suggesting that the cholesterol lowering effect of plant stanols (and sterols) is unrelated to changes in gene expression of NPC1L1, ABCG5, or ABCG8. The study by Field et al. (56) does not exclude the possibility that unknown transporters of cholesterol are regulated by phytosterols.

The fifth possible mechanism, in which intestinal

ACAT-2 is inhibited by phytosterols, is based on the differential affinities of sterols for the enzyme. ACAT isoforms have been shown to have significantly lower substrate specificity for phytosterols than for cholesterol (57-59), resulting in more unesterified phytosterol being available for return to the intestinal lumen (via ABCG5/ABCG8). However, this difference in specificity does not appear to be an important mechanism for lowering cholesterol absorption as the presence of sitosterol along with cholesterol did not inhibit cholesterol esterification (45,57). While the difference in sterol specificity may account for very low plasma concentration of phytosterols in humans, inhibition of ACAT-2 is unlikely a major mechanism for reduction in cholesterol absorption.

SOLUBLE FIBERS

Dietary fiber is defined as consisting of nondigestible carbohydrates and lignin that are intrinsic and intact in plants (60). While dietary fiber is consumed as a part of plant material, functional fiber is used as an added ingredient in manufactured food products. The Institute of Medicine's Food and Nutrition Board has defined fiber accordingly: "dietary fiber" consists of nondigestible carbohydrates that are intrinsic and intact in plants, whereas "functional fiber" consists of isolated nondigestible carbohydrates that have beneficial physiological effects in humans (61).

Fibers are also categorized by their solubility in water. Insoluble fibers are found mainly in cell walls of plants and include cellulose, some hemicelluloses and lignin. Good sources of insoluble fiber are vegetables, legumes, whole wheat (particularly bran), nuts, and seeds. Though insoluble fibers have some capacity to hold water, much of insoluble fibers impact on the gastrointestinal tract is due to added dietary "bulk" that increases both the rate of transit through the gastrointestinal tract and fecal volume. Alternate terms have been suggested by the Institute of Medicine. It was proposed to use "viscous fiber" instead of soluble fiber, and "fermentable fiber" instead of insoluble fiber to describe the physiochemical properties of fiber. Interestingly, previous evidence indicates insoluble fibers have little impact on cholesterol concentrations (62). More recent evidence shows carrot insoluble fiber consumption results in significant reductions in serum triacylglycerol and serum and liver cholesterol, while increasing fecal cholesterol and bile acid excretion (63).

In contrast to insoluble fiber adding to fecal bulk, soluble fiber is attracted to water in the intestine and forms a viscous matrix that in turn leads to a variety of effects on the body. Although soluble fibers are both viscous and fermentable, the independent role of viscosity in reducing cholesterol absorption has been clearly demonstrated (4). The consumption of fiber rich foods, specifically soluble fiber, has been associated with improvements in diabetes (64), plasma LDL concentrations and coronary heart disease (65,66), and gastrointestinal health of patients with intestinal cancers (67). Much of the evidence supporting soluble fibers contribution to improving coronary heart disease is due to their ability to inhibit cholesterol absorption. The hypolipidemic and cardioprotective effects have been documented feeding trials in animal (68,69), human (70,71), and systematic reviews and meta-analyses (72,73). Furthermore, the U.S. Food and Drug Administration has issued statements supporting the consumption of dietary fiber in the prevention of both cancer and coronary heart disease (74,75).

Soluble fibers can be considered both dietary and functional fibers as they are in native foods and used as additives in food products. These include pectin, β-glucans, fructans, gums, and resistant starch (RS) (i.e., resistant to digestion by mammalian enzymes). The pectin family has excellent binding and gel-forming properties and has been used widely in the food industry as a food additive as a thickener, gelling agent, or health supplement. A recent study examining the effect of adding pectin to the diet of male Syrian hamsters on high-cholesterol diet showed significant reductions in plasma and liver lipids (triacylglycerol and cholesterol), and significant increases in fecal lipids (cholesterol, triacylglycerol, and bile acids) (76). The authors concluded that the soluble dietary fiber included in the diet decreased plasma lipids by reducing lipid absorption in the intestine.

