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REVIEWS

Cholesterol-Lowering Nutraceuticals and Functional Foods

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Epidemiological studies have demonstrated that elevated levels of plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) are the major risk factors for coronary heart disease (CHD), whereas high concentrations of plasma high-density lipoprotein cholesterol (HDL-C) and a low ratio of TC to HDL-C are protective against CHD. A relationship between plasma TC and the risk of CHD is well established at concentrations above 240 mg/dL. In addition to the use of three main classes of cholesterol-lowering medications, including HMG-CoA reductase inhibitors, anion-exchange resins, and fibrates, a nutritionally balanced diet that reduces saturated fat and cholesterol intake has traditionally been the first goal of dietary therapy in lowering plasma TC. In recent years, nutraceuticals and functional foods have attracted much interest as possible alternative therapies for lowering plasma TC, especially for hypercholesterolemia patients, whose blood cholesterol level is marginally high (200−240 mg/dL) but not high enough to warrant the prescription of cholesterol-lowering medications. This review summarizes the findings of recent studies on the production, application, efficacy, and mechanisms of popular cholesterol-lowering nutraceuticals and functional foods.

KEYWORDS: ACAT; cholesterol; HDL; HMG-CoA reductase; LDL; lipoproteins; nutraceuticals

INTRODUCTION

Cholesterol has acquired an unsavory reputation for many years due to the strong correlation between the level of blood total cholesterol (TC) and the incidence of coronary heart disease (CHD). Mammals, including humans, require cholesterol for normal metabolism. Cholesterol is an essential cell membrane modulator, a precursor for the synthesis of bile acids (essential for the formation of fat emulsion and absorption in the intestine), and a substrate for the synthesis of steroid hormones, including estrogen and androgen. Finally, vitamin D is derived from a cholesterol derivative, 7-dehydrocholesterol. However, cholesterol is not essentially required in the diet because humans are capable of synthesizing it.

Cholesterol is insoluble in water and is carried in the blood among tissues by four lipoproteins: chylomicron (CM), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). CM is formed in the intestinal lymphatics and transports dietary cholesterol and triacylglycerols (TG) from the intestine to adipose tissue and skeletal muscles (*1*). VLDL is produced in the liver and transports the newly synthesized TG and cholesterol from the liver to adipose tissue and skeletal muscles. LDL, as a major cholesterol carrier in the blood, is formed in plasma when intermediate-density lipoprotein (IDL) acquires cholesteryl ester (CE) from HDL (*2*). LDL provides cholesterol to those tissues that need it. HDL removes excessive cholesterol from peripheral tissues back to the liver and plays a major role in maintaining cholesterol homeostasis in the plasma. In this respect, LDL is considered a "bad" lipoprotein, whereas HDL is often regarded as a "good" lipoprotein (*3*).

Concentrations of circulating LDL cholesterol (LDL-C) and HDL cholesterol (HDL-C) are governed by two proteins, cholesteryl ester transport protein (CETP) and lecithin-cholesterol acyltransferase (LCAT), and two receptors, LDL receptor (LDL-R) and scavenger receptor B class 1 (SR-B1) (*4, 5*) (**Figure 1**). LCAT, which circulates in association with HDL, esterifies the free cholesterol taken up by nascent HDL from the peripheral tissues, maintaining a free cholesterol gradient between peripheral tissues and the HDL particles and promoting the removal of cholesterol from peripheral tissue to the liver (*6*). It is known that high HDL-C is negatively associated with the risk of CHD, whereas LCAT deficiency can lead to a lower level of HDL-C in humans. CETP is a plasma glycoprotein, which mediates a transfer of CE from HDL to LDL or VLDL with an exchange of the equivalent amount of TG. Inhibition of CETP will lower the ratio of LDL-C/HDL-C, and CETP

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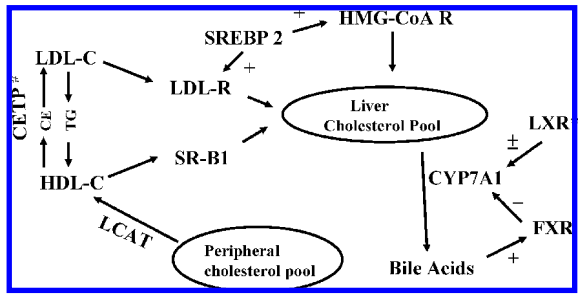

Figure 1. Roles of sterol regulatory element binding protein 2 (SREBP2), liver X receptor (LXR), farnesoid X receptor (FXR), cholesteryl ester transport protein (CETP), lecithin-cholesterol acyltransferase (LCAT), LDL receptor (LDL-R), scavenger receptor B class 1 (SR-B1), 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA R), and cholesterol 7α-hydroxylase (CYP7A1) in cholesterol metabolism. CETP mediates a transfer of cholesteryl esters (CE) from HDL to LDL or VLDL with an exchange of the equivalent triglyceride (TG). LCAT esterifies the free cholesterol and removes cholesterol from peripheral tissue. SR-B1, a HDL receptor, mediates the delivery of HDL cholesteryl esters to the liver and steroidogenic organs. The LDL receptor is responsible for the removal of LDL-C from blood. HMG-CoA R is a key enzyme in cholesterol synthesis. CYP7A1 encoding cholesterol 7α-hydroxylase is a regulatory enzyme in bile acid synthesis. SREBP-2 governs the activation of LDL receptor and HMG-CoA reductase, whereas LXR and FXR regulate the transcription of CYP7A1. +, up-regulation; −, down-regulation; ±, up- or down-regulation in different species. *, LXR activation leads to enhanced expression of CYP7A1 in rodents but not in humans (*9*). #, CETP is absent in rats and mice.

inhibitors have been used as potential new agents to treat CHD (*7*). LDL receptor is responsible for the removal of LDL-C from circulation, whereas the HDL receptor SR-B1 mediates the delivery of HDL CE to the liver and steroidogenic organs. Up-regulation of SR-B1 expression has been linked with decreased HDL levels in hamsters (*8*). A change in the expression of CETP, LCAT, LDL-R, and SR-B1 can lead to an alteration in circulating LDL-C and HDLC levels (**Figure 1**).

Three transcriptional factors, sterol regulatory element binding protein-2 (SREBP-2), liver X receptor (LXR), and farnesoid X receptor (FXR), act in a coordinated manner to govern cholesterol metabolism (*4*). SREBP-2 governs the activation of the transcription for LDL receptor and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, a key enzyme in cholesterol synthesis in the liver, whereas LXR regulates the transcription of CYP7A1 encoding cholesterol 7α-hydroxylase, a key enzyme in bile acid synthesis (**Figure 1**). It should be pointed out that rodents had very different lipoprotein profiles and cholesterol metabolisms compared to humans. In this case, LXR activation leads to enhanced expression of CYP7A1 in rodents but not in humans (*9*). In addition, FXR, a bile acid receptor, plays also an important role in bile acid synthesis (**Figure 1**). Bile acids, which return to the liver via the enterohepatic circulation, activate FXR followed by down-regulation of CYP7A1 (*10*).

