FOOD COMPONENTS THAT REDUCE CHOLESTEROL ABSORPTION

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I. INTRODUCTION

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. The body's cells can synthesize cholesterol when needed, but excess cholesterol cannot be broken down and must be excreted from the body through the bile into the small intestine. 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.

Absorption of cholesterol in the small intestine contributes to maintaining whole-body cholesterol homeostasis, yet the mechanisms of absorption have not been completely defined. For many years it was believed that cholesterol, a normal component of cell membranes, simply diffused through the brush border membrane of enterocytes (Grundy, 1983; Westergaard and Dietschy, 1974). However, the discovery of specific transporters, receptors,

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and enzymes is quickly changing our understanding of how cholesterol and other sterols are absorbed into the body. It now appears that the transport of cholesterol into and out of the enterocyte and other cells is highly regulated and subject to modification by dietary factors including lipids, carbohydrates, and proteins.

From a health standpoint, the efficiency of cholesterol absorption is of great interest because human and animal studies have linked cholesterol absorption with plasma total and low-density lipoprotein (LDL) cholesterol concentration (Carr *et al.*, 1996, 2002; Gylling and Miettinen, 1995; Kesaniemi and Miettinen, 1987; Rudel *et al.*, 1994). So important is this link that a new family of drugs is being developed that blocks the intestinal absorption of cholesterol and, consequently, reduces plasma LDL cholesterol concentration. Experimentation with these drugs has also helped researchers elucidate some of the mechanisms of cholesterol transport at the cellular level (Altmann *et al.*, 2004; Burnett, 2004). One of the drugs, ezetimibe (sold as Zetia® and Vytorin®), received Food and Drug Administration (FDA) approval in October of 2002 and has become an important therapy in managing LDL cholesterol levels. But 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 nonpharmacological means.

This article focuses on specific dietary components—whether naturally occurring or added as food ingredients—known to interfere with the mechanisms of cholesterol absorption. An overview of cholesterol absorption is provided and emphasizes the critical role of bile acids and micelle formation in solubilizing cholesterol for transport to the brush border membrane of enterocytes. Where applicable, information is also included about commercial food ingredients that are specifically used as cholesterol-lowering agents.

II. MECHANISMS OF CHOLESTEROL ABSORPTION

Cholesterol enters the small intestine from two sources: the diet and bile (Figure 1). Dietary intake of cholesterol is about 300 mg/day (Briefel and Johnson, 2004; Ishinaga *et al.*, 2005; Valsta *et al.*, 2004), whereas the bile contributes 800–1400 mg/day (Duane, 1993; Grundy and Metzger, 1972). The liver—not the diet—is therefore the primary source of cholesterol available for 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

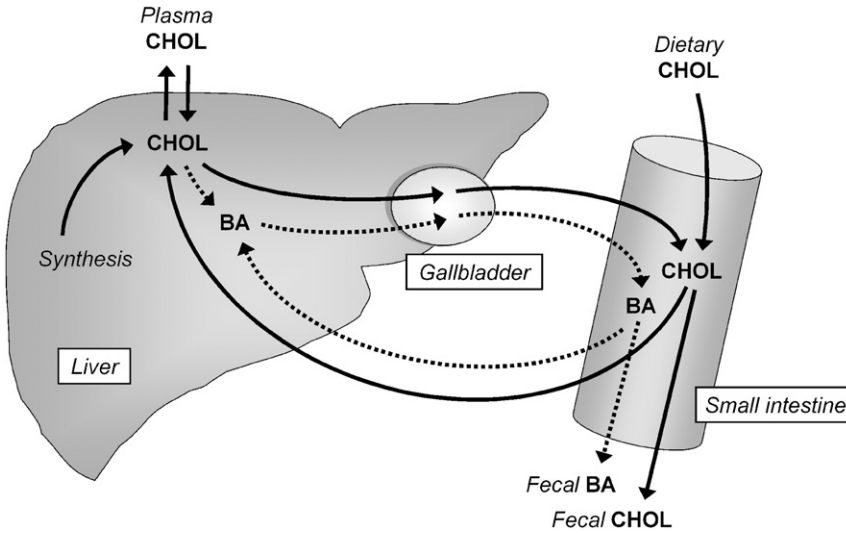

FIG. 1 Movement of cholesterol (CHOL) and bile acids (BA) between the liver and small intestine. CHOL and BA in the liver are secreted into the gallbladder where they are stored temporarily until a fat-containing meal causes their secretion into the intestinal lumen. BA are absorbed with high efficiency (95%) and are recycled back to the liver via the hepatic portal vein. CHOL is absorbed less efficiently (50–60%) and must be incorporated into lipoproteins (chylomicrons) for transport back to the liver via the systemic circulation. Accumulation of CHOL in the liver can promote secretion of CHOL into plasma, thus increasing LDL-CHOL concentration. Loss of CHOL and BA in feces represents the primary route of CHOL elimination from the body.

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.

Some cholesterol entering from the diet may be esterified to various fatty acids, although the extent of esterification is variable. For example, egg yolk cholesterol is about 10% esterified (Bitman and Wood, 1980; Tattrie, 1972); cholesterol in meat and poultry is at least 50% esterified (Kritchevsky and Tepper, 1961). Esterified cholesterol entering the intestinal tract is mostly hydrolyzed by pancreatic enzymes, yielding free cholesterol and fatty acids (Howles *et al.*, 1996). Only unesterified cholesterol is available for absorption.

Biliary cholesterol is entirely unesterified and flows into the small intestine as a component of bile. The other major components of bile are phosphatidylcholine (lecithin) and bile acids. Absorption of cholesterol and other lipids depends on their ability to form micelles within the intestinal lumen.

Micelles are lipid aggregates that form when a critical concentration of lipid from bile (i.e., bile acids, phospholipid, and cholesterol) mixes with lipid from the diet (i.e., triglyceride, phospholipid, and cholesterol). The bile acids act as detergents allowing the lipids to "dissolve" in an aqueous environment, facilitating their delivery to the brush border. Furthermore, the proportion of bile acids, phospholipid, and cholesterol in gallbladder bile must be maintained within a specific range to prevent gallstone formation and to optimize micelle formation in the intestinal lumen (Apstein and Carey, 1996; Yao *et al.*, 2002). Many dietary components that reduce cholesterol absorption do so by binding bile acids or otherwise disrupting their ability to form micelles. Under normal conditions, all of the micelle components—except cholesterol—are transported to the brush border and absorbed with high efficiency (90–100%); cholesterol absorption is typically 50–60% (Matthan and Lichtenstein, 2004). Some evidences suggest that biliary cholesterol, because of its inherent association with bile acids, is absorbed slightly more efficiently than dietary cholesterol (Wilson and Rudel, 1994), although this difference probably has little impact on overall cholesterol balance given the minor contribution of dietary cholesterol.

Bile acids play an important role in cholesterol homeostasis. In addition to being required for micellar solubilization of cholesterol, bile acids are synthesized from cholesterol in the liver (Figure 1). Following micelle formation and delivery of lipid to the proximal intestine, bile acids are reabsorbed in the ileum in a process mediated by the apical sodium-dependent bile acid transporter (ASBT) (Dawson and Oelkers, 1995). Bile acid sequestrants, such as cholestyramine (Questran®) and colestipol (Colestid®), are effective at lowering plasma LDL cholesterol because they promote intestinal bile acid excretion, which limits their reabsorption, causing the liver to use more cholesterol for bile acid synthesis. Increased demand for cholesterol causes the liver to recruit LDL cholesterol from plasma, thus reducing plasma LDL cholesterol concentration. However, micelle formation and cholesterol absorption are not affected by treatment with bile acid sequestrants or pharmacological inhibition of ASBT because the liver compensates by increasing bile acid synthesis (Hui and Howles, 2005; Packard and Shepherd, 1982). In this way, the total bile acid pool size is not diminished even though turnover of bile acids in the enterohepatic circulation may be increased by drugs or dietary factors.