β-Glucans are found in cereal brans, especially oats and barley, and in yeast, the latter being an important commercial source (77). β-Glucans provide thickening properties when used as a food ingredient. A randomized controlled trial was designed to identify the physiological effects of concentrated oat β-glucans in humans. The trail targeted outcomes related to cardiovascular disease risk by measuring serum total cholesterol, LDL-cholesterol, high density lipoprotein (HDL)-cholesterol, triacylglycerol, apolipoprotein A-1, and apolipoprotein B. After the six-week trial was completed, oat β-glucans significantly reduced total cholesterol and LDL-cholesterol (78). Although fructans are soluble in water, they do not have the typical gel-forming or ion- binding properties of other soluble fibers (79). Fructans are primarily known for their ability to support the growth of beneficial intestinal microflora, rather than contributing to the physical characteristics of food products (80).

Gums are consumed as both dietary fiber from legumes, oats, and barley, and as functional fibers. Gums consumed as a functional fiber are extracted from seeds, seaweed, plant exudates, and microbial fermentation and are used for their ability to provide thickening, stability,

emulsification, and glossy appearance to food products (81). Gums have long been shown to reduce plasma cholesterol in humans (82) and animals (83). More recent evidence in rats fed diets enriched with cluster beans for eight weeks found significant reductions in serum total cholesterol and LDL-cholesterol when compared to the respective chow fed controls or high cholesterol diet controls (84). Furthering this point, a confirmatory study feeding gum arabic for six months to a Sudanese patient population with newly discovered hyperlipidemia found significant reductions in total cholesterol, LDL-cholesterol, and triacylglycerol (85).

RSs are found in a wide variety of plant-based foods and are a low calorie, prebiotic dietary fiber. RSs are classified into four categories (RS1, RS2, RS3, and RS4) based on their physiochemical properties. When added as a food ingredient, RSs can impart viscosity to the final product. Several types of RS are commercially available and may be used as functional fibers to improve intestinal health (86). RS4 is of particular interest, as this RS is of the crosslinked type and when added to food, it has little impact on the physiochemical and organoleptic properties final product. Additionally, RS4 has some gastrointestinal side effects such as bloating (87,88). In a double blind controlled crossover trail, 86 men and women were given flour containing RS4. The authors reported significant reductions in total cholesterol, non-HDL cholesterol, LDL cholesterol, and HDL cholesterol (89).

The mechanism of action by which dietary fibers protect against cardiovascular disease has not been fully elucidated. Many hypotheses have been proposed as to how dietary fiber reduces the risk of cardiovascular diseases. Examples of the potential mechanism of action include the interaction with and fermentation of fiber by gut bacteria (90,91), delayed gastric emptying (92), increased muscle glucose transporter type 4 expression and glucose uptake (93), decreased absorption of dietary fats (94), and increased excretion of fecal cholesterol (95). Unfortunately, there is some inconsistency in the published data regarding the effects of soluble fiber. For example, guar gum was reported to decrease cholesterol absorption in some studies (96,97), but not in others (98,99). Similarly, pectin was shown to inhibit cholesterol absorption in some studies (83,96), while others found no effect of pectin on absorption (100,101). The amount of soluble fiber consumed, chemical and physical modification of the fibers, and the composition of the background diet are variables that can affect the research outcomes.

Despite the variable outcomes reported in the literature, viscous soluble fibers consistently showed improvements in cardiovascular disease outcomes. Viscous fibers were shown to increase the thickness of the unstirred water-layer in humans (102,103), as well as reduce the amount of cholesterol appearing in the lymph of cannulated rats (46,96). While others have reported increased bile acid output in hamsters fed psyllium and suggest that increased viscosity of the gastrointestinal contents may have disrupted micelle formation and promoted bile acid excretion (104,105). While the precise mechanism of action of soluble fibers has not been elucidated yet, the most prominent mechanism of action is likely due to the ability of soluble fibers to form a viscous matrix within the gastrointestinal tract that impedes cholesterol uptake in the enterocyte.