CHD induced by atherosclerosis is the main cause of mortality in humans. Elevated levels of plasma TC and LDL-C are the major risk factors for atherosclerosis (**Table 1**), whereas a high concentration of plasma HDL-C and a low ratio of TC to HDL-C are protective against CHD (*11*). In the United States, 30% of the adult population have levels of blood TC higher than 240 mg/dL, whereas in China 19% of the total population have abnormal blood lipids (*12, 13*). Although several factors play an important role in the metabolism of cholesterol, there is no doubt that plasma TC, LDL-C, and HDL-C levels are influenced

Table 1. Desirable Serum Lipoprotein Profile in Humans

lipid	desirable (mg/dL)	borderline (mg/dL)	undesirable (mg/dL)
total cholesterol	<200	200−239	≥240
LDL cholesterol	>130	130−159	≥160
HDL cholesterol	>35		<35

profoundly by diets. In general, plasma TC is raised by dietary cholesterol and saturated and *trans* fatty acids and lowered by monounsaturated and polyunsaturated fatty acids. In recent years there has been considerable interest in the potential for using natural food components as functional foods to treat hypercholesterolemia, especially for patients whose cholesterol level is marginally high (200−240 mg/dL) and does not warrant the prescription of cholesterol-lowering drugs. The aim of this paper is to review the findings of a number of studies conducted in the past few decades on the production, application, efficacy, and mechanisms of popular cholesterol-lowering nutraceuticals and functional foods.

CLASSIFICATION OF CHOLESTEROL-LOWERING NUTRACEUTICALS AND FOODS

Cholesterol-lowering nutraceuticals and functional foods/agents can be classified into five major types: HMG-CoA reductase inhibitors, LDL receptor activators, acyl CoA:cholesterol acyltransferase (ACAT) inhibitors, cholesterol-bile acid absorption inhibitors, and CETP inhibitors.

HMG-CoA Reductase Inhibitors. Inhibition of cholesterol synthesis is the most efficient way to reduce serum cholesterol level. Cholesterol synthesis is a multienzyme pathway in which HMG-CoA reductase mediates the rate-limiting step. The discovery of the statin class of drugs (simvastatin and pravastatin) was a significant advance in the treatment of severe hypercholesterolemia. These drugs inhibit HMG-CoA reductase in the liver. However, side effects are associated with the use of these inhibitors, including rashes and gastrointestinal symptoms (*2*). The mechanism by which some functional foods lower plasma cholesterol is mediated by inhibition and down-regulation on expression of HMG-CoA reductase.

LDL Receptor Activators. Efficient removal of plasma LDL-C is essential for maintaining plasma cholesterol level in a healthy range. Removal of LDL-C from the blood is mediated by receptor-dependent and receptor-independent mechanisms. The former accounts for up to 60−80% of LDL clearance while the latter is responsible for 20−40% cholesterol clearance from the blood. Expression of LDL receptor is a function of cellular free cholesterol. When the cellular cholesterol decreases, the LDL receptor gene is transactivated. In contrast, sufficient cellular free cholesterol down-regulates the LDL receptor gene. Theoretically, up-regulation of LDL-receptor will lead to a lower level of blood cholesterol. A typical example of an LDL receptor activator is a group of phytoestrogens that are found present in soybean (see phytoestrogens below).

ACAT Inhibitors. Two major forms of ACAT, namely ACAT1 and ACAT2, have been identified in mammals. In humans, ACAT2 is important in cholesterol absorption. Reduced absorption of dietary cholesterol can lead to a lower level of blood cholesterol. Intestinal ACAT2 is the primary enzyme responsible for the intracellular esterification of cholesterol. ACAT2 plays an important role in the absorption of cholesterol in the small intestine, before cholesterol is incorporated into CM (*14*). In the liver, this enzyme is partially responsible for the assembly of very low-density lipoproteins (VLDL) prior to secretion into the blood (*15*). TG-rich VLDL particles derived

from the liver are transformed into cholesterol-rich LDL after the removal of their TG by peripheral tissues. Inhibition of ACAT activity therefore lowers the plasma cholesterol level by decreasing cholesterol absorption in the intestine and VLDL production in the liver. Some ACAT inhibitors either isolated naturally from herbs or chemically synthesized have been shown to lower plasma cholesterol levels in both humans and animals (*14*).

Bile Acid Absorption Inhibitors. Bile acids are the major metabolites of cholesterol. Bile acid absorption inhibitors are known as bile acid sequestrants. They bind bile acids in the intestine, prevent their reabsorption, and generate an insoluble complex with bile acids that are excreted in the feces. The increased excretion of bile acids leads to an increase in the synthesis of bile acids from cholesterol in the liver. The lowered level of hepatic cholesterol increases the expression of LDL receptors, which remove the cholesterol from the circulation and decrease the LDL level in the blood. The anion-exchange drugs cholestyramine and colestipol are typical bile acid reabsorption inhibitors, as is dietary fiber (*2*). Ingestion of bile acid inhibitors is usually associated with up-regulation of CYP7A1 encoding cholesterol 7α-hydroxylase in bile acid synthesis.

CETP Inhibitors. A decreased level of plasma HDL-C and an increased level of plasma LDL-C (e.g., the ratio of LDL-C/HDL-C) have been associated with an increased incidence of heart disease. CETP inhibitors prevent the transfer of cholesteryl ester from HDL to TG-rich lipoproteins in exchange for TG, which has the ability to increase the HDL-C level (*16*). Two CETP inhibitors, JTT-705 and torcetrapid, are currently being investigated for their possible utility in the prevention of coronary heart disease. However, their safety has been questioned (*16*). Naturally, some foods (e.g., apple polyphenols) may also have CETP inhibitory activity (*17*). Caution has to be taken when results from rodents are extrapolated to humans because rats and mice lack CETP.

CHOLESTEROL-LOWERING NUTRACEUTICALS AND FUNCTIONAL FOODS

Dietary Fiber. Fibers refer to the edible parts of plants and analogous carbohydrates that are resistant to digestion and absorption in the human small intestine. Dietary fibers can be classified, on the basis of differences in structures, as cellulose, hemicellulose, pectins, gums, mucilage, and lignin. They can also be grouped as soluble fibers and insoluble fibers, depending on their solubility in water. Extensive research has shown that fibers play an important role in cholesterol metabolism by decreasing plasma TC and LDL-C, as demonstrated in a meta-analysis (*18*). In general, most soluble fibers lower plasma total cholesterol more efficiently than water-insoluble fibers by decreasing LDL cholesterol without significantly affecting the HDL-C and TG levels (*19*).