Some dietary factors can also change the bile acid species and, by doing so, alter cholesterol absorption. The liver synthesizes the primary bile acids, cholic and chenodeoxycholic acid. Bacteria in the intestine can convert some of the primary bile acids into secondary bile acids, producing deoxycholic from cholic acid and lithocholic from chenodeoxycholic acid. When certain dietary components alter the intestinal microflora, the rate of secondary bile

acid production can change. 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 (Armstrong and Carey, 1987). The "hydrophobicity index" of bile is a numeric value that quantitatively defines the hydrophilic–hydrophobic balance of bile acids in bile samples (Heuman, 1989). Hydrophobicity index is therefore a useful indicator of the capacity of bile to solubilize cholesterol in the intestinal lumen, a necessary step for cholesterol absorption.

Studies have indicated that cholesterol transport into enterocytes is mediated by a specific transporter, Neiman-Pick C1 Like 1 (NPC1L1) protein (Figure 2). NPC1L1 is highly expressed in the proximal portion of the small intestine and is specifically inhibited by the drug ezetimibe (Altmann *et al.*, 2004). NPC1L1 also transports dietary plant sterols into the enterocyte. Using NPC1L1 knockout mice, Davis *et al.* (2004) demonstrated that intestinal uptake of both cholesterol and plant sterols was significantly reduced compared to wild-type mice. Despite the ability of NPC1L1 to transport cholesterol and plant sterols, less than 1% of dietary plant sterols eventually enter the circulation, whereas 50–60% of intestinal cholesterol

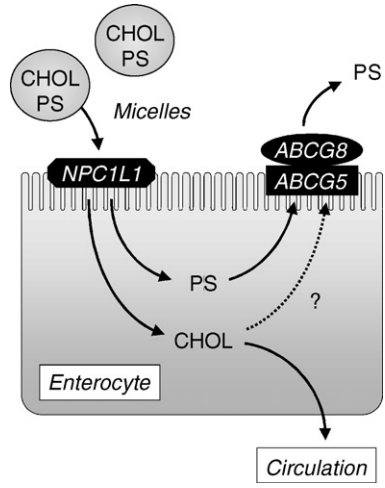

FIG. 2 Transport of cholesterol (CHOL) and plant sterols (PS) in the enterocyte. CHOL, PS, and other lipids are solubilized in micelles that deliver the lipids to the brush border membrane. CHOL and PS are transported into the enterocyte by NPC1L1. Nearly all of the PS are redirected back to the intestinal lumen by the transporters ABCG5 and ABCG8. The extent to which CHOL is transported by ABCG5 and ABCG8 is not known. CHOL within the enterocyte is packaged into lipoproteins (chylomicrons) and secreted into lymph and eventually the bloodstream for transport to the liver.

enters the circulation. It appears that cholesterol and plant sterols are handled differently once inside the enterocyte. Another transport system, involving the proteins ABCG5 and ABCG8 (Berge *et al.*, 2000; Lee *et al.*, 2001a), apparently redirects plant sterols back into the intestinal lumen, but the role of ABCG5 and ABCG8 in cholesterol transport is uncertain (Lee *et al.*, 2001b). The inability (or diminished ability) of cholesterol to interact with ABCG5 and ABCG8 is one possible explanation for the differential absorption rates between cholesterol and plant sterols.

While the discovery of NPC1L1, ABCG5, and ABCG8 represents important milestones in understanding cholesterol absorption, some questions remain. First, cholesterol absorption in NPC1L1 knockout mice was significantly reduced, but not completely eliminated (Davis *et al.*, 2004), suggesting a small proportion of cholesterol is absorbed independent of NPC1L1. What other mechanisms beside NPC1L1 account for cholesterol transport into enterocytes? Second, to what extent does cholesterol interact with ABCG5 and ABCG8, and does this transport system represent an important regulatory pathway in overall cholesterol absorption? Finally, how is cholesterol transported inside the enterocyte? The intracellular trafficking of sterols is an intense area of investigation. While there is still much to be learned, a general understanding of cholesterol absorption is useful when assessing how dietary factors impact cholesterol absorption.

III. FOOD COMPONENTS THAT REDUCE CHOLESTEROL ABSORPTION

A. PLANT STEROLS AND STANOLS

Plant sterols are essential components of cell membranes and are present in all plants. They are structurally similar to cholesterol, with the differences occurring in the side chain attached to the steroid ring (Figure 3). Dozens of plant sterols have been identified, although the most abundant are sitosterol, campesterol, and stigmasterol. Stanols are saturated sterols (i.e., no double bond in the steroid ring) and are much less abundant in nature than the corresponding sterols. Plant stanols comprise about 5–10% of the total sterol/stanol mixture naturally present in the human diet (Andersson *et al.*, 2004; Normén *et al.*, 2001; Phillips *et al.*, 1999; Valsta *et al.*, 2004). Because of their low abundance, stanols are often reported as part of the total ''plant sterol'' content of food or dietary intake. Individuals living in Western societies consume about 200–300 mg plant sterols per day (Andersson *et al.*, 2004; de Vries *et al.*, 1997; Morton *et al.*, 1995; Normén *et al.*, 2001; Phillips *et al.*, 1999; Schothorst and Jekel, 1999; Valsta *et al.*, 2004). Asian

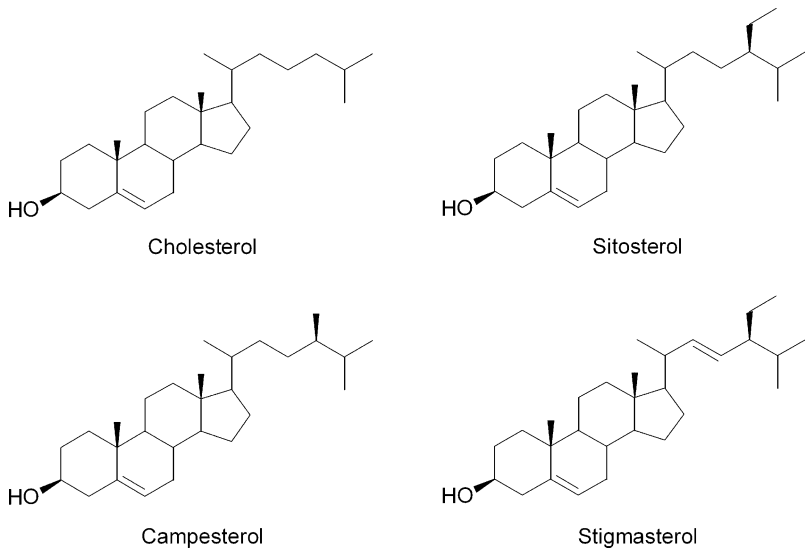

FIG. 3 Structure of cholesterol and common plant sterols.

and vegetarian diets provide higher amounts of plant sterols (Vuoristo and Miettinen, 1994; Zhou *et al.*, 2003). The total plant sterol content of several common foods is provided in Table I. Note that vegetable oils are particularly good sources of plant sterols.

Moderate levels of plant sterols consumed in usual diets probably exert some minimal effect on cholesterol absorption, although higher amounts (1–3 g/day) are needed to produce significant reductions in plasma LDL cholesterol (Law, 2000; Nguyen, 1999). Augmenting the diet with plant sterols is therefore necessary for therapeutic maintenance of LDL cholesterol at desirable levels. Contrary to popular belief, using plant sterols for this purpose is not new. Eli Lilly and Company introduced a plant sterol preparation called Cytellin™ in 1957. Cytellin™ contained free (unesterified) plant sterols suspended in fruit-flavored syrup and was widely prescribed through the 1980s before statin drugs became available. However, the poor solubility of free plant sterols in the intestinal lumen led to inconsistent and confusing results in clinical studies (Ahrens *et al.*, 1957; Denke, 1995; Lees *et al.*, 1977). Moreover, doses of Cytellin™ exceeding 25 g/day were often required to achieve significant LDL cholesterol reduction. Apparently, free plant sterols form highly stable crystals that resist solubilization, even in the presence of bile and dietary lipids. The crystalline nature and poor solubility of free sterols also limited their application in food products.

