Critical Review

Effects on the Human Serum Lipoprotein Profile of β-Glucan, Soy Protein and Isoflavones, Plant Sterols and Stanols, Garlic and Tocotrienols

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ABSTRACT The effects of β-glucan, soy protein, isoflavones, plant sterols and stanols, garlic and tocotrienols on serum lipoproteins have been of great interest the last decade. From a critical review of the literature, it appeared that recent studies found positive as well as no effects of β-glucan from oats on serum LDL cholesterol concentrations. These conflicting results may suggest that the cholesterol-lowering activity of products rich in oat β-glucan depends on factors, such as its viscosity in the gastrointestinal tract, the food matrix and/or food processing. The effects of β-glucan from barley or yeast on the lipoprotein profile are promising, but more human trials are needed to further substantiate these effects. It is still not clear whether the claimed hypocholesterolemic effects of soy can be attributed solely to the isoflavones. Several studies found no changes in serum LDL cholesterol concentrations after consumption of isolated soy isoflavones (without soy protein), indicating that a combination of soy protein and isoflavones may be needed for eliciting a cholesterol-lowering effect of soy. Therefore, the exact (combination of) active ingredients in soy products need to be identified. The daily consumption of 2–3 g of plant sterols or stanols reduces LDL cholesterol concentrations by 9–14%. It has been demonstrated that functional foods enriched with plant sterols and stanols are effective in various population groups, and in combination with cholesterol-lowering diets or drugs. Whether garlic or garlic preparations can be used as a lipid-lowering agent is still uncertain. It is important to characterize the active components in garlic and their bioavailability after ingestion. It is not very likely that tocotrienols from palm oil or rice bran oil have favorable effects on the human serum lipoprotein profile. J. Nutr. 132: 2494–2505, 2002.

KEY WORDS: • *review* • *diet* • *functional foods* • *serum lipoproteins* • *humans*

Coronary heart disease (CHD)[3] is a major health problem in developed countries. Many studies have now shown that elevated concentrations of total or LDL cholesterol in the blood are powerful risk factors for CHD (1), whereas high concentrations of HDL cholesterol or a low LDL (or total) to HDL cholesterol ratio may protect against CHD (2,3).

The composition of the human diet plays an important role in the management of lipid and lipoprotein concentrations in the blood. Reduction in saturated fat and cholesterol intake has traditionally been the first goal of dietary therapy in lowering the risk for cardiovascular disease (4). This may reduce blood total cholesterol concentrations by ~3% (step I diet) or 6% (step II diet) in free-living subjects (5). In recent years, however, the possible hypocholesterolemic effects of several dietary components, such as β-glucan, soy protein, isoflavones, plant sterols and stanols, garlic and tocotrienols, have attracted much interest. In fact, foods, pills and capsules rich in these ingredients are on the market in many countries and may be the basis for new functional foods.

The purpose of this article is to review critically the literature from the last decade about the effects of these dietary components on blood lipids and lipoproteins in humans. The possible mechanisms by which these components may affect lipid and lipoprotein metabolism will also be briefly addressed.

OAT PRODUCTS AND β-GLUCAN

During the last couple of decades, much attention has been given to the role of dietary fibers in the control of lipid and lipoprotein metabolism. Dietary fibers include a variety of plant substances, mainly nonstarch polysaccharides and lignins, which are resistant to digestion by digestive enzymes. They can be classified into two groups based on water solubility. In contrast to water-insoluble fibers, most soluble fibers may lower plasma total cholesterol by a specific effect on LDL cholesterol (6). HDL cholesterol or triacylglycerol concentrations are in general not affected.

Several mechanisms of action for the hypocholesterolemic effect of soluble fibers have been suggested that may depend on the type of fiber. Soluble fibers may increase the binding of bile acids in the intestinal lumen, which leads to a decreased enterohepatic circulation of bile acids and a subsequent increase in the hepatic conversion of cholesterol to bile acids

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[3] Abbreviations used: AGE, aged garlic extract; AHA, American Heart Association; CHD, coronary heart disease; DADS, diallyl disulfide; FDA, Food and Drug Administration; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; NCEP, National Cholesterol Education Program; SAC, S-allylcysteine.

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(7,8). Another suggested mechanism is that the increased viscosity of the food mass in the small intestine because of soluble fibers leads to the formation of a thick unstirred water layer, adjacent to the mucosa. This layer may act as a physical barrier to reduce the absorption of nutrients and bile acids (9,10). Furthermore, soluble fibers may reduce the rate of glucose absorption, leading to a lower glycemic response and lower insulin concentrations. This latter may result in a reduced hepatic cholesterol synthesis (6,8). Finally, it has been shown in animals that production of short-chain fatty acids, such as acetate (11) and propionate (12) after fermentation of soluble fibers by colonic bacteria, inhibits hepatic cholesterol synthesis.

A rich source of soluble fiber is oats. In the early 1960s, De Groot et al. (13) were the first to demonstrate that a daily consumption of 300 g of bread containing 140 g of rolled oats for 3 wk resulted in an 11% decrease serum total cholesterol concentrations in men. A meta-analysis by Ripsin et al. (14), in which 12 trials were included, found that a daily intake of ~3 g of soluble fiber from oat products for 18 d to 3 mo resulted in a modest reduction of total cholesterol concentrations of ~0.13 mmol/L. This reduction was the greatest in men and women with the highest initial total cholesterol concentrations.

Braaten et al. (15) have now demonstrated that the cholesterol-lowering effect of oat products could be attributed to their main soluble fiber component, β-glucan. In this study (15), decreases in LDL cholesterol concentrations of 10% were found in hypercholesterolemic men and women who consumed daily for 4 wk 7.2 g of oat gum containing 5.8 g of β-glucan mixed with a noncarbonated drink or with water. The amount of β-glucan provided by the oat gum is equivalent to the amount found in ~70 g of oat bran. β-glucan from oats is a nonstarch polysaccharide composed of β-(1→ 4)-linked glucose units separated every two to three units by a single β-(1→3)-linked glucose (8). Oat β-glucan is often referred to as mixed linkage β-glucan (16) (**Fig. 1**).

On January 21, 1997, the U.S. Food and Drug Administration (FDA) approved a health claim on food products that "a diet high in soluble fiber from whole oats (oat bran, oatmeal and oat flour) and low in saturated fat and cholesterol may reduce the risk of heart disease." The FDA concluded that at least 3 g/d of β-glucan from oats should be consumed to achieve a clinically relevant reduction in serum total cholesterol concentrations (17,18).

Many of the previous studies did not provide information on the β-glucan content of their experimental products, but, in general, the studies carried out during the last 10 y did. In these studies, to be discussed below, β-glucan was derived from oats (10,15,19–25), barley (26,27) or yeast (28).