Three mechanisms have been suggested to explain the hypocholesterolemic activity of dietary fibers. First, dietary fiber reduces the absorption of cholesterol and reabsorption of bile acids in the intestinal lumen. Greater fecal excretion of bile acids leads to a decreased enterohepatic circulation of bile acids, followed by an increase in conversion of cholesterol to bile acids in the liver and an increase in cholesterol uptake from the circulation (*19, 20*). Second, dietary fiber is associated with reduced insulin secretion because of its low glycemic effect on blood glucose. Most soluble fibers decrease the rate of glucose absorption and attenuate the rise of plasma glucose and insulin levels, leading to a reduced level of cholesterol synthesis in the liver because insulin promotes hepatic biosynthesis of cholesterol (*19, 21*). Third, dietary fiber undergoes fermentation in the colon to produce a series of short-chain fatty acids including acetic, propionic, and butyric acids. These short-chain fatty acids can be absorbed in the colon, and propionate inhibits hepatic cholesterol biosynthesis (*22*). This hypothesis on cholesterol-lowering activity of short-chain fatty acids is, however, controversial (*23*).

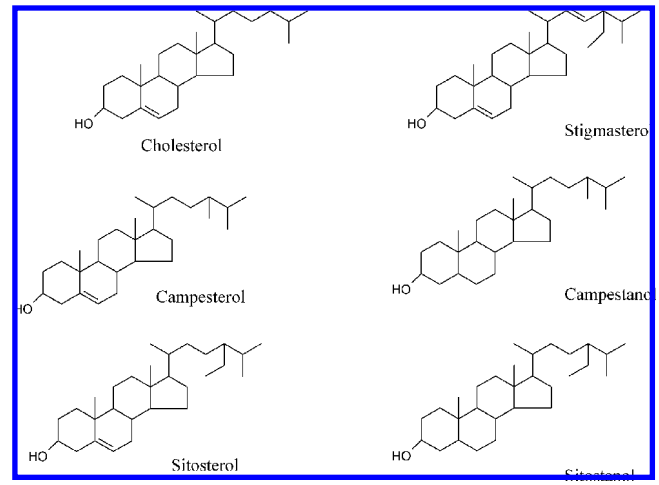

Figure 2. Structures of selected phytosterols.

Phytosterols. Plant sterols and their saturated derivatives, stanols, are a group of cholesterol analogues with different side-chain configurations (**Figure 2**). Mammals synthesize cholesterol, whereas plants synthesize phytosterols. The principal sterols are β-sitosterol, campesterol, and stigmasterol. These phytosterols have been sold as functional cholesterol-lowering nutraceuticals in Europe, the United States, and Australia. A major application for these phytosterols is their addition into spreads and vegetable oils (functional margarine, butter, and cooking oils). It is estimated that the phytosterol intake in humans can reach 160−360 mg/day (*24*), and it has been suggested that the daily consumption of 2 g of phytosterols can effectively lower the cholesterol by 9−14% in humans with little or no effect on HDL-C and TG levels (*25*).

Phytosterols are poorly absorbed in the intestine (**Figure 3**). Humans consume 300−500 mg of cholesterol per day, about half of which is absorbed. The amount of plant and other sterols in diets is comparable to that of dietary cholesterol, but only 5% is absorbed (*26*). The mechanism for differential absorption of cholesterol and phytosterols in the intestine is partially known. First, phytosterols and cholesterol are absorbed into the enterocytes by Niemann−Pick C1 like 1 (NPC1L1), an intestinal cholesterol transporter (**Figure 3**). However, phytosterols are largely prevented from being absorbed because the ATP binding cassette transporters (ABCG5 and ABCG8), which localize in enterocytes, return them to the lumen of the intestine (*27*). Second, ACAT2 is highly expressed in enterocytes in humans and is responsible for the intracellular esterification of cholesterol and its subsequent absorption. ACAT2 prefers to esterify cholesterol rather than sitosterol, and this preference effectively eliminates phytosterols from the absorption process (*26*).

Besides resisting absorption themselves, phytosterols are also inhibitors of intestinal cholesterol absorption, thereby lowering plasma cholesterol levels (*28*). First, phytosterols may cause an effective displacement of cholesterol from micellar binding in the intestine and therefore diminish cholesterol absorption (*29*). Second, phytosterols may affect cholesterol synthesis, as

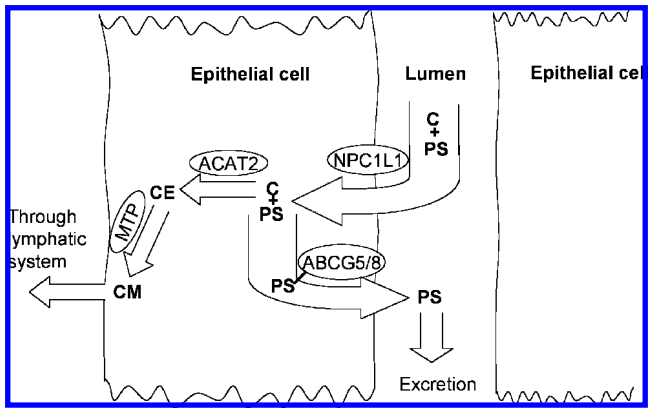

Figure 3. Competition of phytosterols (PS) with cholesterol (C) for absorption in the intestine. Niemann−Pick C1 like 1 (NPC1L1) is responsible for the uptake of both C and PS. ATP binding cassette transporters (ABCG5 and ABCG8) return phytosterols to the lumen of the intestine. Cholesterol acyltransferase 2 (ACAT2) esterifies cholesterol to form cholesteryl ester (CE), which is packed with microsomal triacylglycerols (MTP) into chylomicrons (CM) and transferred into blood through the lymphatic system.

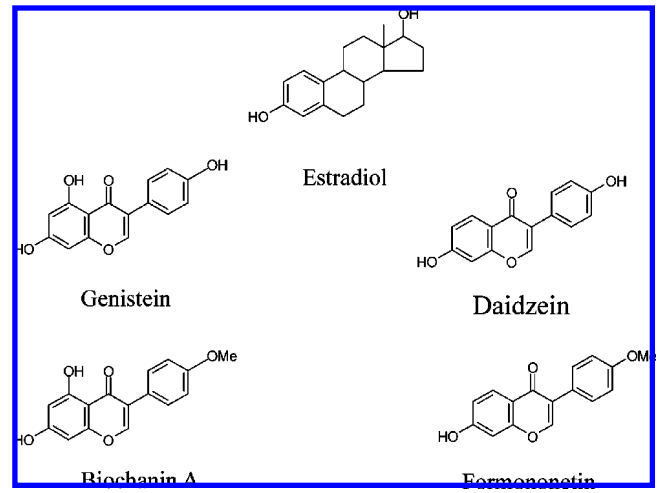

Figure 4. Structures of estrogen and selected phytoestrogens.

β-sitosterol has been shown to decrease cholesterol synthesis by inhibiting HMG-CoA reductase gene expression in CaCo-2 cells (*30*).