TABLE I
STEROL CONTENT OF COMMON PLANT FOODS

	Total plant sterols (mg/100 g edible portion)
Fruits	
Apple	13
Banana	14
Fig	22
Lemon	18
Orange	24
Peach	15
Pear	12
Pineapple	17
Watermelon	1
Vegetables	
Broccoli	39
Carrot	16
Cauliflower	40
Celery	17
Mushroom	18
Olive, black	50
Onion	8
Potato, white	4
Tomato	5
Refined oils	
Canola	250
Chestnut	5350
Coconut	133
Corn	952
Cottonseed	327
Olive	176
Rice bran	1055
Safflower	444
Soybean	221
Sunflower	725
Cereals	
Corn flour	52
Couscous	58
Rice flour	23
Rolled oats	39
Rye flour	86
Whole wheat flour	70
Wheat flour	28
Wheat bran	200
Wheat germ	344

Source: Weihrauch and Gardner (1978); Normén *et al.* (1999, 2002).

In the 1970s, Fred Mattson and colleagues working at Procter & Gamble discovered that esterifying plant sterols with long-chain fatty acids increased their solubility in oil from about 2% to more than 20% and that esterification did not impair their ability to inhibit cholesterol absorption (Jandacek *et al.*, 1977; Mattson *et al.*, 1977, 1982). Armed with this information, researchers at Raisio Group in Finland developed a commercial process of esterifying plant stanols with vegetable oil fatty acids (Miettinen *et al.*, 1996). Their process utilizes stanols, rather than sterols, and is based on studies indicating that free stanols are more effective than free sterols at reducing cholesterol absorption (Becker *et al.*, 1993; Heinemann *et al.*, 1988; Ikeda *et al.*, 1979; Sugano *et al.*, 1977). However, more recent studies using esterified sterols and stanols have established their equal effectiveness at reducing cholesterol absorption and plasma LDL cholesterol concentration (Hallikainen *et al.*, 2000; Jones *et al.*, 2000; Normén *et al.*, 2000; Weststrate and Meijer, 1998). Nevertheless, the plant stanol esters produced by Raisio Group are the main ingredient in Benecol® spreads, yogurts, and other food products. Benecol® spreads were launched in Finland in 1995 and in the United States in 1999 after plant stanol esters were declared ''generally recognized as safe'' (GRAS) for use in vegetable oil-based spreads in amounts not exceeding 20%. The stanol esters used in Benecol® products are made from plant sterols derived from tall oil, a by-product of the wood pulp industry. The sterols are isolated and purified, chemically hydrogenated to stanols, then esterified with vegetable oil fatty acids (Hicks and Moreau, 2001). A similar spread, Take Control®, is made by Unilever and contains plant sterol esters as the active ingredient. The plant sterols in Take Control® are derived from soybean oil and are used directly for esterification, eliminating the need and expense of converting sterols to stanols. The retail price of Take Control® spread is consequently lower than Benecol® spread.

The food and nutraceutical industries have responded to increasing consumer demand for products containing plant sterols. Ingredient companies, including Cargill (makers of CoroWise™) and Archer-Daniels-Midland (makers of CardioAid™), now offer a range of free and esterified plant sterol compounds that are used in a variety of foods available to consumers worldwide. Both companies have also developed technologies of emulsifying plant sterols for use in beverages and water-based foods. CoroWise™ plant sterols are found in Minute Maid® Premium Heart Wise™ orange juice and GNC's Heart Advance™ dietary supplement. CardioAid™ ingredients are available in a variety of spreads, dairy products, and dietary supplements. Another company, Forbes Medi-Tech, developed an ingredient called Reducol™ that contains a mixture of plant sterols and stanols derived from tall oil. Reducol™ is the active ingredient in Naturemade's Cholest-Off™

dietary supplement. Forbes Medi-Tech received approval in November of 2004 to market Reducol™ as a food ingredient in Europe.

Several clinical studies have been conducted in recent years, which indicate a dose of 1–3 g of plant sterol (or stanol) esters reduces plasma LDL cholesterol concentration up to 15% compared to placebo (Law, 2000; Nguyen, 1999; O'Neill *et al.*, 2005). It appears that a dose–response relationship is continuous up to about 2 g of plant sterol esters per day, with no further reductions in LDL cholesterol above that dose (Law, 2000). Accordingly, the National Cholesterol Education Program now recommends 2 g/day of plant sterol (or stanol) esters as a therapeutic option for reducing plasma LDL cholesterol concentration (National Institutes of Health, 2002). Furthermore, in response to a request by Cargill, the FDA agreed in February of 2003 to allow a heart health claim for a broad range of foods and beverages. The FDA-approved claim states that foods containing at least 0.4 g plant sterol/stanol (or 0.65 g sterol/stanol esters) per serving, consumed twice a day with meals for a daily total of at least 0.8 g plant sterol/stanol (or 1.3 g sterol/stanol ester), as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease.

The cholesterol-lowering properties of dietary plant sterols have been known for decades (Best *et al.*, 1954; Peterson, 1951; Pollak, 1953), due specifically to reductions in cholesterol absorption. Inverse correlations between plant sterol intake and cholesterol absorption have been reported in animals (Carr *et al.*, 2002; Ntanios and Jones, 1999) and humans (Ellegård *et al.*, 2000). The exact mechanism by which plant sterols inhibit cholesterol absorption is unclear, and several mechanisms of action have been proposed, including: (1) competition with cholesterol for solubilization in micelles within the intestinal lumen, (2) cocrystallization with cholesterol to form insoluble crystals, (3) interaction with digestive enzymes, and (4) regulation of intestinal transporters of cholesterol.

First, dietary mixed micelles play a key role in dietary lipid absorption, acting as vehicles that transport both lipophilic and amphiphilic compounds toward the intestinal wall. Solubilization of cholesterol in mixed micelles is necessary for cholesterol transit to the brush border membranes of enterocytes. Cholesterol not dissolved in micelles will form a separate oil phase within the intestinal lumen, making it generally unavailable for absorption (Hofmann and Small, 1967). Using model bile solutions *in vitro*, Ikeda and coworkers reported that cholesterol solubility was significantly decreased in the presence of sitosterol (Ikeda and Sugano, 1983; Ikeda *et al.*, 1988, 1989a). They further demonstrated that sitosterol, infused with cholesterol into rat intestinal tracts as an artificial "bile" mixture, significantly reduced cholesterol absorption *in vivo* (Ikeda *et al.*, 1988). Armstrong and Carey (1987) 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 plant sterols causes cholesterol to be displaced from the micelle (Jesch and Carr, 2005). Heinemann *et al.* (1991) 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.* (2002) 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.* (2004a) found that 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 (Mel'nikov *et al.*, 2004a). While there is general agreement that plant sterols 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 (Yao *et al.*, 2002).

The second proposed mechanism whereby plant sterols reduce cholesterol absorption is cocrystallization of cholesterol with plant sterols. The concept that cocrystallization would render cholesterol unavailable for absorption has been considered for some time, but the data are quite limited. Christiansen *et al.* (2003) 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 was 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 coprecipitated from the water–acetone solution, the total sterol solubility decreased with increasing proportions of sitosterol, suggesting that the more hydrophobic sitosterol promotes cocrystallization with cholesterol. Under more realistic conditions, Mel'nikov *et al.* (2004b) examined the cocrystallization 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 cocrystallization of plant sterols and cholesterol may not occur to a great extent *in vivo* and would not be a major contributor in reducing cholesterol absorption (Mel'nikov *et al.*, 2004b).