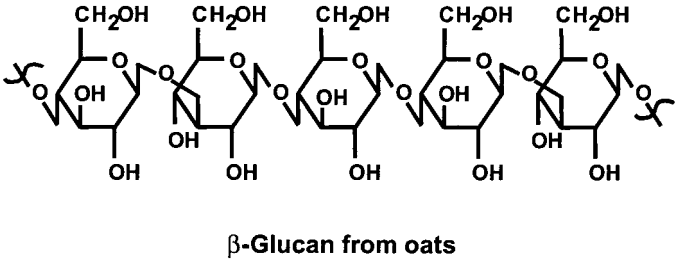


FIGURE 1 Chemical structure of β-glucan from oats. β-glucan from oats is a nonstarch polysaccharide composed of β-(1→ 4)-linked glucose units separated every two to three units by a single β-(1→3)-linked glucose (8). Oat β-glucan is often referred to as mixed linkage β-glucan (16).

Davidson et al. (19) found in hypercholesterolemic men and women consuming a National Cholesterol Education Program (NCEP) step I diet, a decrease in serum LDL cholesterol concentrations of 10, 16 and 12% with increasing doses of 3.6, 4.0 and 6.0 g of β-glucan from 84 g of oatmeal, or from 56 or 84 g of oat bran, respectively. Surprisingly, the highest dosage of β-glucan led to an intermediate hypocholesterolemic response. As such, a dose-response effect was not established. Oat bran and oatmeal were incorporated into hot cereals, low-fat muffins and low-fat shakes, which were consumed for 6 wk. Uusitupa et al. (20) examined the effects of β-glucan from oat bran on serum lipids and lipoproteins in mildly to moderately hypercholesterolemic men and women. During the study, subjects followed an American Heart Association (AHA) step I diet. The estimated intake of oat bran, which was mostly consumed with juice, yogurt, porridge or a dessert, ranged from 20 to 66 g/d and provided 3.3–11.0 g of β-glucan. After an intervention of 8 wk, serum LDL cholesterol concentrations were nonsignificantly lowered by 3%. After 4 wk, however, LDL cholesterol concentrations were significantly lowered by 6%. Subjects who consumed at least 6.9 g of β-glucan showed the greatest decline in serum LDL cholesterol concentrations, which was 8% after 4 wk and 4% after 8 wk; again, only the reductions at 4 wk were significant. Based on these findings, it was suggested that the hypocholesterolemic effect of β-glucan from oat bran might diminish with time. Uusitupa et al. (29), therefore, investigated whether this attenuation could be explained by changes in the synthesis and/or absorption of cholesterol. However, no changes after 4 and 8 wk were found in the cholesterol-standardized serum concentrations of cholesterol precursors, which reflect the endogenous cholesterol synthesis, and in those of cholestanol and plant sterols, which reflect the intestinal absorption of cholesterol. Behall et al. (23) supplemented the diet of mildly hypercholesterolemic men and women for 5 wk with 50–75 g/d of two different oat fiber extracts, in which the amount of β-glucan was either low or high. The background diet provided 0.8 g/d of β-glucan and the two fiber extracts 0.8–1.2 or 5.1–7.6 g/d of β-glucan. Plasma LDL cholesterol concentrations decreased ~15 and 21% from prestudy values, respectively. The 7% difference in LDL cholesterol between subjects who consumed the low and high β-glucan diets was not statistically significant but the 6% difference in total cholesterol was. The oat fiber extract was given in a variety of food products including muffins, cakes, brownies, waffles, gelatins, yogurts, spaghetti sauces and meat loaf. Furthermore, Önning et al. (24) found that daily consumption of 750 mL of oat milk, devoid of insoluble fiber but providing 3.8 g/d of β-glucan for 5 wk lowered serum LDL cholesterol by 6% in moderately hypercholesterolemic men. The oat preparation consisted of 50% commercial oat flakes and 50% heat-treated, dry-milled oat bran. The insoluble fibers were separated by decanting.

Mackay and Ball (21) did not demonstrate in hypercholesterolemic men and women consuming a low-fat diet a decline in plasma LDL cholesterol concentrations after a daily intake of 55 g of two different types of oat bran providing low amounts of either 1.9 or 3.0 g of β-glucan for 6 wk. Unlike in most other studies, however, plasma HDL cholesterol concentrations increased after both doses of β-glucan. Oat bran was given in a variety of food products, such as muffins, porridge, pancakes and scones.

Törrönen et al. (22) also did not observe a decrease in serum LDL cholesterol concentrations after consumption of 37–75 g/d of an oat bran concentrate providing, respectively,

5.6–11.2 g/d of β-glucan for 8 wk in mildly to moderately hypercholesterolemic men. A dose of 11 g/d of β-glucan from the oat bran concentrate is equivalent to the amount provided by ~170 g of oat bran. The oat bran concentrate, which was produced by removing nonfiber compounds by milling in a cold-water suspension, was incorporated into bread. During the baking and frozen storage of bread, the β-glucan content as well as its molecular weight were unchanged. As already mentioned, soluble fibers, such as β-glucan, seem to increase the viscosity of the intestinal contents, leading to an increased unstirred water layer adjacent to the mucosa. Törrönen et al. (22) suggested that the lack of effect in their study could be attributable to a poor solubility of β-glucan, which may have prevented an increase in the viscosity in the intestine. Beer et al. (10) investigated the effects of daily intake of 14.4 g of oat gum containing 9 g of β-glucan, which was served in an instant whip, on the serum lipid and lipoprotein profile in normocholesterolemic men. This daily dosage of oat gum containing 62% β-glucan is equivalent to ~150 g of oat bran. After a treatment period of 2 wk, serum concentrations of total cholesterol, LDL cholesterol and triacylglycerol were unaffected, whereas the HDL cholesterol concentration rose. The investigators suggested that the attenuation of the cholesterol-lowering effect might be explained by the low solubility and moderate molecular weight of the oat gum, and, therefore, a low viscosity in the intestine.

A recent study of Lovegrove et al. (25) was conducted to investigate the effects of the minimal recommended dose by the FDA of 3 g/d of β-glucan on fasting lipoproteins and postprandial triacylglycerols. In this study, men and women with mild to moderate hypercholesterolemia consumed cereals with low-fat yogurt or low-fat milk that provided for a treatment period of 8 wk 20 g/d of oat bran concentrate, which consisted of 3 g/d of β-glucan. No changes were found in fasting plasma concentrations of total cholesterol, LDL cholesterol and triacylglycerol, whereas a decline in concentrations of HDL cholesterol was observed. There was no beneficial effect of 3 g/d of β-glucan from oat bran concentrate on postprandial plasma triacylglycerol responses, which were measured at the start of the study and at the end of the treatment period.