A number of animal studies have indicated that the intake of phytosterols can be beneficial. Stigmasterol at 0.5% of diet suppressed intestinal cholesterol and bile acid absorption and decreased the plasma TC level (*31*), leading to 4−9-fold accumulation of stigmasterol in the liver and the consequent suppression of HMG-CoA reductase activity by 4-fold in WKY rats and by 1.8-fold in Wistar rats and the suppression of CYP7A1 by 1.6-fold in WKY rats and by 3.5-fold in Wistar rats (*31*). In a gerbil experiment, a 5:1 ratio of phytosterols/cholesterol was found to effectively block cholesterol absorption when the dietary cholesterol load was moderate (*32*). It was found that free phytosterols dissolved in fat were as effective as esterified sterols and stanols in lowering plasma and liver cholesterol levels, and all were equally effective in blocking cholesterol absorption (*32*). A study of mice found that dietary plant sterols and stanols inhibited cholesterol absorption in the intestinal lumen, but that the effect was independent of LXR (*33*). Dietary intake of plant sterols has been shown to inhibit cholesterol absorption and lower plasma LDL-C in guinea pigs (*34*) and to prevent the development of aortic foam cells in hamsters (*35*).

The cholesterol-lowering activity of phytosterols is also pronounced in humans. When children with severe familial hypercholesterolemia (TC < 370 mg/dL) were given 2 g of sitosterol three times a day for 3 months followed by 0.5 g sitostanol three times a day for an additional 7 months, LDL-C was reduced by 20% and there was a significant increase in fecal excretion of neutral sterols, indicating inhibition of intestinal cholesterol absorption (*36*). In a randomized double-blind placebo-controlled cross-over study, plant sterol-enriched spread reduced blood TC, LDL-C, and apolipoprotein B by 7−10% compared with the control spread in 42 healthy adult volunteers (*37*). It has been shown that plant sterol esters and stanol esters in margarine were equally effective in lowering TC and LDL-C in normocholesterolemic and mildly hypercholesterolemic volunteers (*38*). Accumulated data also suggest that the cholesterol-lowering activity of dietary sterol esters is less marked in longer term than in short-term studies, whereas plant stanol esters maintain their efficacy (*39*). Vegetable oils are rich in phytosterols, and published data suggest that they may account for part of the cholesterol-lowering activity of these oils (*40*). Gremaud et al. (*41*) used stable isotope tracers to study the effect of plant sterols on cholesterol absorption and synthesis in 12 mildly hypercholesterolemic subjects and found that cholesterol absorption dropped dramatically without a consistent, concomitant increase in cholesterol synthesis. It is evident that foods with plant sterols or stanols and their esters lower plasma cholesterol levels. A meta-analysis of 41 trials that assessed the efficacy of plant sterols and stanols concluded that the intake of 2 g/day could reduce LDL-C by 10%. However, higher intakes have little additional effect (*42*).

It should be pointed out that phytosterols may be a risk factor for CHD under certain physiological conditions. Weingärtner et al. (*43*) evaluated the vascular effect of diet supplementation with plant sterol esters, finding plant sterol esters impaired endothelial function, aggravated ischemic brain injury, and enhanced atherogenesis in mice. It was further found that plant sterol accumulated in aortic valves in patients with aortic valve stenosis (AS), raising the possibility that phytosterols could be a risk factor of AS (*44*). Particularly, hyperabsorption of sterols may result in sitosterolemia or phytosterolemia, leading to premature coronary artery diseases in patients with extremely rare genetic defects in the ABCG5 and ABCG8 genes (*45*). Additional studies are needed to confirm these adverse effects and uncover the underlying mechanism associated with intake of phytosterols.

Phytoestrogens. Phytoestrogens are compounds found in plants and have weak estrogenic activity by binding to estrogen receptor and initiating some estrogen-dependent transcription (**Figure 4**). Phytoestrogens have been claimed to have benefits for heart, bone, breast, and general menopausal health (*46*). There is some evidence to suggest that phytoestrogens can reduce blood cholesterol level by inhibiting cholesterol synthesis and increased expression of the LDL receptors (*47*). Major classes of phytoestrogens include isoflavones, flavones, flavanones, comestans, lignans, and stibenes (*48*). Isoflavones have been extensively studied and are mainly found in soybean (genistein, daidzein, glycitein, and their glycosides). Soy isoflavones are the most consumed phytoestrogens in humans. Extensive research has focused on the role of soy phytoestrogens on the plasma cholesterol level in animals (*49, 50*).

Soy phytoestrogens are hypocholesterolemic in most animal studies. The hypocholesterolaemic activity of soy phytoestrogens

is mediated by their stimulating effect on LDL receptor like natural estrogen. Dietary isoflavones have been shown to reduce plasma cholesterol and atherosclerosis in C57BL/6 mice but not in LDL receptor-deficient mice (*51*). In HepG2 cells, incubation with isoflavonoids, formononetin, biochanin A, and daidzein caused significant elevations in LDL receptor activity (*52*). This stimulating effect on LDL receptor is probably mediated by its effect on SREBP2, which regulates expression of both LDL receptor and HMG-CoA reductase. In phytoestrogen-treated HepG2 cells, a mature form of SREBP2 was increased, as were LDL receptor and HMG-CoA reductase (*53*).

The results of randomized clinical trials in humans, however, have been inconsistent. Some clinical trials have shown that soy phytoestrogens reduce plasma TC and LDL-C in hypercholesterolemic subjects (*54, 55*). However, it has been demonstrated that soy isoflavones had no dose-dependent effect (*56*) or had no significant effect on blood cholesterol (*57*). In normocholesterolemic subjects, some clinical trials demonstrated that consumption of soy protein with a high isoflavone content significantly decreased plasma LDL-C compared with the same soy intake with a low isoflavone intake (*58, 59*). In contrast, other trials found no difference in blood cholesterol between a high-isoflavone and a low-isoflavone diet (*54, 60*). To evaluate more precisely the effect of soy isoflavones on plasma TC and LDL-C concentrations, Zhuo et al. (*61*) performed a meta-analysis of eight clinical trials, concluding that with the same soy protein intake, a high-isoflavone diet had greater hypocholesterolemic activity than a low-isoflavone diet and that soy isoflavones had an LDL-C lowering effect independent of soy protein. However, Zhan and Ho (*62*) performed a meta-analysis of 23 randomized control trials and found that soy protein containing isoflavones significantly reduced plasma TC, LDL-C, and TG and increased HDL-C, whereas tablets containing extracted isoflavones had no effect on these parameters. In this regard, one study found soy protein fortified with isoflavones had greater LDL-C lowering activity than soy protein with isoflavone removed (*54*). Further studies are needed to investigate the interaction of soy isoflavones with soy protein or the synergistic action of these two components in their contribution to the hypocholesterolemic activity of soy products.

Policosanol. Policosanol refers to a group of aliphatic primary alcohols with a chain of 24−34 carbons isolated from sugar cane, rice bran, beeswax, sorghum kernel, and wheat germ. It has been shown that policosanol modifies favorable cholesterol metabolism in various cell cultures and reduces plasma TC and LDL-C in animal models and humans. Fibroblast cells have been used to investigate the underlying mechanism by which policosanol reduces plasma cholesterol. Policosanol was shown to be an HMG-CoA reductase inhibitor when it was incubated in fibroblast cells (*63*) and was also found to increase LDL receptor activity (*64*) and to inhibit the absorption of bile acids (*65*).