PHOSPHOLIPIDS

Phospholipids are a group of amphipathic molecules in which long acyl chains form a hydrophobic region, while a hydrophilic region is formed by a phosphate-containing "head" group. The acyl chains can be highly variable; phospholipids from animals tend to be more saturated than those of plant origin. Molecules that comprise the polar head group are typically choline, ethanolamine, serine, or inositol, although choline is mostly frequently found in both plant and animal phospholipids. Because of their unique chemical structure, phospholipids spontaneously form lipid bilayers and are therefore the primary structural components of cell membranes. In addition to their role in cell membranes, phospholipids are a major component of bile and participate in micelle formation and cholesterol solubilization. Phosphotidylcholine (PC) is the primary biliary phospholipid and, when secreted into the intestine, can exceed dietary phospholipid amounts by as much as 5 to 1 (106). Food sources naturally rich in PC include egg yolks, muscle foods, peanuts and soybeans. Naturally occurring soybean PC contains 10～20% saturated fatty acids, whereas native egg yolk PC contains 40～50% saturated fatty acids. Food manufacturers frequently use purified PC (also called lecithin) as a food ingredient because of its excellent emulsifying properties.

Sphingomyelin (SM) is another phosphoslipid found mostly in animal tissues; therefore, good food sources of SM include egg yolks, muscle foods, and milk and dairy products. SM is similar to PC to the extent they share the same phosphate-choline head group and have long chain, hydrophobic acyl moieties, but SM differs in several ways from naturally occurring PC. Whereas the backbone of PC is a glycerol base, the backbone of SM is a sphingoid base, which increases the polarity of SM and allows for stronger intra- and inter-molecular hydrogen bonding (107). The main acyl chain of SM is usually longer than the fatty acyl chains of PC and is frequently saturated. These characteristics contribute to a stronger interaction between cholesterol and SM in cell membranes compared to other phospholipids (108,109).

The ability of PC to inhibit cholesterol absorption was first demonstrated in experimental animals (110,111) and later in humans given intraduodenal PC infusion (112). Kesaniemi and Grundy (113) also found a small but significant reduction in cholesterol absorption in hyperlipidemic patients fed soy PC. Koo and colleagues (114,115) have shown that egg PC inhibits cholesterol absorption in lymph duct cannulated rats, and that egg PC is more effective at reducing absorption than soy PC. In fact, soy PC did not interfere with cholesterol absorption but rather produced a slight increase in absorption relative to no-PC controls. These researchers suggested that the higher degree of fatty acid saturation in egg PC compared to soy PC may have caused greater disruption of micellar solubilization of cholesterol. This hypothesis was further supported in rats that had lower rates of cholesterol absorption when fed hydrogenated (i.e., fully saturated) PC compared to native PC, whether from soy (116) or egg (115).

SM also inhibits cholesterol absorption in experimental animals, and this effect has been shown to be dose-dependent (106,116,117). Noh and Koo (118) further reported that milk SM was a more potent inhibitor than egg SM, suggesting that a higher degree of saturated and long chain length of milk SM may be important factors. Eckhardt et al. (106) indicated that milk SM compared to egg PC was more effective in reducing cholesterol solubility in micelles and in limiting cholesterol uptake in Caco-2 cells. They also observed that milk SM significantly reduced cholesterol absorption in mice, whereas egg PC had no effect. On an equal molar basis, it appears that SM is a more effective inhibitor of cholesterol absorption than PC. Despite considerably more dietary and biliary PC compared to SM in the intestine under normal conditions, the addition of SM to the diets of experimental animals was still able to reduce cholesterol absorption in the presence of endogenous PC, indicating a higher potency of SM relative to PC (106,116). Feeding mice SM (119) or a mixture of sphingolipids (120) resulted in lower cholesterol absorption and an added benefit of protecting against liver steatosis (non- alcoholic fatty liver disease). Human studies, however, are limited and the efficacy of dietary SM is still uncertain (121,122).