Two studies have reported the effects on plasma lipoproteins of β-glucan from barley. McIntosh et al. (26) demonstrated that plasma LDL cholesterol concentrations were lowered by 7% in mildly hypercholesterolemic men, who consumed ~170 g of barley containing 8 g/d of β-glucan for 4 wk. Barley bran was incorporated into bread, spaghetti and cookies, whereas barley flakes were given in muesli. Bourdon et al. (27) examined the postprandial cholesterol and triacylglycerol responses in men to two different pastas that were made from either barley flour enriched with β-glucan during processing or barley flour naturally high in β-glucan. Both pastas provided 5 g of β-glucan and were served as a part of a low-fat meal. The postprandial plasma cholesterol concentrations were lowered at 30 min and at 4 h after the β-glucan-containing meals. No favorable effects were found on postprandial plasma triacylglycerol responses.

Like oats and barley, yeast (*Saccharomyces cerevisiae*) from bakeries or breweries is also a rich source of β-glucan. The yeast contains 85% of β-glucan after processing, which is composed of a β-(1→6)-branched, β-(1→3)-linked linear glucose polysaccharide. In contrast to β-glucan from oat products, yeast-derived β-glucan has a low viscosity, and its solubility in water is low. Therefore, it does not gel when added to liquids (8). Nicolosi et al. (28) were the first to report that an intake of 15 g/d of β-glucan from yeast mixed in orange juice for 8 wk in hypercholesterolemic obese men tended to lower plasma concentrations of LDL cholesterol by 8%, whereas those of HDL cholesterol tended to increase by 9%. Plasma triacylglycerol concentrations were unaffected. As already discussed, it has been suggested that the high water solubility and high viscosity of the β-glucan from oats may be of importance for its cholesterol-lowering effect (9,10). Unlike oat β-glucan, yeast-derived β-glucan has a low viscosity, and its solubility in water is low. Still, this type of β-glucan may lower serum LDL cholesterol concentrations. It is, therefore, not likely that the mechanism of action of yeast-derived β-glucan can be explained by the increased intestinal viscosity and must be due to other mechanisms, such as binding of the soluble fiber to bile acids, reduced serum insulin concentrations or increased production of short-chain fatty acids (8).

In short, recent studies showed positive as well as no effects of oat β-glucan on LDL cholesterol. One of the suggested mechanisms of action of oat β-glucan is that it increases intestinal viscosity. If true, the food matrix of the oat products may influence the viscosity and bioavailability in the gastrointestinal tract. To explain the observed discrepancies, therefore, it is necessary to further clarify the hypocholesterolemic mechanism of the different β-glucan preparations. In addition, more human trials are needed to confirm the potentially favorable effects of β-glucan from barley or yeast on the lipid and lipoprotein profiles.

SOY PROTEIN AND ISOFLAVONES

There is great interest in the beneficial effects on the plasma lipid and lipoprotein profiles of soy protein, a component of traditional Asian foods. Also, in Western diets, various types of soy protein-containing products, such as soybeans, soymilk, tofu (soybean curd), tempeh (a cake of cooked soybeans), miso (soybean paste) and soy sauce, are used. Based on a review of 26 studies, Carroll (30) concluded that replacement of 13–20% of total energy from dietary animal protein by soy protein for 3–6 wk reduced total cholesterol concentrations by ~20% in men and women with hypercholesterolemia. The soy protein diets had no effect on HDL cholesterol concentrations, whereas triacylglycerol concentrations tended to decrease, especially in subjects with hypertriglyceridemia. The effects of soy protein diets on plasma lipids and lipoproteins in men and women with normal cholesterol concentrations were less consistent. More moderate cholesterol reductions ranging from 1 to 12% occurred in normocholesterolemic subjects. Carroll (30), therefore, suggested that men and women with hypercholesterolemia were more responsive to soy protein diets than normocholesterolemic persons.

In a meta-analysis of 38 trials, Anderson et al. (31) investigated the effects of soy protein on serum lipid concentrations in men and women. Soy protein was used in the form of isolated soy protein or textured soy protein. In most of the studies, the control and soy-containing diets differed by < 10% in amounts of total fat and saturated fat. It was estimated that an average consumption of 47 g/d soy protein, in place of animal protein, lowered total cholesterol, LDL cholesterol and triacylglycerol concentrations by ~9% (0.60 mmol/L), 13% (0.56 mmol/L), and 11% (0.15 mmol/L), respectively. No changes were found in serum HDL cholesterol concentrations. The changes in serum total and LDL cholesterol concentrations, however, depended upon the initial serum total cholesterol concentration. Men and women with normal cholesterol concentrations (< 5.17 mmol/L) and those with mild hypercholesterolemia (5.17–6.59 mmol/L) both had nonsignificant decreases of ~3 and 4%, respectively. In con-

trast, men and women with moderate hypercholesterolemia (6.70–8.61 mmol/L) had significant reductions of ~7% and those with severe hypercholesterolemia (> 8.66 mmol/L) had marked decreases of ~20%. Changes in LDL cholesterol concentrations followed a similar pattern. Thus, as already mentioned by Carroll (30), the cholesterol-lowering effects of soy protein were greatest in persons with the highest initial cholesterol concentrations.

The possible mechanisms of the hypocholesterolemic effect of soy protein have been thoroughly described in several reviews (32–35). Based on studies with cell cultures, animals or humans, suggested mechanisms include increases in LDL receptor activity, increases in the synthesis and fecal excretion of bile acids, and a suppression of cholesterol absorption. Furthermore, Forsythe (36) suggested that in animals, the hypocholesterolemic effect of soy protein results from increases in plasma thyroxine concentrations. In humans, however, results of hormonal thyroid status were inconsistent (34).

On October 26, 1999, the U.S. FDA (37,38) approved a health claim that "25 g of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease." Of interest is which component of the soy protein-containing products in the diet may be responsible for the observed changes in blood lipids. It has been suggested that phytoestrogens, or plant estrogens, found in soy may account for 60–70% of the effects seen (31). The phytoestrogens genistein and daidzein, known as isoflavones, are structurally related to the mammalian estrogen, estradiol (39) (**Fig. 2**). The hypocholesterolemic effects of soy phytoestrogens could be explained by their weak estrogenic activity through binding of isoflavones to estrogen receptors (35,39,40). In general, LDL cholesterol increases and HDL cholesterol decreases in postmenopausal women because of a decline in estrogen. Postmenopausal administration of a low dose of conjugated estrogens of 0.625 mg/d for 3 mo indeed lowered concentrations of LDL cholesterol by 15% and increased those of HDL cholesterol by 16% (41). If phytoestrogens actually contribute to the effects of soy on plasma lipids, it is noteworthy that the meta-analysis of Anderson et al. (31) did not suggest any effect of soy on HDL cholesterol.