A number of animal studies have found that policosanol lowers cholesterol levels. When monkeys were orally given 0.25−25 mg of policosanol/kg of body weight for 54 weeks, a persistent reduction in blood cholesterol was seen (*66*). The administration of 5 mg of policosanol/kg of body weight to rabbits lowered plasma TC by 29% and LDL-C by 43% and raised HDL-C by 15%, without affecting TG (*67*). When hamsters were fed diets containing 0, 0.38, 0.75, and 1.5 g of policosanol/kg, serum TC was reduced by 15−25%, whereas HDL-C was raised by 7−17% (*65*). However, two other animal studies did not observe a cholesterol-lowering effect (*68, 69*).

Most clinical randomized, double-blind crossover studies have found that policosanols are significantly hypocholesterolemic.

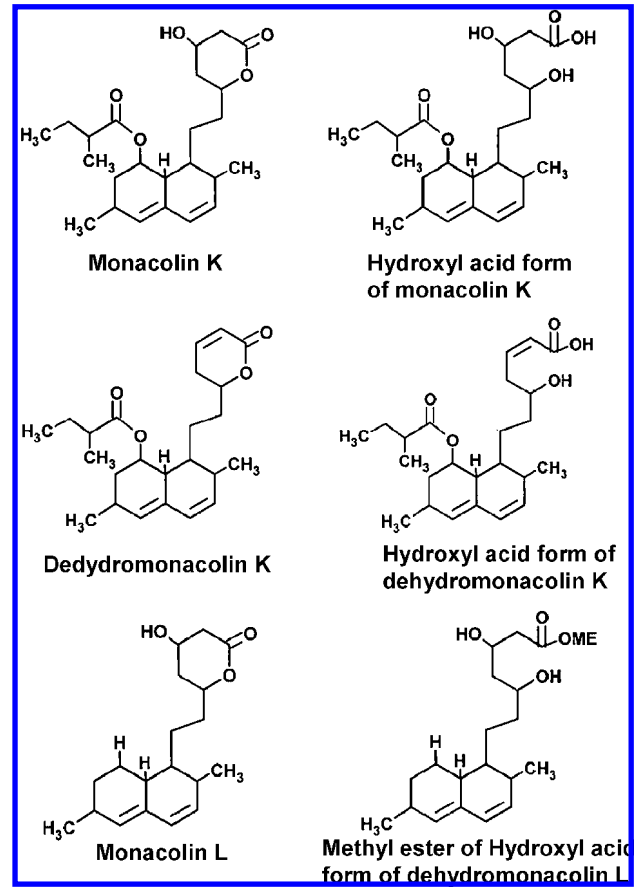

Figure 5. Structures of monacolin K and its derivatives.

Policosanol at doses of 5−40 mg/day was effective in reducing TC and LDL by 16−27% and elevating HDL-C by 17% in type II hypercholesterolemia patients (*70*−*73*). Policosanols at doses of 10 mg/day reduced plasma TC by 17.5% and LDL-C by 21.8% and elevated HDL-C by 11.3% in non-insulin-dependent diabetes mellitus (NIDDM) hypercholesterolemia patients (*74*). Policosanol therapy also helped to lower blood cholesterol and blood pressure in hypercholesterolemic elderly individuals taking β-blockers (*75*). Gouni-Berthold and Berthold (*76*) have reviewed a number of placebo-controlled lipid-lowering studies on policosanol and concluded that policosanol at doses of 10−20 mg was able to lower TC by 17−21% and LDL-C by 21−29% and to increase HDL-C by 8−15%. However, three other studies failed to find any cholesterol-lowering activity associated with the intake of policosanol mixture (*77*−*79*).

Red Yeast Rice and Monacolins. Red yeast rice (Hongqumi in Chinese; known as Koji or Akakoji in Japan) is a fermented rice, which acquires its bright reddish purple color when polished rice is cultivated with the mold *Monoscus purpureus*. Red yeast rice is sold in China as a red colorant for Peking duck and as an ingredient in rice wine. Interestingly, it has for centuries been hailed by practitioners of traditional Chinese medicine as a drug that can help to improve the circulation of the blood. Four brands of medicine derived from red yeast rice are currently marketed in China: Zhitai, Cholestin, Hypocol, and Xuezhikang. The major active compounds in red yeast rice are mainly monacolin K (or lovastatin) and its monacolin-related substances (**Figure 5**), all of which are HMG-CoA reductase inhibitors (*80*). Red yeast rice contains also sterols, isoflavones, and monounsaturated fatty acids, all of which have a hypocholesterolemic activity (*80*). Inhibition of HMG-CoA reductase was observed in HepG2 cells treated with Cholestin, leading to

a decrease in cholesterol synthesis by 31−54% and a decrease in secretion of both free cholesterol and CE by 14−33% (*81*).

Cholesterol-lowering activity has been demonstrated in rabbits, quails, and chickens. In casein-induced hypercholesterolemia rabbit models, treatment with red yeast rice for 30 days at doses of 0.4 and 0.8 g/kg/day significantly lowered serum TC and the TC/HDL-C ratio (*82*). When rabbits were given a diet containing 0.5% cholesterol, intake of red yeast rice at 0.8 g/kg/day for 40 days reduced not only serum TC and TG but also lesions in the aorta (*82*). Similarly, rabbits given 0.4 or 1.45 g of Cholestin/kg/day and maintained on a semipurified diet containing 0.25% cholesterol had serum TC reduced by 25−40% (*83*). When quails were given doses of 0.1, 0.2, and 0.4 g/day, red yeast rice reduced serum TC by 29−38% and TG by 34−40% (*82*). When red yeast rice was added to the diet of broiler chicken, there was a reduction of TC, TC/HDL-C, and LDL-C/HDL-C (*84*).

More than 90 randomized trials have examined the cholesterol-lowering activity of three preparations from red yeast rice in humans, and Liu et al. (*85*) conducted a meta-analysis of these trials to assess the effectiveness and safety of three red yeast rice preparations in blood lipids in primary hyperlipidemia. It was found that red yeast rice treatment resulted in a significant reduction of plasma TC levels (weighted mean difference, −0.91 mmol/L; 95% confidence interval, −1.12 to −0.71) and plasma TG (−0.41 mmol/L, −0.6 to −0.22) and LDL-C levels (−0.73 mmol/L, −1.02 to −0.043) and a concomitant increase in serum HDL-C level (0.15 mmol/L, 0.09 to 0.22) (*85*). The favorable effect of red yeast rice preparations on lipid modification was comparable to that of statin drugs and HMG-CoA reductase inhibitors and better than that of nicotinate and fish oils (*85*). It seems reasonable to conclude that the intake of red yeast rice has a short-term benefit for the blood lipoprotein profile, although further research is needed to establish its long-term effect and assess whether there are any risks associated with its long-term use.