There appears to be multiple mechanisms by which dietary phospholipids inhibit cholesterol absorption. *In vitro* studies have suggested that intact (undigested) PC disrupts micelle formation by interfering with enzymatic hydrolysis of dietary lipids. Studies have indicated that the presence of PC on the surface of lipid emulsions hinders the hydrolysis of triglycerides (123,124), which also inhibits cholesterol uptake into IEC-6 intestinal cells (125). The addition of phospholipase A2 *in vitro* was able to overcome the inhibitory action of PC, resulting in increased absorption of cholesterol into Caco-2 cells (126). Because PC is rapidly hydrolyzed by digestive enzymes under normal conditions, relatively large amounts of dietary PC are likely needed to inhibit cholesterol absorption. By comparison, dietary SM is digested slowly and may affect cholesterol in two ways. First, SM binding to cholesterol in the intestinal lumen may prevent cholesterol from incorporating into micelles. The preferential affinity of cholesterol for SM is well-documented (127-129), and lack of micellarization results in increased fecal cholesterol excretion. Second, the strong association between cholesterol and SM in the enterocyte membrane may prevent cholesterol from interacting with its key transport protein, NPC1L1, as reported in intestinal cell lines (130) and animal studies (131).

STEARIC ACID

Stearic acid is an 18-carbon saturated fatty acid present in virtually all edible fats and oils, primarily as a constituent of triacylglycerol molecules. Common fats/oils in Western diets having the highest percentage of stearic acid are beef fat (20%) and cocoa butter (33%). Several tree nuts and seeds indigenous to West Africa, India, and Southeast Asia contain relatively high percentages of stearic acid, including dhupa, illipe, kokum, mango kernel, sal, and shea (132). The use of sheanut oil (shea butter) and sal oil has expanded into Europe and Japan as cocoa butter substitutes in chocolate making. These stearic acid-rich oils are also used in cosmetics, candles, and other industrial products worldwide. Sheanut oil contains about 38% stearic acid and sal oil contains about 34% stearic acid.

It is generally accepted worldwide that consumption of saturated fatty acids (SFA) raises LDL cholesterol concentration and increases the risk of atherosclerotic diseases. The World Health Organization, the European Food Safety Authority, the Dietary Guidelines for Americans, and many other organizations recommend limiting intake of SFA. However, stearic acid is unique among dietary SFA because it does not raise plasma cholesterol levels, as originally observed in the 1960s by Keys et al. (133) and Hegsted et al. (134). When replacing *trans* fatty acids or other saturated fatty acids in the diet, stearic acid lowers LDL cholesterol (135). The neutral or cholesterol lowering effect of dietary stearic acid has been observed repeatedly in animal and human studies (135-137). Despite these observations, the American Heart Association acknowledges that there is no simple way to incorporate specific information about stearic acid into dietary guidelines because stearic acid is generally found with other saturated fatty acids in foods and because the content of specific fatty acids is not provided to consumers (138).

Animal studies have demonstrated that the cholesterol-lowering effect of stearic acid is mediated by reducing cholesterol absorption. Feldman et al. (139,140) were the first to demonstrate a reduction in cholesterol absorption using three different methods to quantify cholesterol absorption (i.e., plasma isotope ratio method, fecal dual isotope method, and lymph duct cannulation), and in each case absorption was significantly decreased by stearic acid. Other studies using lymph duct cannulated rats fed stearic acid-enriched diets showed significant reductions in cholesterol absorption (141,142). Schneider et al. (143) used a more realistic dietary approach by feeding hamsters the NIH-07 cereal-based diet specifically enriched in single fatty acids (stearic, palmitic, oleic, linoleic, or *trans* fatty acids); cholesterol absorption was significantly reduced in hamsters fed stearic acid compared to the other fatty acids. As a result of lower cholesterol absorption, fecal cholesterol excretion was increased in rats and hamsters fed stearic acid diets (143-145).