A third possible mechanism involves the interaction of plant sterol esters with digestive enzymes, although the extent of interaction is still uncertain. On one hand, Nissinen *et al.* (2002) 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 plant sterol esters with digestive enzymes would also preclude dietary cholesterol esters from hydrolysis, further limiting cholesterol absorption. In contrast, it has been suggested that plant sterol esters are poorly hydrolyzed by digestive enzymes (Trautwein *et al.*, 2003). If the plant sterol 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 lumen where cholesterol absorption is much less efficient. Further research is clearly needed to resolve this issue.

A fourth proposed mechanism involves the regulation of the intestinal transporters, NPC1L1, ABCG5, and ABCG8. As described earlier (Figure 2), NPC1L1 resides in the brush border membrane and transports both cholesterol and plant sterols into the enterocyte (Davis *et al.*, 2004; Salen *et al.*, 2004), whereas ABCG5 and ABCG8 transport plant sterols and possible cholesterol back to the intestinal lumen (Lee *et al.*, 2001b). In this way, plant sterols could compete with cholesterol for binding to NPC1L1, although direct evidence for this is lacking. It is also possible that plant sterols could inhibit gene expression of NPC1L1 or, conversely, enhance expression of ABCG5 and ABCG8, which could promote cholesterol efflux if cholesterol is transported by ABCG5 and ABCG8. However, Field *et al.* (2004) 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.* (2004) does not exclude the possibility that unknown transporters of cholesterol are regulated by plant sterols.

B. SOLUBLE FIBER

Consumption of foods rich in fiber is associated with reduced plasma LDL cholesterol concentration, diminished glycemic response, and improved bowel function. These physiological responses lead to reductions in risk of coronary heart disease, diabetes, and intestinal cancers. In 2002, dietary reference intakes (DRI) were established for fiber, ranging from 30–36 g/day for adult males and 21–29 g/day for adult females (Institute of Medicine, 2002). The actual fiber intake for American adults appears to be

much lower, with estimated intakes of 17 g/day for males and 13 g/day for females (Institute of Medicine, 2002).

Fiber has traditionally been defined as nondigestible carbohydrates of plant origin, although nondigestible carbohydrates from nonplant sources may be considered "fiber." The fiber content of several common foods is provided in Table II. Because fiber can be consumed as an inherent component in native foods or as an added ingredient in manufactured foods, 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 (Institute of Medicine, 2002). These definitions recognize the diversity of fibers in the human food supply and allow for flexibility in

TABLE II
FIBER CONTENT OF COMMON PLANT FOODS

	Total fiber (g/100 g edible portion)	Soluble fiber (% of total fiber)
Fruits		
Apple	2.4	26.2
Banana	2.6	22.1
Orange	2.2	36.8
Peach	1.5	28.8
Pear	3.1	17.7
Pineapple	1.4	11.2
Vegetables		
Broccoli	2.6	30.9
Carrot	2.8	32.2
Corn	2.7	11.3
Potato, white	2.4	34.0
Tomato	1.2	13.8
Legumes		
Kidney beans	6.4	20.2
Navy beans	10.5	25.3
Lentils	7.9	9.3
Pinto beans	9.0	23.7
Cereals		
Bread, white	2.4	33.3
Rolled oats	9.4	34.0
Rice, brown	3.4	23.7
Wheat flour	2.7	29.8
Whole wheat flour	12.2	14.5

Source: Anderson and Bridges (1988); US Department of Agriculture (2005).

incorporating new fiber sources and ingredients into consumer products with specific functionality in mind.

Fibers may also be categorized according to their solubility in water. Insoluble fibers are found mainly in cell walls of plants and include cellulose, some hemicelluloses, and lignin. Generally speaking, good sources of insoluble fiber are vegetables, legumes, whole wheat (particularly bran), nuts, and seeds. Cellulose-based ingredients are frequently used as thickening agents, as fat replacers, or simply to reduce calories in food products. The moderate water-holding capacity of insoluble fibers and their ability to add dietary "bulk" results in increased fecal volume and faster transit time through the colon, thus promoting laxation. However, insoluble fibers generally have little or no impact on cholesterol absorption (Gallaher and Schneeman, 2001). A possible exception is chitosan, the deacetylated form of chitin, found in the exoskeleton of crustaceans and in certain fungi. Although chitosan is not derived from plants, it is a nondigestible carbohydrate and may be considered a functional fiber because of its ability to lower plasma LDL cholesterol concentration (Ylitalo *et al.*, 2002). Studies in rats have indicated that dietary chitosan inhibits cholesterol absorption (Gallaher *et al.*, 2000; Vahouny *et al.*, 1983). A study in humans indicated that a supplement containing equal amounts of chitosan and a soluble fiber (glucomannan) promoted cholesterol excretion (Gallaher *et al.*, 2002), but cholesterol absorption efficiency was not directly measured nor was it possible to isolate the independent effects of chitosan.

Most water-soluble fibers, in contrast to insoluble fibers, have the ability to inhibit cholesterol absorption and subsequently lower plasma LDL cholesterol concentration, as confirmed in meta-analyses of studies involving several types of soluble fiber (Brown *et al.*, 1999; Castro *et al.*, 2005). Many soluble fibers are considered both dietary and functional fibers because they are abundant in native foods and they are used frequently as additives in food products. These include pectin, β-glucans, fructans, gums, and resistant starch (i.e., resistant to digestion by mammalian enzymes). Pectin is actually a family of related compounds that have high-binding and gel-forming properties (Thakur *et al.*, 1997). Good sources of pectin include apples, citrus fruits, and strawberries. The majority of pectin used commercially is extracted from citrus peel or apples, and is used in food products that require gelling such as jellies, icings, frozen foods, and fat-reduced foods. β-Glucans are found in cereal brans, especially oats and barley, and in yeast, the latter being an important commercial source (Bell *et al.*, 1999). β-Glucans provide thickening properties when used as a food ingredient. Fructans are soluble in water but do not have gel-forming or ion-binding properties (Schneeman, 1999). The major fructans—oligofructose and inulin, and consumed mainly in wheat and onions—are known for their ability to support the growth of

beneficial intestinal microflora, rather than contributing to the physical characteristics of food products (Boeckner *et al.*, 2001). Gums are used extensively as functional fibers in the manufacture of food products, although they are also consumed as dietary fibers in legumes, oats, and barley. Gums are derived from a variety of sources including seeds, seaweed, plant exudates, and microbial fermentation and are used for their ability to provide thickening, stability, emulsification, and glossy appearance to food products (Pszczola, 2003). An example of a specialized gum is the dietary supplement, Benefiber® (Novartis Consumer Health, Inc.), containing partially hydrolyzed guar gum, which prevents it from thickening when mixed with liquids. Resistant starches, found in a wide range of plant-based foods, can impart viscosity to foods when added as an ingredient. Several types of resistant starch are commercially available and may be used as functional fibers to improve intestinal health (Kendall *et al.*, 2004). Another soluble fiber, psyllium, is purely a functional fiber obtained from the husk of plantago seeds. It has a very high water-holding capacity and forms viscous solutions when mixed with water. Psyllium is the main component of Metamucil® (Procter & Gamble) and is used for its laxative properties.