The effects of isoflavones on serum lipids and lipoproteins are, however, controversial. Several studies have reported a cholesterol-lowering effect of soy isoflavones. In these studies, triacylglycerol concentrations were unchanged. Cassidy et al. (42) demonstrated that plasma total cholesterol concentrations were lowered by 9% in premenopausal women who consumed 60 g/d of textured soy protein containing 45 mg of conjugated isoflavones for 1 mo. The textured soy protein was incorporated into various food items including bologna, chicken casserole and vegetable curry. Because of small sample size, LDL cholesterol, HDL cholesterol and triacylglycerol concentrations could not be measured. Potter et al. (43) showed that an intake of 40 g/d of isolated soy protein consisting of 56 or 90 mg of isoflavones for 6 mo decreased plasma non-HDL (LDL + VLDL) cholesterol and increased HDL cholesterol concentrations in hypercholesterolemic postmenopausal women consuming a NCEP step I diet. Isolated soy protein was given in a variety of food products, such as breads, muffins, drinks, milks and soups. Another finding in this study was that the concentration of mononuclear cell LDL receptor mRNA, which is related to that in the liver (44), rose after consumption of both doses of soy isoflavones, indicating an up-regulation of hepatic LDL receptor activity. Also, Washburn et al. (45) observed that consumption of 20 g of soy protein powder containing 34 mg/d of phytoestrogens for 6 wk decreased serum LDL cholesterol concentrations 7% in perimenopausal, normocholesterolemic women. The powders were mixed in various ways with milk, orange juice, yogurt or cereals. Crouse et al. (46) also suggested that the cholesterol-lowering effect of soy protein depends on its content of isoflavones. In their study, moderately hypercholesterolemic men and women consuming a NCEP step I diet consumed a beverage with 25 g of isolated soy protein, which provided 3, 27, 37 or 62 mg/d of isoflavones for 9 wk. The virtually isoflavone-depleted soy protein (3 mg isoflavones) as well as isolated soy protein containing only 27 mg isoflavones had no favorable effects on the plasma lipid and lipoprotein profile. Isolated soy protein with the highest isoflavone content (62 mg) reduced LDL cholesterol concentrations 6%. After consumption of isolated soy protein providing 37 mg of isoflavones, however, decreases in LDL cholesterol concentrations of 8% were found only in the men and women with the highest initial LDL cholesterol concentrations. Similarly, Teixeira et al. (47) demonstrated that an intake of 20 g/d of isolated soy protein containing a low content of isoflavones (38 mg) reduced plasma total and non-HDL (LDL + VLDL) cholesterol concentrations in moderately hypercholesterolemic men consuming an NCEP step I diet by 2 and 3%, respectively. At intakes of 30, 40 or 50 g of isolated soy protein providing 57, 76 or 95 mg of isoflavones, plasma non-HDL cholesterol concentra-

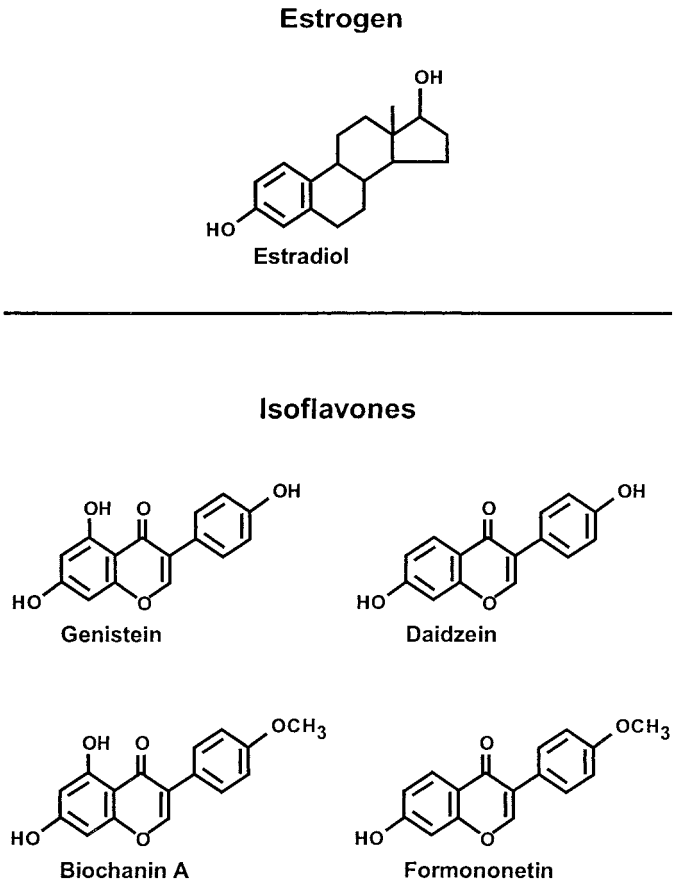


FIGURE 2 Chemical structures of isoflavones. The isoflavones, which are structurally related to the estrogen, estradiol, include genistein and daidzein and their methyl ether derivatives biochanin A and formononetin, respectively (40). Genistein and daidzein are predominantly present in soy, whereas red clover contains also higher amounts of biochanin A and formononetin. After ingestion, biochanin A and formononetin can be converted to genistein and daidzein, respectively (58).

tions declined by 3, 2 and 5%, respectively. Also, plasma total cholesterol concentrations were significantly lowered, except on the diet providing 40 g of isolated soy protein. The isolated soy proteins were incorporated into various baked products and ready-to-mix beverages, which were consumed for 6 wk. Another study by Takatsuka et al. (48) examined the effects of soymilk on serum lipids in premenopausal, normolipidemic Japanese women. The investigators observed that daily consumption of ~350 mL soymilk, which provided 15 g soy protein and 96 mg of isoflavones, for 60 d decreased serum total cholesterol concentrations 5%. Very recently, Wangen et al. (49) found that plasma LDL cholesterol concentrations in normocholesterolemic and mildly hypercholesterolemic postmenopausal women were lowered 7% after a daily intake of 85 g of isolated soy protein beverage powder containing a high isoflavone content (132 mg) for 93 d. A lower dose of soy isoflavones (65 mg) had no effects on plasma lipids and lipoproteins. Another very recent trial (50) demonstrated that in postmenopausal women with moderate hypercholesterolemia, the use of 42 g/d of isolated soy protein powder providing 80 mg of isoflavones for 12 wk lowered plasma LDL cholesterol concentrations by 0.3 mmol/L compared with 42 g of isolated soy protein without isoflavones. Surprisingly, the decrease in LDL cholesterol due to 42 g/d of milk protein did not differ from isolated soy protein powders that were mixed with juice, water or soup.