Schmidt and Gallaher (146) reported that cholesterol solubilization was decreased within the intestinal contents of rats fed stearic acid-enriched diets. One possible mechanism of action of stearic acid is its ability to interfere with micelle formation through its incorporation into phospholipids. Unlike most of the other food components that inhibit cholesterol absorption, stearic acid is relatively well absorbed into the bloodstream and body tissues. Wang and Koo (147,148) reported that, after absorption, stearic acid was preferentially incorporated into hepatic and biliary phospholipids compared to other dietary fatty acids. Cohen and Carey (149) demonstrated that micelle stability and cholesterol solubility were impaired when micellar phospholipids contained stearic acid compared to unsaturated fatty acids. Another possible mode of action may involve alterations in the bile acid species present in the enterohepatic circulation. We have shown that dietary stearic acid decreased the proportion of secondary bile acids in the gallbladder compared to primary bile acids, thus decreasing the overall hydrophobicity index (150). Similarly, Hassel et al. (151) reported significantly lower proportions of secondary bile acids in feces of hamsters fed stearic acid. Secondary bile acids are more hydrophobic than primary bile acids and their diminished presence in the enterohepatic circulation can decrease the efficiency by which micelles solubilize cholesterol (40). It is also possible the stearic acid exerts some regulatory effect on cholesterol transport into (or within) the enterocyte, although this has not been reported. Nevertheless, dietary stearic acid most likely inhibits cholesterol absorption through systemic mechanisms rather than disrupting micelle formation through physical interactions within the intestinal lumen.

OTHER CHOLESTEROL-LOWERING FOOD COMPOUNDS

Several other compounds in the food supply have been identified as having cholesterol-lowering properties. Their mechanisms of action are poorly understood and may not be directly involve reduced cholesterol absorption. Nevertheless, we felt these compounds should be mentioned in this review.

Alkylresorcinols

Wheat alkylresorcinols were recently found to increase cholesterol excretion by 39.6% and decrease blood cholesterol concentration by 30.4% in mice fed a high fat and high sucrose diet designed to induce obesity and glucose intolerance (152). The same group completed a follow-up to that study with the aim of identifying the possible mechanism of action wheat alkylresorcinols have on increasing fecal cholesterol excretion. The follow-up study confirmed wheat alkylresorcinols effects on increasing cholesterol excretion and *in vitro* studies revealed a dose dependent decrease in micellar cholesterol solubility in model bile (153).

Policosanols

Consuming policosanols was a promising therapy for dyslipidemia. An early clinical trial examining the efficacy and tolerability of 10 mg/d policosanol in elderly patients (154) and a follow up clinical trial by the same group in patients with non-insulin-dependent diabetes mellitus and elevated serum total and LDL cholesterol (155) found serum total cholesterol was reduced by 16.4 and 17.5%, respectively, and serum LDL cholesterol was reduced by 17.5 and 21.8%, respectively. Since these early studies, there has been limited evidence to support policosanols effectiveness in treating dyslipidemia and trials delivering twice the dose (20 mg/d) found no effect on reducing serum cholesterol concentration (156).

Guggulsterone

Guggulsterone (also known as guggul) has been used in Ayurvedic medicine and is classified as a plant steroid. A variety of *in vitro* and *in vivo* studies has been performed using guggul with consistent mechanistic and clinical results. A recent clinical trial registered with the Clinical Trial Registry of India found consumption of fresh guggul found significant reductions in serum total cholesterol, triglyceride and very LDL cholesterol (157). The mechanism of action for guggul's lipid lowering efficacy has largely been isolated to agonistic effects on the farnesoid X receptor (FXR). Wild type and FXR null mice were fed a high cholesterol diet supplemented with guggul, while the wild type mice saw significantly lower accumulation of hepatic cholesterol; the FXR null mice re-

alized no effect of guggul consumption (158). This led the investigators to hypothesize that the mechanism of action is the agonistic behavior of guggul on FXR.