While pectin and certain gums have received much attention regarding their impact on cholesterol absorption, information regarding β-glucans, resistant starches, and other gums is still emerging. The wide range of commercial soluble fibers available for research has also contributed to inconsistent results in the scientific literature. For example, guar gum was reported to decrease cholesterol absorption in some studies (Ebihara and Schneeman, 1989; Vahouny *et al.*, 1988) but not in others (Evans *et al.*, 1992; Miettinen and Tarpila, 1989). Similarly, pectin was shown to inhibit cholesterol absorption in some studies (Kelley and Tsai, 1978; Vahouny *et al.*, 1988), while others found no effect of pectin on absorption (Fernandez *et al.*, 1994; Mathé *et al.*, 1977). 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 these variables, it appears that the primary attribute of soluble fibers that inhibit cholesterol absorption is the ability to form a viscous matrix when hydrated. Many water-soluble fibers become viscous in the small intestine (Eastwood and Morris, 1992). It is believed that increased viscosity impedes the movement of cholesterol, bile acids, and other lipids and hinders micelle formation, thus reducing cholesterol absorption and promoting cholesterol excretion from the body. Consumption of viscous fibers was shown to increase the thickness of the unstirred water layer in humans (Flourie *et al.*, 1984; Johnson and Gee, 1981) and reduce the amount of cholesterol appearing in the lymph of cannulated rats (Ikeda *et al.*, 1989b; Vahouny *et al.*, 1988). Turley *et al.* (1991, 1994) reported that

bile acid output was increased in hamsters fed psyllium, suggesting that the increased viscosity may have disrupted micelle formation by promoting bile acid excretion. In contrast, Favier *et al.* (1998) reported that fecal cholesterol excretion was increased in rats fed guar gum, whereas excretion of bile acids was not affected, indicating that viscosity can specifically effect cholesterol independent of bile acids. Isolating the effects of viscosity is somewhat hampered due to other properties of soluble fiber that influence cholesterol metabolism (i.e., fermentation, inhibition of digestive enzymes, and direct binding of cholesterol and bile acids). One tool that has proven useful in this regard is hydroxypropyl methylcellulose (HPMC), a cellulose derivative that imparts viscosity in the small intestine but, unlike other soluble fibers, is resistant to fermentation (Gallaher *et al.*, 1993). We have demonstrated in hamsters and rats that increased viscosity due to HPMC feeding was inversely correlated with cholesterol absorption efficiency and promoted fecal cholesterol excretion (Carr *et al.*, 1996, 2003).

Another possible mechanism involves the direct binding of cholesterol or bile acids, which could alter micelle formation and decrease the ability of cholesterol to incorporate into micelles. To varying degrees, soluble fibers appear to bind cholesterol (Eastwood and Mowbray, 1976; Lund, 1984) and other lipids that comprise micelles, including phospholipid and triglyceride (Vahouny *et al.*, 1980, 1981). Story and Kritchevsky (1976) reported that soluble fibers have the ability to bind primary and secondary bile acids (and their taurine and glycine conjugates). However, Gallaher and Schneeman (1986) demonstrated that while soluble fibers bound significant quantities of bile acids *in vitro*, lipid solubilization and the ability to form micelles was not impaired. The notion of lipid binding as a means of reducing cholesterol absorption may seem attractive, but its contribution is likely to be small relative to the effects of viscosity. Both psyllium and HPMC increased the intestinal contents viscosity and reduced cholesterol absorption, but neither appeared to bind bile acids (Carr *et al.*, 2003; Turley *et al.*, 1991). From a different point of view, Ellegård *et al.* (1997) indicated that oligofructose and inulin—soluble fibers that apparently do not bind bile acids nor do they become viscous—predictably had no effect on cholesterol absorption. It is possible that the binding ability of soluble fibers *in vitro* is greater than that within the physiological environment of the small intestine where viscosity is likely the major factor in preventing micelle formation.

Oligofructose and inulin are known to lower plasma LDL cholesterol, but their mode of action does not involve inhibiting cholesterol absorption (Beylot, 2005; Kaur and Gupta, 2002). As mentioned earlier, oligofructose and inulin are not viscous fibers but rather serve as excellent fuel sources for beneficial intestinal bacteria, particularly lactobacilli and bifidobacteria (Boeckner *et al.*, 2001). In this way, changes in intestinal microflora induced

by oligofructose and inulin have been shown to alter the bile acid profile and promote fecal bile acid excretion (Levrat *et al.*, 1994; Trautwein *et al.*, 1998). Bile acids normally circulate within the enterohepatic circulation, so any loss of bile acids requires the liver to increase synthesis. The result is an increase in the utilization of hepatic cholesterol, the substrate for bile acid synthesis, and a parallel increase in recruitment of cholesterol from the plasma via LDL receptors (Fernandez, 2001). The relative impact of soluble fibers on this mechanism versus cholesterol absorption is uncertain.

C. SAPONINS

Saponins are a group of naturally occurring compounds found mainly in plants but are also present in lower marine animals and some bacteria (Francis *et al.*, 2002; Price *et al.*, 1987). They appear to act in the defense system of plants by repelling insects and inhibiting the growth of bacteria and mold. Saponins consist of a hydrophobic triterpenoid or steroid moiety linked to one or more hydrophilic carbohydrate molecules. Figure 4 illustrates the basic structure of representative triterpenoid and steroid saponins, where the R group represents carbohydrate linkages that often include rhamnose, xylose, arabinose, galactose, glucose, or glucuronic acid. The amphiphilic nature of saponins—having both polar and nonpolar regions within its chemical structure—provides the molecules with excellent emulsifying properties and the ability to form soap-like foams, thus giving rise to their name.

Their unique structure and surface activity not only appears largely responsible for their beneficial biological effects but also for the long-held belief that saponins are toxic. Some saponins are strongly hemolytic and are

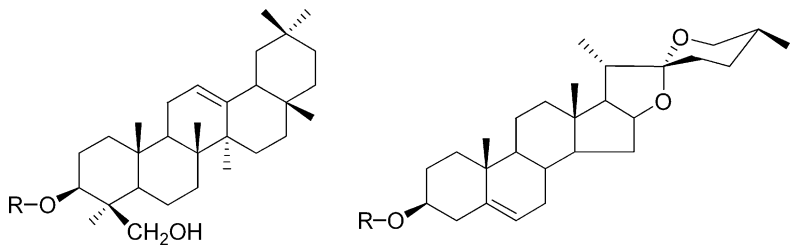

FIG. 4 Structure of representative saponins found in soybeans (triterpenoid) and eggplant (steroid). The R group represents one or more carbohydrate linkages that may contain rhamnose, xylose, arabinose, galactose, glucose, or glucuronic acid.

known toxicants in certain fish and cold-blooded animals (Francis *et al.*, 2002; Price *et al.*, 1987). Their bitter taste in high concentrations has undoubtedly fueled the suspicion of their antinutritional qualities (Liener, 1994). However, the saponins traditionally consumed by humans and other mammals appear to be safe (Oakenfull and Sidhu, 1990), although it seems likely that saponins from unusual sources, or common saponins administered in purified form or in high doses, could produce undesirable effects.

Saponins are present in most plants and are particularly abundant in legumes and alfalfa (Table III). They are also consumed, albeit in smaller amounts, with many herbs, spices, and tea. Triterpenoid saponins are generally predominant in food crops (e.g., legumes, sugar beets, spinach), while steroid saponins are more common in herbs and spices. Botanicals have long been used by ancient cultures for food and medicinal purposes, and it is now believed that many healthful properties of plants are due to saponins. Among the many health benefits attributed to saponins are reduced plasma cholesterol levels, inhibition of cancer cell growth, lower blood glucose response, reduced incidence of kidney stones, immune stimulation, anti-inflammation, and antioxidant properties (Francis *et al.*, 2002; Lacaille-Dubois and Wagner, 1996; Shi *et al.*, 2004).

The cholesterol-lowering ability of saponins was first observed in the 1950s and has since been confirmed in a number of species including humans (reviewed in Oakenfull and Sidhu, 1990). Saponins have also been shown to decrease the development of arterial atherosclerosis (Koo, 1983; Malinow

TABLE III
SAPONIN CONTENT OF COMMON PLANT FOODS

	Total saponins (g/100 g edible portion)
Alfalfa sprouts	8.0
Asparagus	1.5
Black-eye peas	0.03
Chickpeas	3.6
Kidney beans	4.1
Lentils	0.5
Navy beans	4.0
Oats	1.3
Peanuts	1.6
Pinto beans	4.1
Soybeans	5.6
Spinach	4.7
Sugar beets	5.8

Source: Price *et al.* (1987); Oakenfull and Sidhu (1989).

et al., 1978; Sautier *et al.*, 1979). A number of pharmaceutical and nutraceutical companies have developed saponin-based compounds for the purpose of lowering plasma LDL cholesterol concentration. To date, the FDA has not granted approval for a saponin-containing prescription drug intended for this purpose. However, a saponin extract from alfalfa called Esterin® (Innovative Product Solutions Group) is available over-the-counter and is the main ingredient in the dietary supplements Cholestaid™, Cholesterin™, and CholestSorb™.