Not all studies have reported a hypocholesterolemic effect of isoflavones. Gooderham et al. (51) showed that plasma total and HDL cholesterol concentrations did not change in normocholesterolemic men who consumed 60 g of an isolated soy protein supplement providing 131 mg/d of isoflavones for 28 d. These findings were confirmed by Nestel et al. (52), who investigated the effects of a daily intake of 80 mg isolated soy isoflavones (tablet) for 5–10 wk on plasma lipids in a study with postmenopausal and perimenopausal women. Furthermore, Jenkins et al. (53) demonstrated that daily consumption of breakfast cereals containing 36 g of soy protein and 168 mg of isoflavones for 3 wk had no beneficial effects on serum lipids and lipoproteins in a trial with hyperlipidemic men and postmenopausal women. Another trial by Simons et al. (54) examined the effects of a tablet of 80 mg/d of soy isoflavones, as part of a diet low in saturated fat and cholesterol, on the plasma lipid and lipoprotein profile in postmenopausal women with normal lipid concentrations. After treatment for 8 wk, plasma lipid and lipoprotein concentrations were not affected. In a recent study by Hsu et al. (55), there were no changes in the plasma lipoprotein profile of postmenopausal women after a daily intake of 150 mg of pure isoflavones for 6 mo. This study, however, lacked a control group. Also, four trials with isolated isoflavones from red clover did not demonstrate a cholesterol-lowering effect (56–59). Isoflavones from red clover differ from soy in that red clover contains not only the isoflavones genistein and daidzein, which are predominantly present in soy, but also high amounts of the isoflavone precursors biochanin A and formononetin (Fig. 2). After ingestion of isoflavones by humans, these compounds are converted to genistein and daidzein, respectively (58). Samman et al. (56) observed that consumption of tablets containing 86 mg/d of isoflavones from red clover for ~2 mo did not influence plasma lipids in premenopausal, normocholesterolemic women. Another study by Nestel et al. (57) also reported no effects of tablets containing 40 or 80 mg/d of red clover isoflavones for 5 wk on plasma lipids in postmenopausal women. Similarly, Howes et al. (58) showed that the lipoprotein profile was not affected by tablets containing 45 or 90 mg/d of isoflavones from red clover for 4 wk by postmenopausal women with mild to moderate hypercholesterolemia. A 6-mo trial by Clifton-Bligh et al. (59) showed that serum LDL cholesterol concentrations were not changed after daily consumption of 28.5, 57 or 85.5 mg of isoflavones from red clover in postmenopausal women. A possible shortcoming of this study was that it was not placebo-controlled. In addition, an 8-wk trial by Hodgson et al. (60) with tablets providing 55 mg of isoflavones from subterranean clover did not report beneficial effects on serum lipids and lipoproteins in men and postmenopausal women. Like red clover, subterranean clover contains the isoflavones predominantly present in soy (genistein and daidzein) as well as the isoflavones biochanin A and formononetin.

Based on the inconsistent findings about the effects of isoflavones on lipids and lipoproteins, it remains uncertain whether isoflavones are responsible for the proposed hypocholesterolemic effects of soy. In several studies, serum lipoprotein concentrations were unaffected by consumption of isolated soy isoflavones (without soy protein) indicating that both soy protein and isoflavones may be needed to elicit a cholesterol-lowering effect. Furthermore, in most of the studies that reported a lack of effect, isoflavones isolated from soy or from red clover were given in tablet form. In general, results among studies with soy products are variable, although it should be noted that the most consistent cholesterol-lowering effects have been found in subjects with high plasma cholesterol concentrations. Also, other components in soy may be hypocholesterolemic. Lovati et al. (61,62) observed in studies with cell cultures that a 7S globulin fraction from soybeans enhanced the activity of the LDL receptor, suggesting that this component may be a cholesterol-lowering agent. The 7S globulin is a major storage protein of soybeans. In contrast to in vitro media, however, soy protein is completely digested in vivo. Accordingly, we assume that this 7S globulin will not be absorbed intact from the human intestine. Thus, its role in vivo remains speculative. Other components present in soy, such as various amino acids, fiber, saponins or a combination of components, have also been postulated to be active compounds (32). It seems, therefore, important to identify the active components in soy products and to identify population groups who may benefit from them. Because various commercial soy proteins and soy-based products differ markedly in composition, it is difficult to substantiate that soy protein intake leads to a reduction in blood cholesterol concentrations. Rather, evidence should be obtained on specific soy protein sources or soy-based products to support consumer benefit claims.

PLANT STEROLS AND STANOLS

At present, a variety of food products enriched with plant sterols and stanols are on the market in many countries. For incorporation into foods, plant sterols and stanols are often esterified with fatty acids to improve their fat solubility. One gram of plant sterols or stanols is equivalent to ~1.6 g of esterified plant sterols or stanols. This is often referred to as 1 g plant sterols (or stanols) provided as its fatty acid esters or 1 g plant sterol (or stanol) esters. Plant sterols, of which campesterol, β-sitosterol and stigmasterol are the most abundant in nature, are structurally related to cholesterol, but they have a different side-chain configuration. Saturation of the sterols with hydrogen leads to the formation of plant stanols, such as campestanol and sitostanol (63) (**Fig. 3**).

Because the human body can not synthesize plant sterols, these components are derived only from the diet. They are usually present in vegetable oils, nuts, cereals and beans, and

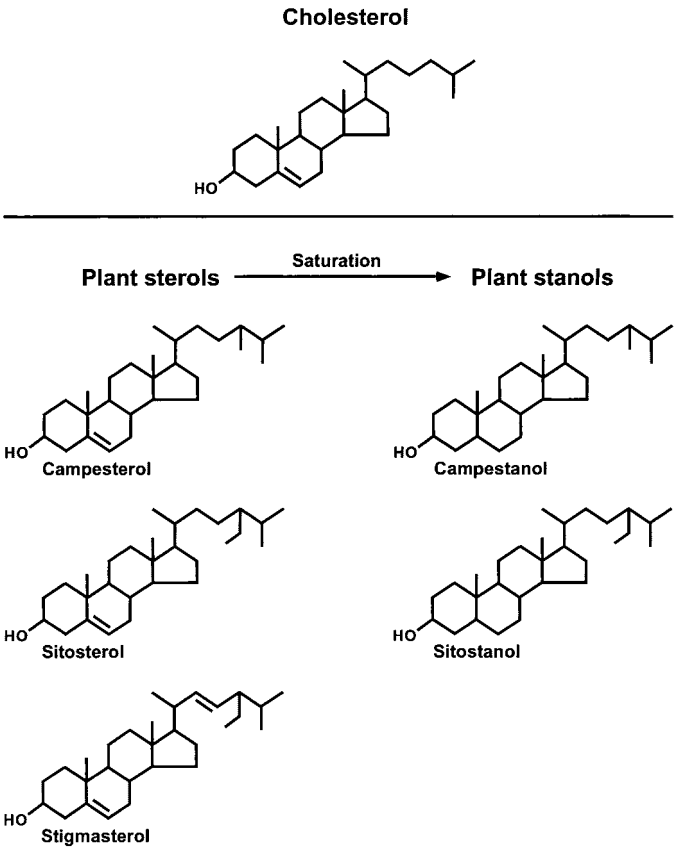


FIGURE 3 Chemical structures of plant sterols and stanols. The plant sterols, campesterol, sitosterol and stigmasterol are structurally related to cholesterol but they vary in side chain. Saturation of these plant sterols with hydrogen leads to the formation of the plant stanols, campestanol and sitostanol. Note that saturation of stigmasterol also leads to the formation of sitostanol (63).

are consumed in Western diets in an amount of ~160 to 360 mg/d (63). Plant stanols are less abundant in these dietary sources than plant sterols. Compared with cholesterol, plant sterols and stanols are absorbed less and their biliary excretion is faster. Consequently, their serum levels are very low. The different plant sterols and stanols are not equally absorbed. This was demonstrated in a study by Heinemann et al. (64) in which the intestinal absorptions of cholesterol and different plant sterols and stanols were compared in healthy men, using an intestinal perfusion technique over a 50-cm segment of the upper jejunum. Absorptions of cholesterol, campestanol, campesterol, stigmasterol and sitosterol were 33, 13, 10, 5 and 4%, respectively. Absorption of sitostanol is minimal.