Tea Catechins. The cholesterol-lowering activity of tea catechins has been extensively investigated. Tea, derived from the leaves of *Camellia sinensis*, is the world′s most popular and widely consumed beverage. On the basis of distinct manufacturing processes, tea can be classified into three main types: green tea, oolong tea, and black tea. Green tea is nonfermented and is a major beverage consumed in Asian countries, especially in China and Japan. Black tea generally refers to the fermented products, which are more popular in North America and Europe. Oolong tea is a partially fermented type of tea, the production and consumption of which are confined to Mainland China and Taiwan. Green tea catechins (GTCs) are scarcely changed in green tea, but by the process of fermentation in black tea they are oxidized and polymerized to the "pigments" called theaflavins (TF) and thearubigins (TR). Four GTC derivatives, namely, (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin-gallate (EGCG), have been extensively studied for their wide range of biological and pharmacological properties (**Figure 6**). However, the biological activities of theaflavin (TF1), theaflavin-3-gallate (TF2A), theaflavin-3′-gallate (TF2B), and theaflavin-3,3′-digallate (TF3) from oolong and black tea have not been thoroughly examined (*86*).

GTCs have been shown to lower plasma cholesterol in several animal models and to alter cholesterol metabolism favorably in cell cultures. Although the mechanisms responsible for the cholesterol-lowering activity of GTCs are not yet fully understood, some evidence suggests that they reduce blood cholesterol

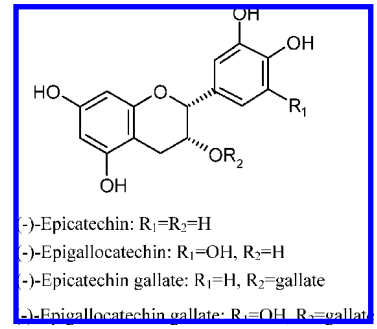

Figure 6. Structure of green tea catechins.

level probably by the following mechanisms. First, they up-regulate the LDL receptor mediated by activation of SREBP 2 (*87, 88*). It has been claimed that EGCG was the active ingredient, which was able to increase LDL receptor activity by 3-fold and protein by 2.5-fold when it was incubated with HepG2 liver cells (*89*). In rats fed a diet containing 2% GTCs, LDL receptor binding activity and protein were increased by 2.7- and 3.4-fold, respectively (*90*). Similarly, LDL receptor activity and protein could be up-regulated by 80 and 70%, respectively, in rabbits fed a hypercholesterolemic-GTC diet (*91*). Second, GTCs reduce the plasma cholesterol level by increasing fecal bile acid and cholesterol excretion. In hamsters fed a 0.1% cholesterol diet, GTCs not only decreased both plasma TC and TG but also increased excretion of both neutral and acidic sterols (*92*). A similar effect was observed in rats fed tea extracts (*93*). Third, GTCs have been shown to inhibit cholesterol synthesis in rabbits (*91*) but not in rats (*93*).

Data from studies of the cholesterol-lowering effects of GTCs in humans are not consistent. Epidemiological observations indicate that tea consumption is associated with reduced levels of plasma TC and LDL-C in Japanese (*94*) and Norwegian subjects (*95*). One study demonstrated that theaflavin-rich tea extract at a dose of 375 mg a day effectively reduced TC and LDL-C in mild to moderate hypercholesterolemia subjects (*96*). Another study found that GTCs were able to attenuate the postprandial increase in plasma TG following a fat load (*97*). However, some studies did not observe a favorable effect. The results from a cross-sectional study did not demonstrate that drinking green tea was associated with changes in any of the lipid levels (*98*). One study found that consumption of 900 mL of green or black tea/day by 45 volunteers for 4 weeks did not affect serum lipid concentration (*99*). As the evidence for the cholesterol-lowering activity of tea in humans is mixed, further additional clinical randomized, double-blind crossover studies are needed to clarify the issue.

Grape Polyphenols. The beneficial effects of polyphenols in red wine are relatively well-established. In many countries, a high intake of saturated fats strongly correlates with a high risk of coronary heart disease, but not in France and some regions where wine consumption is high. This paradox has been attributed to the anti-LDL oxidation activity and antiatherogenic effect of wine polyphenols (*100*). The polyphenols present in grape and its seed are mainly hydroxycinnamic acid, flavonols, anthocyanins, catechins, and proanthocyanidins (*101*). In addition, favorable modification of lipoproteins by decreasing the LDL-C/HDL-C ratio and LDL-C oxidation has been also claimed to be responsible for the reduced risk of coronary heart disease associated with moderate consumption of red wine (*102*).

The hypocholesterolemic activity of grape polyphenols has been demonstrated in rats (*103*) and hamsters (*104*). Although they could not lower plasma lipids, grape polyphenols were

capable of attenuating atherosclerosis in rabbits (*105*) and ovariectomized guinea pigs (*106*). In postmenopausal women, lyophilized grape powder decreased plasma LDL cholesterol and apolipoproteins B and E (*107*). When the diets of both healthy subjects and hemodialysis patients were supplemented with red grape juice, there was a significant decrease in LDL-C and apolipoprotein B-100 concentration and an increase in the concentration of HDL-C and apolipoprotein A-I (*108*). In other studies on human subjects, purple grape juice or grape polyphenols did not affect the plasma cholesterol level but were able to reduce the susceptibility of LDL to oxidation and improve the endothelial function (*109, 110*).

Several mechanisms have been proposed to explain the cholesterol-lowering activity of grape polyphenols. One possibility is that grape polyphenols increase fecal bile acids and reduce cholesterol absorption, as rats given diets containing 2% grape monomer and polymer of anthocyanins had a greater output of fecal acidic and neutral sterols (*103*). Indeed, expression of the key enzyme controlling bile acid synthesis, CYP7A1, was up-regulated in rats given grape seed polyphenol extract (*111*). A second possibility is that the cholesterol-lowering activity of grape polyphenols is mediated by regulating expression of LDL receptor. When HepG2 cells were incubated with dealcoholized wine extract, the mRNA of LDL receptor gene was significantly increased (*112*). In HepG2 and HL-60 cells, red grape juice increased the level of the active form of SREBP, and this was accompanied by greater mRNA expression of LDL receptor (*113*).

Garlic. Garlic is widely regarded as a cholesterol-lowering functional food ingredient, and results from animal trials support this notion. There is evidence that allicin is responsible for the cholesterol-lowering effect of garlic (*114*). Garlic powder has been shown to suppress serum TC and TG in rats (*115*), whereas aged garlic extracts decreased TC by 15% and TG by 30% in rats fed a high-cholesterol diet (*116*). Interestingly, raw garlic had a pronounced effect in reducing plasma TC and TG levels, whereas boiled garlic had little effect (*117*). When garlic powder was added into the diet at 1% level, it not only decreased plasma TC and LDL-C but also increased HDL-C concentration in rabbits (*118*), thus lowering favorably the LDL-C/HDL-C ratio.