Saponins appear to lower plasma LDL cholesterol concentration by interfering with cholesterol absorption. Studies in rats and monkeys fed naturally occurring saponins exhibited significant reductions in cholesterol absorption efficiency and an increase in fecal cholesterol excretion (Malinow *et al.*, 1981; Nakamura *et al.*, 1999; Sidhu *et al.*, 1987). Decreased bile acid absorption and increased excretion has also been reported in animals fed saponins (Malinow *et al.*, 1981; Nakamura *et al.*, 1999; Stark and Madar, 1993). One possible mechanism of action for decreased cholesterol absorption is the ability of saponins to form insoluble complexes with cholesterol (Gestetner *et al.*, 1972; Malinow *et al.*, 1977). In an effort to isolate the specific properties of saponins, Malinow (1985) prepared a variety of synthetic saponins in which the complex carbohydrate moieties of native plant saponins were replaced with simplified carbohydrates such as glucose or cellobiose. One of these synthetic saponins, tiqueside (Pfizer, Inc.), can effectively precipitate cholesterol from micelle solutions *in vitro* and inhibit cholesterol absorption in a variety of animals (Harwood *et al.*, 1993) and in humans (Harris *et al.*, 1997). But despite ample data showing the formation of a saponin/cholesterol complex *in vitro*, there is essentially no definitive evidence that complexation occurs in the intestinal lumen (Morehouse *et al.*, 1999).

Another possible mechanism involves the effect of saponins on micelle formation. Saponins are known to alter the size or shape of micelles (Oakenfull, 1986; Oakenfull and Sidhu, 1983), an observation that is consistent with decreased bile acid absorption (Stark and Madar, 1993) and increased fecal bile acid excretion (Malinow *et al.*, 1981; Nakamura *et al.*, 1999). Saponins may also directly bind bile acids (Oakenfull and Sidhu, 1989), which would presumably interfere with micelle formation and decrease cholesterol absorption. Other studies have found that saponins decrease the absorption of fat-soluble vitamins (Jenkins and Atwal, 1994) and triglycerides (Han *et al.*, 2002; Okuda and Han, 2001), indicating decreased micelle formation. However, direct evidence showing impaired micelle formation *in vivo* is lacking. Moreover, Harwood *et al.* (1993) reported no change in bile acid absorption or interruption of the enterohepatic circulation of bile acids in hamsters fed tiqueside, despite significant reductions in cholesterol absorption.

Because of their amphiphilic nature and high surface activity, saponins could affect cholesterol absorption by interacting with cholesterol and other lipids in the brush border membrane of enterocytes. *In vitro* studies have indicated that the permeability of the intestine can be affected by saponin treatment (Alvarez and Torres-Pinedo, 1982; Johnson *et al.*, 1986). Onning *et al.* (1996) reported that saponins altered the permeability of albumin in rat intestine *in vitro*, but the transport of glucose was not affected. Furthermore, when given *in vivo* in the same study, the saponins did not affect albumin transport across the intestinal membrane. Most common food-derived saponins are unlikely to affect intestinal cell membranes to a significant extent. Even though the detergent action of specific hemolytic saponins is responsible for their toxicity, these hazardous saponins are generally not part of the human food supply or they are consumed in negligible amounts (Francis *et al.*, 2002; Price *et al.*, 1987). The synthetic saponin, tequeside, did not alter cholesterol or bile acid absorption in everted sacs after pretreatment with saponins, indicating no significant change in enterocyte transport function (Sidhu *et al.*, 1987). Tequeside is synthesized from food-based saponins and is probably more representative of saponins commonly consumed by humans. Tequeside is also well tolerated by humans and no toxicological side effects have been observed (Harris *et al.*, 1997).

The full extent to which saponins reduce cholesterol absorption requires further study. Because of the large number of saponins present in the food supply, it is possible that all of the mechanisms discussed earlier contribute to reduced cholesterol absorption. Unlike plant sterols in which their mode of action is relatively well defined, there are probably multiple effects of saponins within the intestinal tract, including their ability to interact with other dietary constituents and the ability of some saponins to be absorbed systemically. The regulatory effects of saponins on cellular cholesterol transport have not been examined.

D. SOY PROTEIN

Replacing animal proteins in the diet with plant proteins—primarily soy protein—is known to lower plasma cholesterol concentration in both animals and humans (Forsythe *et al.*, 1986). In a large cross-study of 4838 Japanese men and women, Nagata *et al.* (1998) observed a significant trend for decreasing total cholesterol concentration with an increasing intake of soy products. A meta-analysis of 38 clinical studies indicated that an average intake of 47 g soy protein per day was associated with a 13% decrease in plasma LDL concentration (Anderson *et al.*, 1995). Based largely on the meta-analysis, the FDA approved a health claim for the association between dietary soy protein and reduced risk of coronary heart disease in October of

1999 in response to a petition submitted by Protein Technologies International, Inc. The FDA determined that a daily intake of 25 g of soy protein was required to elicit a clinically significant reduction in plasma LDL cholesterol and thus allows the following health claim: "25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease."

Studies in monkeys (Greaves *et al.*, 2000), rabbits (Huff and Carroll, 1980), and rats (Nagata *et al.*, 1982; Vahouny *et al.*, 1984) have demonstrated significant reductions in cholesterol absorption due to dietary soy protein. In each of these studies, soy protein was compared to equivalent amounts of casein. Greaves *et al.* (2000) also compared soy protein to casein enriched with either isoflavone extract or conjugated equine estrogen, but only the soy protein caused a decrease in cholesterol absorption. Increased fecal cholesterol excretion has also been reported in experimental animals fed soy protein (Beynen, 1990; Potter, 1995). One human study is in agreement with the animal data (Duane, 1999), although another reported no change in fecal cholesterol output in hypercholesterolemic patients treated with soy protein (Fumagalli *et al.*, 1982).

Investigators originally focused on the amino acid content of soy protein, with its low lysine/arginine ratio compared to casein, as being responsible for increased fecal cholesterol excretion. Sugano *et al.* (1984) suggested that the low lysine/arginine ratio may alter pancreatic secretions (hormones and digestive enzymes), which in turn could influence cholesterol absorption. However, Gibney (1983) was unable to show any effect of the lysine/arginine ratio on cholesterol kinetics in rabbits, and the precise mechanisms involving lysine and arginine have not been subsequently defined. Furthermore, rats fed amino acid mixtures that mimic soy protein exhibited no change in cholesterol absorption or fecal cholesterol excretion relative to dietary casein, but that intact soy protein caused an inhibition in cholesterol absorption and greater fecal cholesterol output (Nagata *et al.*, 1982; Tanaka *et al.*, 1984). The latter studies have led to the belief that the specific amino acids of soy protein probably have little impact on cholesterol absorption, raising the possibility that nonprotein constituents within commercial soy protein preparations are responsible for the cholesterol-lowering action previously observed in animal and human studies.

Soy protein preparations contain a variety of biologically active compounds including saponins, fibers, trypsin inhibitors, and isoflavones (Potter, 2000). Hamsters and rats fed ethanol-extracted soy protein isolates had no ability to lower plasma cholesterol compared to intact soy protein isolates (Lucas *et al.*, 2001; Ni *et al.*, 1999). Extraction with ethanol is a treatment that would remove saponins, isoflavones, and other phytochemicals from the protein. Although one study showed that ethanol washing did not

diminish the cholesterol-lowering ability of soy protein isolate (Fukui *et al.*, 2004), it seems likely that the relatively high saponin content of soybeans (Table III) could contribute to the inhibition of cholesterol absorption. Soy protein "isolates" are the most highly refined soy protein products available to consumers and contain about 90% protein. The saponin content of commercial isolates ranges from 0.3 to 2.3 g/100 g (Fenwick and Oakenfull, 1981; Ireland *et al.*, 1986). Soy protein "concentrates" contain about 70% protein and retain most of the soybean fiber, which may also contribute to inhibition of cholesterol absorption, but the saponin concentration is undetectable (Ireland *et al.*, 1986).