Plant sterols and stanols have a greater affinity for micelles than cholesterol because of their greater hydrophobicity. Therefore, they can easily displace intestinal cholesterol from the micelles, reducing intestinal cholesterol absorption (65). This reduction results in a compensatory increase in endogenous cholesterol synthesis (66) and in higher LDL-receptor expression (67). The net overall effect is that circulating LDL cholesterol concentrations are lowered after consumption of plant sterol or stanol-containing foods.

Ikeda et al. (68) assumed that the cholesterol-lowering action is the greatest for the least absorbable plant sterols. Because the absorbability of sitostanol is lower than that of campestanol (64), a greater hypocholesterolemic effect can be expected after consumption of plant stanol ester mixtures in which the amount of sitostanol is higher than that of campestanol. However, daily consumption of 2–4 g of two different plant stanol ester mixtures consisting of different proportions of sitostanol and campestanol reduced serum concentrations of LDL cholesterol to the same extent (69–71). Furthermore, it was demonstrated in men and women with ileostomies that plant sterol and stanol esters at doses of 1.5 g/d for 3 d reduced intestinal cholesterol absorption similarly (72). In fact, serum LDL cholesterol concentrations were lowered similarly after a daily intake of ~2 g of plant sterol or stanol esters for 4 wk in hypercholesterolemic men and women consuming a low-fat diet (73).

It is not necessary to consume plant stanols at each meal or simultaneously with dietary cholesterol to obtain the maximal cholesterol-lowering effect (74). In the study with normocholesterolemic and mildly hypercholesterolemic men and women (74), daily intake for 4 wk of 2.5 g of plant stanols once at lunch was as effective in lowering serum LDL cholesterol concentrations as when divided over three meals (0.42 g at breakfast, 0.84 g at lunch, and 1.25 g at dinner, proportional to the dietary cholesterol intake). This indicates that replacement of intestinal cholesterol from the micelles is not the only mechanism by which plant stanols lower LDL cholesterol. Plant sterols and stanols are effective when consumed as part of a diet low in fat and cholesterol (69,75). Thus, not only dietary cholesterol but also biliary cholesterol absorption in the intestine is suppressed. Additionally, plant sterols and stanols are effective in combination with cholesterol-lowering drugs such as statins (76).

Not all plant sterols are potent cholesterol-lowering agents. Sierksma et al. (77) showed that plasma lipid and lipoprotein concentrations were not affected in men and women after daily intake for 3 wk of 3.3 g of 4,4-dimethylsterols from sheanut oil, such as α-amyrin and lupeol, which were incorporated into spreads. These findings were consistent with their previous study (78). Because the molecular structures of 4,4-dimethylsterols are less comparable with cholesterol than those of the hypocholesterolemic 4-desmethylsterols, like sitosterol and campesterol, a possible explanation for the lack of effect of 4,4-dimethylsterols could be that they do not displace intestinal cholesterol from the micelles.

In a recent meta-analysis of 14 studies, Law (79) concluded that ~2 g of plant sterols or stanols lowered serum LDL cholesterol concentrations by 9–14%. Little or no effect was observed on HDL cholesterol or triacylglycerol concentrations. There were no further significant decreases in serum LDL cholesterol concentrations at higher doses. In most of the included studies, plant sterols or stanols were added to margarine and in the other trials, these compounds were incorporated into mayonnaise, olive oil or butter. However, these components are also effective in low-fat products (80,81).

Currently, the U.S. FDA has under review two requests for the allowance of a health claim for plant sterols and stanols. This far, the agency has concluded that "based on the totality of the publicly available scientific evidence, plant sterol and stanol esters may reduce the risk of CHD" (82). A final decision is expected in 2002.

In general, plant sterols and stanols have no adverse effects as demonstrated in extensive safety evaluation studies (65). However, the absorption of fat-soluble components other than cholesterol, such as vitamins and antioxidants, might be reduced as well. Like cholesterol, carotenoids and tocopherols are transported by lipoproteins. Because the number of LDL particles decreases in the circulation after consumption of plant sterols or stanols, the plasma concentrations of carotenoids and tocopherols also decrease. For this reason, these

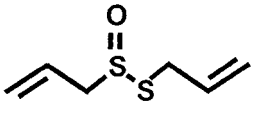
antioxidants are often standardized to plasma lipid concentrations. Recently, we summarized the results from randomized placebo-controlled trials about the effects of plant sterols or stanols on α- plus β-carotene and α-tocopherol concentrations (83). The absolute plasma concentrations of α- plus β-carotene as well as of α-tocopherol decreased after plant sterol or stanol intake. After standardization to LDL cholesterol, however, α-tocopherol concentrations were not affected, whereas those of α- plus β-carotene were still reduced in almost all trials. Note that levels of carotenoids and tocopherols were still within the normal ranges. Plasma concentrations of retinol (vitamin A), 25-hydroxy-vitamin D and vitamin K were unaffected by dietary plant sterols and stanols.

Thus, the efficacy of plant sterols and stanols as cholesterol-lowering agents has been well established. Extensive safety evaluation studies have not revealed any adverse effects. However, more information should be obtained if consumption of plant sterols and stanols, and of functional foods in general, may cause unexpected side effects in the longer term. Because plant sterol and stanols can displace cholesterol from the intestinal micelles, they can reduce intestinal cholesterol absorption. Although this reduction increases endogenous cholesterol synthesis and LDL-receptor expression, the net overall effect is that serum LDL cholesterol concentrations are lowered by 9–14% after consumption of ~2–3 g of plant sterols or stanols. No further decreases occur at higher doses. Functional foods enriched with plant sterols or stanols are effective in various population groups of all ages, including children (84,85), and also in combination with cholesterol-lowering drugs.

GARLIC

A large number of intervention trials with garlic (*Allium sativum*) have been published. Allicin, which causes the characteristic garlic odor, is believed to be the active lipid-lowering compound in garlic (**Fig. 4**). Through crushing, cutting or chewing the garlic clove, the enzyme alliinase comes into contact with the odorless alliin, which is then converted into allicin. Approximately 3.7 mg of allicin can be produced from 1 g of crushed raw garlic (86,87). The mechanisms underlying the possible lipid-lowering action of garlic are not well understood. Animal studies have suggested that garlic supplemented diets may inhibit the synthesis of cholesterol and fatty acids in the liver (88).