Cyclodextrins

Cyclodextrins are reported to have beneficial effects on a variety of health outcome measures from body weight control to allergy suppression. A recent mechanistic study examining the effects of α-cyclodextrin on micelle formation in fed-state simulated intestinal fluid found precipitation of cyclodextrin bound lecithin (159). The results suggest that α-cyclodextrin disrupts cholesterol absorption by precipitating lecithin from bile salt micelles within the intestinal lumen. Mice fed a high cholesterol diet treated with β-cyclodextrin exhibited a reduction in atherosclerotic plaque size and cholesterol crystal deposition in the sub-endothelial space, which promoted plaque regression (160). Further study is needed to determine the efficacy of cyclodextrin in managing atherosclerosis in humans.

Probiotics

Probiotics are microorganisms that, when consumed, promote a beneficial health effect such as *Bifidobacterium animalis* and *Lactobacillus acidophilus*. A meta-analysis of randomized controlled trials examining the effects of probiotics on their lipid lowering effects published between 2000 and 2014 included 15 studies and 788 total subjects (161). The meta-analysis revealed significant reductions in serum total and LDL cholesterol when consuming probiotics as fermented milk or yogurt when compared to consuming probiotics in a capsule. The mechanism for these effects can be attributed, at least partially, to the decrease in intestinal cholesterol absorption through the co-precipitation of cholesterol with deconjugated bile salts, intestinal conversion of cholesterol to coprostanol, and the inhibition of the expression of the intestinal sterol transporter NPC1L1 (162).

Saponins

Saponins are found mainly in plants but are also present in lower marine animals and some bacteria. Saponins possess a wide range of biological activities and some are known to be toxic. However, the human diet contains appreciable amounts of saponins, particularly in legumes and alfalfa sprouts. Dietary saponins are poorly absorbed, thus suggesting their cholesterol-lowering action occurs in the intestine. One possible mechanism of action is the ability of saponins to form insoluble complexes with cholesterol in the intestinal lumen (163,164). Another possibility is direct binding of saponins to bile acids, which would disrupt micelle formation and, consequently, cholesterol absorption. However, Harwood et al. (165) reported no change in bile acid absorption or interruption of the enterohepatic circulation of bile acids in hamsters fed saponins, despite significant reduction in cholesterol absorption.

CONCLUSION

Many cholesterol-lowering compounds are naturally present in the human food supply. Each of these compounds can be isolated, purified, and subsequently used as additives in food products and dietary supplements designed specifically for reducing plasma LDL cholesterol concentration. This review focused on compounds that lower plasma cholesterol by inhibiting cholesterol absorption in the small intestine, including phytosterols, soluble fibers, phospholipids, and stearic acid. All of these compounds appear to exert their effects by interfering with micellar solubilization of cholesterol within the intestinal lumen. This can be the result of displacing cholesterol from the micelle, binding or precipitating cholesterol, impeding the movement of cholesterol by forming a viscous matrix, inhibiting digestive enzymes, binding bile acids, and decreasing their participation in micelle formation, or downregulating cholesterol transporters within the enterocyte. Stearic acid also appears to work systemically by incorporating into hepatic and biliary phospholipids, which destabilizes micelles and reduces cholesterol solubility. These compounds are attractive to food and nutraceutical companies because, in most cases, they are regulated as foods and not drugs. Most of the compounds work entirely within the intestine and are poorly absorbed, if at all, thus significantly reducing (or eliminating) the risk of toxicity. Some also contribute important functional properties when added to foods, such as emulsification, improved texture, calorie reduction, and in the case of soluble fibers, provide prebiotic action. In view of these desirable characteristics, manufacturers are likely to develop a greater diversity of food and nutraceutical products in the coming years giving consumers more choices to manage their plasma cholesterol levels through nonpharmacological means.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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