Another consideration is the effect of partially digested soy protein on cholesterol metabolism. Sugano *et al.* (1990) demonstrated that a soy protein hydrolysate (partially digested by porcine pepsin or microbial proteases) fed to rats decreased plasma cholesterol levels and promoted fecal cholesterol excretion to a greater extent than intact soy protein. Using the Caco-2 intestinal cell model, Nagaoka *et al.* (1997) reported that soy protein hydrolysate directly inhibited the absorption of micellar cholesterol. The same investigators later found that rats infused with soy protein hydrolysate had significantly lower rates of cholesterol absorption and increased fecal excretion of cholesterol compared to rats fed intact soy protein (Nagaoka *et al.*, 1999). In additional experiments, Nagaoka *et al.* (1999) showed that soy protein hydrolysate decreased the micellar solubility of cholesterol *in vitro*. These studies suggest that digestion products of soy protein may directly interfere with micelle formation.

Soy protein intake has also been reported to increase bile acid excretion in animals (Beynen *et al.*, 1990; Huff and Carroll, 1980; Kuyvenhoven *et al.*, 1989; Nagata *et al.*, 1982; Wright and Salter, 1998), although the effect was only marginal in a human study (Duane, 1999). As with soluble fibers and saponins, increased bile acid excretion may indicate a disruption in micelle formation, leading to reduced cholesterol solubilization and absorption, although this has not been definitively established. Lin *et al.* (2004) reported an additive effect of soy protein and plant sterol esters in promoting the excretion of both cholesterol and bile acids when fed simultaneously to hamsters, indicating the benefit of consuming a variety of cholesterol-lowering plant-based foods.

E. PHOSPHOLIPIDS

Phospholipids are a group of amphiphilic molecules in which long acyl chains form a hydrophobic region, while the presence of a hydrophilic region is due to a phosphate-containing "head" group. Figure 5 illustrates two types of phospholipids, phosphatidylcholine (PC) and sphingomyelin (SM),

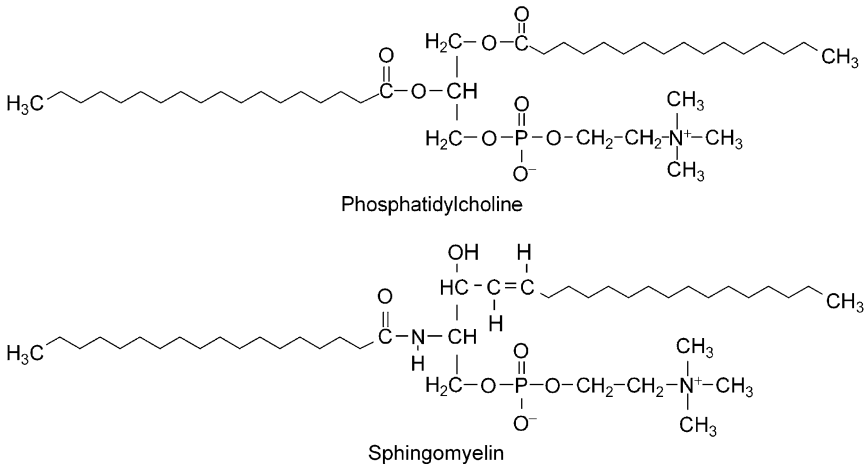

FIG. 5 Phospholipids present in the food supply.

found throughout nature and thus in the food supply. Various molecules can comprise the polar head group, although choline is frequently found in both plant and animal phospholipids. The long chain acyl moieties can be highly variable, although phospholipids from animals tend to be more saturated than those of plant origin. Because of their unique chemical structure, phospholipids spontaneously form lipid bilayers and are therefore the primary structural components of cell membranes. Most phospholipids are synthesized in the body but may also be consumed in diet. In addition to their role in cell membranes, phospholipids (specifically PC) are an important component of bile and are required for micelle formation and cholesterol solubilization. Because of biliary secretion, the principle phospholipid in the intestinal lumen is PC, which can exceed dietary amounts by as much as 5 to 1 (Eckhardt *et al.*, 2002). Food sources rich in PC include egg yolks, muscle foods, peanuts, and soybeans. SM is most abundant in egg yolks, muscle foods, soybean, and milk and dairy products. Food manufacturers frequently use purified PC (also called lecithin) as a food additive because of its excellent emulsifying properties. The PC found in commercial products is most often derived from soybeans and, to a lesser extent, egg yolk.

Dietary SM and PC are known to inhibit cholesterol absorption in experimental animals, resulting in lower plasma cholesterol levels (Imaizumi *et al.*, 1992; Jimenez *et al.*, 1990; O'Brien and Corrigan, 1988; Wilson *et al.*, 1998). Inhibition of cholesterol absorption was observed in humans infused

intraduodenally with purified soy PC (Beil and Grundy, 1980). Kesaniemi and Grundy (1986) also found a small but significant reduction in cholesterol absorption in hyperlipidemic patients fed soy PC. Koo and colleagues 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 (Jiang *et al.*, 2001; Koo and Noh, 2001). In fact, soy PC did not interfere with cholesterol absorption but rather produced a slight increase in absorption relative to no-PC controls (Jiang *et al.*, 2001). These researchers suggested that the higher degree of fatty acid saturation in egg PC compared to soy PC may have caused greater disruption in the 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, irrespective of whether the PC was from soy (Nyberg *et al.*, 2000) or egg (Jiang *et al.*, 2001). Naturally occurring soy PC contains 10–20% saturated fatty acids (SFA), whereas native egg PC contains 40–50% SFA.

In vitro studies using intestinal cells have suggested that intact PC—that is, PC that has not been hydrolyzed by digestive enzymes—exerts an inhibitory effect on micelle formation and, hence, cholesterol solubility. Young and Hui (1999) reported an inhibitory effect of PC on cholesterol uptake in IEC-6 intestinal cells when PC was incorporated into lipid emulsions. In another study, the addition of phospholipase A_2 eliminated the inhibitory action of PC, resulting in increased absorption of cholesterol into Caco-2 cells (Homan and Hamelehle, 1998). Studies have indicated that the presence of PC on the surface of lipid emulsions hinders the hydrolysis of triglycerides (Borgström, 1980; Patton and Carey, 1981), which also inhibits cholesterol uptake into intestinal cells (Young and Hui, 1999). These data indicate that proper digestion of dietary lipids is necessary to maximize micelle formation and cholesterol solubilization and that PC can inhibit digestive enzymes. The data further suggest that saturated egg PC is a more potent inhibitor of lipases than unsaturated soy PC mainly because saturated PC is a poor substrate for phospholipase A_2 and is not readily hydrolyzed (Jiang *et al.*, 2001; Kinkaid and Wilton, 1991). Still unknown is the effect of PC fatty acid chain length on micellar solubility of cholesterol.

SM also inhibits cholesterol absorption in experimental animals, and this effect has been shown to be dose dependent (Eckhardt *et al.*, 2002; Noh and Koo, 2003; Nyberg *et al.*, 2000). However, there is little information regarding how SM from different food sources affects cholesterol absorption. Noh and Koo (2004) reported that milk SM was a more potent inhibitor than egg SM, suggesting that the higher degree of saturation and longer chain length of milk SM may be important factors, although further investigation is needed.