Although the results from two earlier meta-analyses (89,90) suggested a hypocholesterolemic effect of garlic, they did not provide strong evidence for the usefulness of garlic as a hypocholesterolemic agent because of methodological shortcomings of many of the studies included. Not all the included trials provided information about the subjects' dietary intakes and body weights, or about the comparability of the smell or taste of the placebo and garlic preparations. In the meta-analysis performed by Warshafsky et al. (89), only 5 randomized, placebo-controlled trials of the 28 identified trials were included. The authors suggested that a cholesterol-lowering effect of ~9% (0.59 mmol/L) could be achieved by a daily consumption of ~1.5–3 g of fresh garlic for 2–6 mo. According to the second meta-analysis of 16 trials (90), garlic, in powder or nonpowder form, consumed for 1–3 mo, reduced serum total cholesterol concentrations 12% (0.77 mmol/L). After consumption of nonpowdered garlic preparations, serum total cholesterol concentrations declined by 15% (0.99 mmol/L), whereas a daily dose of 600–900 mg of dried garlic powder, in which the allicin content was standardized, lowered cholesterol 8% (0.51 mmol/L). A dose of 600–900 mg of dried garlic powder is equivalent to 1.8–2.7 g of fresh garlic. Based on data from eight trials with dried garlic powder preparations (600–900 mg/d), serum triacylglycerol concentrations decreased by 13%, whereas HDL cholesterol concentrations were not affected.



Allicin

FIGURE 4 Chemical structure of allicin from garlic. Allicin is believed to be the active lipid-lowering compound in garlic. After crushing, cutting or chewing the garlic clove, the enzyme alliinase acts on alliin to produce allicin (86,87).

Neil et al. (91) performed a 6-mo trial with 115 men and women to investigate the effect of a daily intake of 900 mg of dried allicin-standardized garlic powder on serum cholesterol concentrations. Plasma lipids and lipoproteins were not affected. When they included the results of this study in their previous meta-analysis (90), the overall cholesterol-lowering effect of garlic was reduced to 0.65 mmol/L. The authors of a subsequent meta-analysis (92) excluded 9 of the 16 trials in the meta-analyses of Silagy and Neil (90), which were not placebo-controlled, blind or involved normocholesterolemic subjects, and included 5 new trials. This recent meta-analysis suggested that garlic, used as allicin-standardized garlic powder (600–900 mg/d), garlic oil or spray-dried garlic, for 2–10 mo reduced total cholesterol concentrations ~4–6% (0.41 mmol/L) in hypercholesterolemic persons, which is much smaller than the effects reported in previous meta-analyses (89–91).

In the most recent meta-analysis of 45 studies, Ackermann et al. (93) concluded that garlic decreased total cholesterol concentrations ~0.19 mmol/L after an intervention of 4–6 wk and 0.44 mmol/L after 8–12 wk, whereas no changes were observed after 20–24 wk. This may suggest that the effect is transient. The decreases in LDL cholesterol and triacylglycerol concentrations paralleled those in total cholesterol concentrations. HDL cholesterol concentrations were unchanged. In 22 included trials, dehydrated alliin-standardized (1.3 g/100 g) garlic preparations were used. The other studies tested preparations that were standardized to a minimum of 0.3% allicin and 4.6 mg of alliin per supplement or used various nonstandardized garlic preparations.

Over the last 3 y, six well designed trials (94–99) did not confirm the favorable effects of garlic on lipid and lipoprotein concentrations. These studies were all randomized, double-blinded, placebo-controlled experiments. Thus, Isaacsohn et al. (94) investigated the effects of a daily dose of 900 mg allicin-standardized garlic powder on plasma lipids and lipoproteins in moderately hypercholesterolemic men and women. After 12 wk, plasma concentrations of total, LDL and HDL cholesterol, and of triacylglycerol were not affected. These findings were confirmed by Berthold et al. (95) who examined the effects of a daily dose of garlic oil of 10 mg, which corresponds to ~4–5 g of fresh garlic, for 12 wk on serum lipoproteins in a study of moderately hypercholesterolemic men and women. In addition, they performed a fecal balance and a double-isotope continuous feeding method at the end of each 12-wk treatment to measure cholesterol absorption and synthesis, which remained unchanged during the study. Another study by Superko and Krauss (96) showed no

effects of a daily dose of 900 mg allicin-standardized garlic powder for 12 wk on plasma lipoproteins, LDL and HDL subclass distribution or postprandial triacylglycerols in hypercholesterolemic subjects. Similarly, Zhang et al. (97) demonstrated that the plasma lipoprotein profile of trained male runners with a low coronary risk profile who used 12.3 mg garlic oil daily for 16 wk did not change. The study by Gardner et al. (98) also did not demonstrate any reduction in lipids and lipoproteins. In this study, a daily dose of 500 or 1000 mg allicin-standardized garlic powder was given for 12 wk to moderately hypercholesterolemic men and women. Zhang et al. (99) also did not find favorable effects of garlic oil capsules delivering daily 8.2 mg of allyl sulfide, a derivative of allicin, for 11 wk in men and women at low risk for CHD. Compared with men, however, the women had increased plasma HDL cholesterol concentrations. The investigators suggested that any beneficial effect of garlic on plasma lipids might differ between men and women.

In contrast, a recently published trial (100) showed that in mild to moderate hypercholesterolemic men and women consuming a low-fat diet for 12 wk, an enteric-coated garlic powder supplement decreased plasma LDL cholesterol concentration 7%. Because of the instability of allicin, most preparations are designed to produce allicin enzymatically from alliin after consumption, and contain fixed amounts of alliin and alliinase. However, alliinase can be deactivated in the stomach (93). In the study of Kannar et al. (100), however, enteric-coated tablets, which were standardized to produce 9.6 mg/d of allicin, were used to protect the enzyme alliinase from deactivation by the low gastric pH. This may lead to an adequate enzymatic production of allicin from alliin in the intestinal tract after consumption.

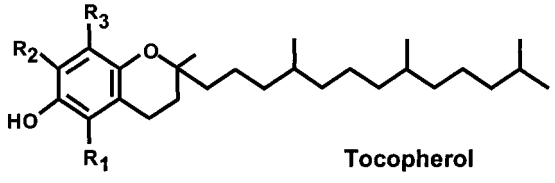
In summary, the usefulness of garlic as an effective lipid-lowering agent is still a matter of dispute. Apart from one trial (100), other very recent trials have not shown such effects. Allicin has been suggested to be one of the major sulfur-rich components in garlic that may contribute to its hypocholesterolemic effect. The reason for the lack of effect of garlic on the lipid and lipoprotein profile in recent well designed studies is unclear. It is possible that the allicin release from the garlic preparations, despite standardization, was not optimal (99). Garlic preparations are often designed to release allicin enzymatically from alliin after consumption. It seems that the enzyme alliinase quickly denaturates at a low gastric pH (93). However, allicin and some of its metabolites, such as ajoene and diallyl disulfide (DADS), have never been found in human blood after consumption of garlic products, including enteric-coated products that protect the enzyme alliinase against deactivation by stomach acid. Furthermore, aged garlic extract (AGE) products contain no alliin or allicin. S-allylcysteine (SAC), which is a major water-soluble organosulfur compound in AGE, can be measured in the blood. For this reason, the stable SAC present in AGE could be a hypocholesterolemic active agent as suggested in some animal and human trials (87,88). Another possible explanation for the lack of effect could be that other components, such as saponins (101), may account for the lipid-lowering properties of garlic. Future studies should try to characterize the possible active constituents and their availability after ingestion as well as to elucidate the mechanism to explain the possible favorable effects of garlic on lipids and lipoproteins.