Randomized controlled trials in humans have produced conflicting results. Stevinson et al. (*119*) conducted a meta-analysis of 13 trials and found that garlic was able to reduce blood cholesterol compared with the placebo. However, the effect of garlic versus placebo on blood cholesterol became insignificant when the meta-analysis was performed on only the six trials of the highest methodological quality (*119*). Similarly, in an analysis of 10 studies, Alder et al. (*120*) found that 6 trials demonstrated the cholesterol-lowering activity of garlic. However, some of these randomized controlled trials had methodological shortcomings, including short duration, lack of power analysis, and lack of the control of diet as a confounding variable (*120*).

The underlying mechanism by which garlic and its active ingredients lower blood cholesterol has been investigated in cell culture and in animals. Principally, garlic inhibits HMG-CoA reductase. The water-soluble organosulfur compounds *S*-allylcysteine, *S*-ethylcysteine, and *S*-propylcysteine have been shown to reduce cholesterol synthesis by deactivating HMG-CoA reductase via enhanced phosphorylation, but did not change the levels of mRNA or the amount of the enzyme in cultured rat hepatocytes (*121*). In HepG2 cells, allyl mercaptan, a major metabolite of garlic compounds, effectively inhibited cholesterol synthesis at concentrations as low as 5 μg, and its inhibition was concentration dependent (*122*). To a lesser extent, garlic also inhibits CETP activity and modifies the LDL-C/HDL-C ratio. The effect of garlic supplementation on CETP activity, together with its antiatherosclerotic effect, has been studied in cholesterol-fed rabbits, and it was found that CETP activity was significantly reduced in the garlic-supplemented group compared to the control group (*118*). Thus, garlic not only reduced atherosclerosis lesions but also altered the ratio of LDL-C/HDL-C (*118*).

Buckwheat. Buckwheat has received attention because of its favorable hypocholesterolemia and hypotensive activity. Both buckwheat herb and seeds are rich in rutin and contain some quercetin and quercitrin (*123*). It has been claimed that buckwheat protein is the active ingredient responsible for cholesterol-lowering activity (*124*). In rats fed a diet containing 20% buckwheat protein, there was a 32% reduction in serum cholesterol compared with the same amount of casein (*125*). Both buckwheat flour and buckwheat protein were equally effective in reducing plasma TC by 32% (*126*). It has been shown that buckwheat protein is more effective than soybean protein in lowering plasma cholesterol in hamsters fed a high-cholesterol diet (*124*). Buckwheat sprout has been also found to reduce plasma TC, TC/HDL-C, and LDL-C/HDL-C in hamsters (*127*).

Information on the cholesterol-lowering activity of buckwheat in humans is scarce, however. In a study of the relationship of oat and buckwheat intake and cardiovascular disease risk factors in 850 subjects, He et al. (*128*) found that buckwheat intake (100 g/day) was associated with a reduction in plasma TC (−0.07 mmol/L) and LDL-C (−0.06 mmol/L). One epidemiological study investigating the effect of lifetime consumption of buckwheat as a staple food in Inner Mongolia suggests that buckwheat is a preventative factor for hypertension, hyperlipidemia, and hyperglycemia (*129*). When 12 volunteers replaced part of their cereal intake at lunch by a preparation made from 100 g of sieved buckwheat flour for a period of 4 weeks, plasma HDL-C increased by 30% and the HDL-C/TC ratio increased by 27%.

It has been suggested that the cholesterol-lowering activity of buckwheat is mediated by its influence on fecal excretion of bile acid and neutral sterols. Tomotake et al. (*124*) investigated the effect of buckwheat protein on plasma cholesterol, gallbladder bile composition, and fecal steroid excretion compared with casein and soybean proteins in hamsters, demonstrating that buckwheat protein lowered significantly plasma TC and hepatic cholesterol but caused greater excretion of both neutral and acidic sterols than the other two proteins. Buckwheat protein is poorly digested, and its poor digestibility is at least partially responsible for the increased level of steroid excretion (*130*).

Rice Bran Oil (RBO). A number of studies in both humans and animals have demonstrated that RBO is more effective than other vegetable oils in reducing plasma TC. Rats fed RBO at 10% level for a period of 8 weeks showed significantly lower levels of TC, LDL-C, and VLDL-C in both cholesterol-containing and cholesterol-free diets (*131*). Compared with peanut oil, RBO appeared to decrease LDL-C and VLDL-C but to increase HDL-C more effectively (*132*). In hamsters, a reduction in plasma TC was significantly correlated to the level of rice bran oil added in the diet (*133*). Compared with coconut and canola oil, RBO decreased not only plasma TC and LDL-C but also atherosclerosis in hamsters fed an atherogenic diet (*134*). Similarly, RBO has been shown to lower serum TC and LDL-C in monkeys (*135*). In hyperlipidemia patients, a cross-over design experiment found that the use of RBO reduced plasma

TC and TG level (*136*). Similarly, a randomized cross-over trial in 26 healthy, moderately hypercholesterolemia humans found that RBO lowered TC (*137*). However, the cholesterol-lowering activity of RBO was no better than that of canola and corn oil (*138*).

The hypocholesterolemic activity of RBO is probably attributable to its several active constituents, including γ-oryzanol, ferulic acid, tocotrienols, and phytosterols (*139*). Both γ-oryzanol and ferulic acid decreased blood cholesterol, but γ-oryzanol more effectively lowered LDL-C and raised HDL-C levels (*140*). The major forms of phytosterols in RBO are β-sitosterol, campesterol, and their derivatives (*141*). Phytosterols may also be partially responsible for the hypocholesterolemic activity of RBO, as they lower the plasma cholesterol level by inhibiting cholesterol absorption (see earlier discussion). Apart from the known α-, β-, γ-, and δ-tocopherols and tocotrienols, RBO contains two unique tocotrienols, desmethyltocotrienol and didesmethyltocotrienol (*142*). Both γ-oryzanol and tocotrienols are HMG-CoA inhibitors by down-regulation of cholesterol synthesis (*142, 143*). In addition, the two active ingredients have been shown to activate LDL receptor and to increase fecal neutral sterol and bile acid excretion via up-regulation of CYP7A1 encoding cholesterol 7α-hydroxylase (*144*).