SM is similar to PC to the extent that they share the same phosphatecholine head group and have long chain hydrophobic moieties (Figure 5), but SM differs in several ways from naturally occurring PC. The backbone of SM is a sphingoid base, whereas the backbone of PC is a glycerol base, which increases the polarity of SM and allows for stronger intra- and intermolecular hydrogen bonding (Yeagle *et al.*, 1976). 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 (Demel *et al.*, 1977; McIntosh *et al.*, 1992). Eckhardt *et al.* (2002) 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. Dietary intake of SM in humans is estimated to be 0.3–0.4 g/day (Vesper *et al.*, 1999), whereas PC intake is about 1–2 g/day (Åkesson, 1982). When taking into account biliary secretion of PC, there is considerably more PC than SM in the intestinal lumen under normal conditions. Addition of SM to the diets of experimental animals was still able to reduce cholesterol absorption in the presence of normal biliary PC, indicating a higher potency of SM relative to PC (Eckhardt *et al.*, 2002; Nyberg *et al.*, 2000). Further evidence of the effectiveness of SM is indicated by the case of the 88-year-old "egg man," who habitually ate 25 eggs/day, yet had normal plasma cholesterol levels (Kern, 1991). Eggs contain large amounts of SM (about 0.17% by weight), which could interact with cholesterol and decrease its availability for micellar solubilization. Foods that have little or no cholesterol but high SM, such as soybeans, have a greater potential to bind endogenous cholesterol, thus preventing its absorption.

F. STEARIC ACID

Stearic acid is a long chain SFA present, to varying degrees, in virtually all edible fats and oils. Table IV provides the fatty acid composition of fats and oils commonly consumed by humans. The most abundant food sources of stearic acid in the American diet are beef fat and cocoa butter (chocolate). Cocoa butter is valued by chocolate manufacturers because it remains solid at room temperature but dissolves quickly at body temperature, a unique characteristic of chocolate that is due largely to stearic acid. During the last few decades as cocoa butter prices and supplies have fluctuated, food companies began looking for alternative oils that could provide equivalent amounts of stearic acid in order to retain the desirable physical characteristics. Several

TABLE IV
FATTY ACID COMPOSITION (%) OF EDIBLE FATS AND OILS

	Medium chain fatty acids (8:0–12:0)[a]	Myristic acid (14:0)	Palmitic acid (16:0)	Palmit-oleic acid (16:1)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)	Others
Beef fat (tallow)	0.9	3.7	24.9	4.2	18.9	36.0	3.1	0.6	7.7
Butter oil	8.3	10.0	26.2	2.2	12.1	25.0	2.2	1.4	12.6
Canola oil	–	0.1	4.0	0.2	1.8	56.1	20.3	9.3	8.2
Chicken fat	0.1	0.9	21.6	5.7	6.0	37.3	19.5	1.0	7.9
Cocoa butter	–	0.1	25.4	0.2	33.2	32.6	2.8	0.1	5.6
Coconut oil	58.7	16.8	8.2	–	2.8	5.8	1.8	0.1	5.8
Corn oil	–	0.1	10.9	0.2	1.8	24.2	58.0	0.7	4.1
Olive oil	–	–	11.0	0.8	2.2	72.5	7.9	0.6	5.0
Palm oil	0.1	1.0	43.5	0.3	4.3	36.6	9.1	0.2	4.9
Palm kernel oil	54.2	16.4	8.1	–	2.8	11.4	1.6	–	5.5
Peanut oil	–	0.1	9.5	0.1	2.2	46.8	32.0	–	9.3
Pork fat (lard)	0.3	1.3	23.8	2.7	13.5	41.2	10.2	1.0	6.0
Safflower oil	–	0.1	4.3	0.1	1.9	14.4	74.6	0.4	4.2
Soybean oil	–	0.1	10.3	0.2	3.8	22.8	51.0	6.8	5.0

[a]Abbreviations represent the number of carbon atoms (left side of colon) followed by the number of double bonds.
Source: US Department of Agriculture (2005).

tree nuts and seeds indigenous to West Africa, India, and Southeast Asia were found to be rich in stearic acid, including dhupa, illipe, kokum, mango kernel, sal, and shea (Bhattacharyya, 2002). Shea nut oil (shea butter) and sal oil have proven to be the most feasible and are mainly used in Europe and Japan as cocoa butter substitutes. These stearic acid-rich oils are also used in cosmetics, candles, and other industrial products worldwide, in addition to providing a local source of cooking oil. Shea nut oil contains about 38% stearic acid and sal oil contains about 34% stearic acid. Hydrogenating vegetable oils can increase the stearic acid content by converting unsaturated 18-carbon fatty acids to stearic acid, but the process also yields undesirable *trans* fatty acids.

The impact of dietary fatty acids on plasma cholesterol levels has been studied for decades, summarized by the classic prediction equations of Keys *et al.* (1965) and Hegsted *et al.* (1965) and later refined to focus on LDL cholesterol (Hegsted *et al.*, 1993; Howell *et al.*, 1997). The purpose of the equations is to predict changes in plasma LDL cholesterol concentration in response to changes in dietary intake of SFA, polyunsaturated fatty acids (PUFA), and cholesterol. Dietary monounsaturated fatty acids are believed to have "neutral" effects on plasma cholesterol and are excluded from the equations. One common feature of all the prediction equations is that the strongest determinant of plasma cholesterol concentration is SFA (increases plasma cholesterol) followed by PUFA (decreases cholesterol), while dietary cholesterol has relatively little effect on plasma cholesterol concentrations (in contrast to dietary recommendations to limit cholesterol intake). The strong influence by SFA has led to the well-known recommendation from the National Cholesterol Education Program, the American Heart Association, and other health organizations to limit SFA intake (Krauss *et al.*, 2000; National Institutes of Health, 2002). But despite the blanket condemnation of SFA, the original data of Keys *et al.* (1965) and Hegsted *et al.* (1965) clearly showed that stearic acid was unique among dietary SFA because it did not raise plasma cholesterol levels. The neutral or cholesterol-lowering effect of dietary stearic acid has since been confirmed repeatedly in animal and human studies (Grundy and Denke, 1990; Kris-Etherton and Yu, 1997; Sanders, 2003).

Feldman *et al.* (1979a,b) were the first to demonstrate a reduction in cholesterol absorption due to dietary stearic acid in rats. The investigators used 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 (Chen *et al.*, 1989; Ikeda *et al.*, 1994). Using a more realistic dietary approach,

we conducted a study in hamsters fed NIH-07 cereal-based diet specifically enriched in single fatty acids (stearic, palmitic, oleic, linoleic, or *trans* fatty acids). Cholesterol absorption was similar among hamsters fed palmitic, oleic, linoleic, or *trans* fatty acids, however, cholesterol absorption was significantly reduced in hamsters fed stearic acid compared to the other fatty acids (Schneider *et al.*, 2000). Dietary stearic acid has also been shown to increase fecal cholesterol excretion in rats and hamsters (Imaizumi *et al.*, 1993; Kamei *et al.*, 1995; Schneider *et al.*, 2000).

Schmidt and Gallaher (1997) 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 (1993a,b) reported that after absorption, stearic acid was preferentially incorporated into hepatic and biliary phospholipids compared to other dietary fatty acids. Cohen and Carey (1991) 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 (Cowles *et al.*, 2002). Similarly, Hassel *et al.* (1997) 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 (Armstrong and Carey, 1987). 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.

IV. CONCLUSIONS

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

chapter focuses only on those compounds known to lower plasma cholesterol by inhibiting cholesterol absorption in the small intestine, including plant sterols and stanols, soluble fibers, saponins, soy protein, phospholipids (SM and PC), and stearic acid. All of these compounds—except, perhaps, stearic acid—appear to exert their effects mainly 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 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. The FDA currently allows a heart health claim for certain products containing plant sterols or soy protein, while the American Heart Association endorses certain products rich in soluble fiber. Many of these compounds also contribute important functional properties when added to foods, such as emulsification, improved texture, and calorie reduction. Some soluble fibers may function as beneficial prebiotics that promote intestinal health. Furthermore, 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. 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.

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