Hawthorn Fruit. The bright red berries of the hawthorn (*Crataegus*) have a long history of medicinal use in both China (where hawthorn fruit is popularly known as shanzha) and Europe. Zhang et al. (*145*) examined the hypolipidemic activity of hawthorn fruit in three groups of New Zealand white rabbits fed a high-cholesterol diet supplemented with 2.0% hawthorn fruit powder and found that there was a 23% reduction in blood TC and a 22% reduction in blood TG. In addition, hawthorn fruit supplementation led to 51% less cholesterol accumulation in the aorta of rabbits (*145*). Similarly, significant reductions in plasma TC by 10% and in TG by 13% were also observed in hamsters fed a diet supplemented with 0.5% hawthorn fruit ethanolic extract (*146*). In a trial on 30 hyperlipidemic humans who consumed hawthorn fruit drinks, serum TC, TG, and apo-B decreased by 15, 10, and 8%, respectively, whereas HDL-C remained unchanged (*147*). In a randomized double-blinded, placebo-controlled, cross-over design, 73 mildly hypercholesterolemic patients were asked to take a 250-mL hawthorn drink or placebo drinks three time a day for 4 weeks. Compared with the control group, there was a 7.8% reduction in TC and a 12.4% reduction in LDL-C in the hawthorn group (*148*). The active ingredients in hawthorn fruits responsible for hypocholesterolemic activity remain unknown, but the flavonoids in shanzha fruit have been suggested. HPLC analysis found that hawthorn fruit was rich in epicatechin, chlorogenic acid, hyperoside, isoquercitrin, protocatechuric acid, rutin, and quercetin (*149*).

The mechanisms by which dietary hawthorn fruit decreases serum cholesterol may involve complex interactions of cholesterol metabolisms. First, hawthorn fruit reduces cholesterol synthesis. Rajendran et al. (*150*) followed cholesterol synthesis by measuring the incorporation of [^{14}C]acetate into the liver cholesterol in rats fed a diet supplemented with hawthorn ethanolic extract. It was found that supplementation of hawthorn ethanolic extract led to 33% suppression in cholesterol biosynthesis in rats. Second, hawthorn fruits have probably an intestinal ACAT inhibiting activity. Supplementation of hawthorn fruit ethanolic extract was associated with a lower intestinal ACAT activity (*145, 146*), suggesting that inhibition of cholesterol absorption of dietary cholesterol is at least partially mediated by down-regulation of intestinal ACAT activity. Third, supplementation of hawthorn fruit in diet significantly increased liver CYP7A1 activity and was accompanied by a greater excretion of bile acids (*146*). Lastly, hawthorn fruit has been shown to up-regulate LDL receptor in HepG2 cells (*151*). Evidence suggests that hawthorn fruit lowers blood cholesterol by a combination of several mechanisms, including reducing cholesterol synthesis, increasing LDL receptor activity, and increasing bile acid excretion.

Fermented Milk. The cholesterol-lowering activity of fermented milk has also attracted considerable attention. Mann and Spoerry (*152*) were the first to report a hypocholesterolemic activity of fermented milk in Kenya′s Maasai tribe. Thereafter, the cholesterol-lowering activity of certain fermented milk and dairy products with some species of *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Streptococcus* has been demonstrated in rats (*153*), hamsters (*154*), and pigs (*155*). Most human trials have confirmed the cholesterol-lowering properties of fermented milk (*156*−*159*). However, one human study found that fermented milk had little or no effect on blood cholesterol (*160*), whereas one report on hamsters observed that fermented skimmed milk had no effect on serum cholesterol, but lowered serum TG (*161*). Existing evidence from animals and human studies suggests that fermented milks have a moderate cholesterol-lowering activity (*162, 163*).

Live bacteria in fermented dairy products such as yogurt, acidophilus milk, and kefir are probably responsible for the observed cholesterol-lowering activity. First, some strains of lactobacilli and bifidobacteria may survive in passage through the acidic stomach, particularly when they are ingested together with milk or other foods (*164*). The live probiotic *lactobacilli* and *bifidobacteria* may alter the gut bacterial content and colonization (*165*). Second, these probiotic bacteria in the large colon may ferment unabsorbed carbohydrates to produce short-chain fatty acids, including propionate, which lowers cholesterol by inhibiting HMG-CoA reductase (*162*). Third, live *lactobacilli* and *bifidobacteria* cells may bind and absorb cholesterol, reducing cholesterol absorption in the intestine (*165, 166*). Fourth, these probiotic bacteria may reduce the reabsorption of bile acids through enterohepatic circulation. Bile acids, consisting for the most part of cholic and deoxycholic acids produced in the liver, are in conjugated form and enter the small intestine, where they are partly absorbed and directed back to the liver. Live *lactobacilli* and *bifidobacteria* cells can hydrolyze the conjugated bile acids, excrete them more rapidly, and reduce the extent to which they are reabsorbed (*158*). For these reasons, fermented milk products are regarded as potentially effective cholesterol-lowering functional foods.

Seaweed. There is also growing interest in researching and developing marine functional foods. One popular marine organism, which could be potentially developed as a cholesterol-lowering agent, is seaweed. The hypocholesterolemic effects of several algae have been demonstrated in cholesterol-fed rats (*167*) and rabbits (*168*). In one study, ethanol extracts of five seaweed species (*Solieria robusta*, *Iyengaria stellata*, *Colpomenia sinuosa*, *Spatoglossum asperum*, and *Caulerpa racemosa*) were found to decrease significantly blood TC, LDL-C, and TG levels (*169*). The active ingredients remain unexplored, but polysaccharides from red and brown algae appear to be responsible for cholesterol-lowering activity (*169*). The action mechanism of these compounds remains unclear, but it is speculated that their hypocholesterolemic effects are associated with inhibition of cholesterol absorption by seaweed polysaccharides. In addition, algae are rich in sterols, which may compete with cholesterol for absorption and reduce cholesterol absorption like other phytosterols (see previous discussion).

CONCLUSION

Cholesterol-lowering nutraceuticals and functional foods play an important role in reducing the risk of coronary heart disease by improving the plasma lipoprotein profile. The action mechanisms for favorable modification of plasma lipids vary with individual nutraceuticals and functional foods. In addition to the nutraceuticals and foods discussed above, soybean protein, almond, fish oil, flaxseed, black rice, licorice, and ginseng oil have been also claimed to possess cholesterol-lowering activity. Future studies could profitably focus on the interaction of the active ingredients with the expression of the genes involved in cholesterol metabolism. The synergistic effects of nutraceuticals on the regulation of blood cholesterol at more than one metabolic site should be tested to develop effective cholesterol-lowering functional foods.

ABBREVIATIONS USED

ABCG, ATP binding cassette transporters; ACAT, acyl CoA:cholesterol acyltransferase; CE, cholesteryl ester; CETP, cholesteryl ester transport protein; CHD, coronary heart disease; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG; epigallocatechin gallate; FXR, farnesoid X receptor; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; IDL, intermediate-density lipoprotein; GTC, green tea catechins; LCAT, lecithin-cholesterol acyltransferase; LDL, low-density lipoprotein; LDL-C, LDL cholesterol; LDL-R, LDL receptor; LXR, liver X receptor; NIDDM, non-insulin-dependent diabetes mellitus; NPC1L1, Niemann−Pick C1 like 1; RBO, rice bran oil; SR-B1, scavenger receptor B class 1; SREBP, sterol regulatory element binding proteins; TC, total cholesterol; TF 1, theaflavin; TF2A, theaflavin-3-gallate; TF2B, theaflavin-3′-gallate; TF3, theaflavin-3,3′-digallate; TG, triacylglycerol; TR; thearubigins; VLDL, very low-density lipoprotein.

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