TOCOTRIENOLS

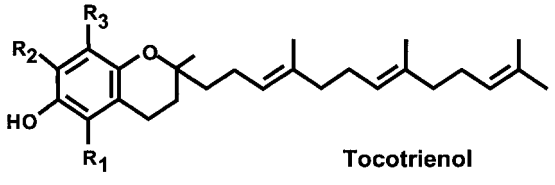
Tocopherols and tocotrienols are two subgroups of the vitamin E family, which consist of a chromanol ring attached to a phytyl side chain. The tocotrienols differ only from the tocopherols in that their phytyl side chain contains three double bonds. The α-, β-, γ- and δ-tocopherols and corresponding tocotrienols are based on the number and position of methyl groups on their chromanol ring (102) (**Fig. 5**). Tocopherols are commonly found in most vegetable oils. The tocotrienols are less abundant in nature than the tocopherols, but are present in palm oil and rice bran oil (103,104).

The tocopherols, of which α-tocopherol has the greatest vitamin E activity, have no effects on plasma concentrations of total, LDL, HDL and VLDL cholesterol, and triacylglycerol (105). Unlike tocopherols, tocotrienols have been suggested to lower cholesterol concentrations in the blood, possibly because of an inhibition of the activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in endogenous cholesterol synthesis, as demonstrated in animals (106–109). The mechanism of this inhibitory action by tocotrienols may involve a reduced HMG-CoA reductase protein synthesis rate and an increased degradation rate as found in human hepatoma HepG2 cells (110). The different tocotrienol subtypes seem to possess various degrees of hypocholesterolemic activity. δ-Tocotrienol and γ-tocotrienol were claimed to be more active than α-tocotrienol in inhibiting HMG-CoA reductase, whereas β-tocotrienol has a very low effect (108,111).

Results concerning the effects of tocotrienols from palm oil on circulating lipids and lipoproteins in humans are not consistent. The studies published by Tan et al. (112) and Qureshi



Tocopherol



Tocotrienol

Compound	R_1	R_2	R_3
α-Tocopherol / α-Tocotrienol	CH_3	CH_3	CH_3
β-Tocopherol / β-Tocotrienol	CH_3	H	CH_3
γ-Tocopherol / γ-Tocotrienol	H	CH_3	CH_3
δ-Tocopherol / δ-Tocotrienol	H	H	CH_3

FIGURE 5 Chemical structures of tocopherols and tocotrienols. The tocotrienols differ from the tocopherols in that they have unsaturated phytyl side chains. R_1, R_2 and R_3 are rest-groups on the chromanol ring. Four subtypes of tocopherols and corresponding tocotrienols have been identified as: α-(5, 7, 8-trimethyl), β-(5, 8-dimethyl), γ-(7, 8-dimethyl) and δ-(8-methyl) based on the number and position of methyl groups on the chromanol ring (102).

et al. (113,114) have shown a hypocholesterolemic effect of tocotrienols from palm oil in men and women. In these studies, no changes were found in serum HDL cholesterol and triacylglycerol concentrations. Tan et al. (112) demonstrated that daily ingestion of a tocotrienol-rich fraction from palm oil resulted in lowered serum concentrations of LDL cholesterol in both normocholesterolemic and hypercholesterolemic men and women. A shortcoming of this study, however, is that they did not use a control group. Qureshi et al. (113) conducted a study in which hypercholesterolemic men and women used 200 mg of a tocotrienol-rich fraction from palm oil containing 30–40 mg of α-tocopherol, 24–30 mg of α-tocotrienol, 70–80 mg of γ-tocotrienol and 50–60 mg of δ-tocotrienol per day. After 4 wk, serum LDL cholesterol concentrations tended to be 8% lower. When 3 poor responders of 15 participants in the tocotrienol group were excluded from the statistical analysis, a significant 28% decrease was found. The validity of excluding these subjects from the statistical analysis, however, is doubtful. Daily supplementation with 200 mg of the specific γ-tocotrienol fraction from palm oil to the three poor responders and four new hypercholesterolemic subjects for 4 wk decreased LDL cholesterol 27%. The authors suggested that γ-tocotrienol might be the most effective cholesterol-lowering compound in palm oil. Note, however, that these changes of the γ-tocotrienol group were not compared with those of a control group. Another trial by Qureshi et al. (114) also demonstrated a 13% decline in serum LDL cholesterol concentrations after the daily supplementation with a tocotrienol-rich fraction from palm oil, which consisted of 40 mg of α-tocopherol, 48 mg α-tocotrienol, 112 mg of γ-tocotrienol and 60 mg of δ-tocotrienol for 4 wk in men and women with hypercholesterolemia consuming an AHA step I diet. When 200 mg of γ-tocotrienol from palm oil per day was given for 4 wk, serum total cholesterol concentrations declined by 13%.

In contrast, various other research groups did not find any change in the serum lipid and lipoprotein profile after supplementation with tocotrienols from palm oil. In the study of Wahlqvist et al. (115), men and women with hypercholesterolemia used supplements containing a tocotrienol-rich fraction from palm oil in increasing doses from 60, 120, 180 up to 240 mg/d. Each dose was given for 4 wk and consisted of 30% of α-tocopherol, 23% of α-tocotrienol, 31% of γ-tocotrienol and 16% of δ-tocotrienol. No changes were found in serum concentrations of total, LDL and HDL cholesterol, or triacylglycerol. These findings were confirmed by Tomeo et al. (116) who examined the effects of daily supplementations with a tocotrienol-rich fraction from palm oil in increasing doses from 224, 280 up to 336 mg for 18 mo on serum lipids and lipoproteins in hyperlipidemic men and women with carotid atherosclerosis. Each dose of the tocotrienol-rich fraction from palm oil contained 29% α-tocopherol and 71% γ- plus α-tocotrienols. Furthermore, Mensink et al. (117) found no changes in serum lipid and lipoprotein concentrations in mildly hypercholesterolemic men who received supplements containing a tocotrienol-rich fraction from palm oil consisting of 83 mg of α-tocopherol, 40 mg of α-tocotrienol, 5 mg of β-tocotrienol, 68 mg of γ-tocotrienol and 25 mg of δ-tocotrienol per day for 6 wk. O' Byrne et al. (118) supplemented hypercholesterolemic men and women with 250 mg of purified α-, γ-, or δ-tocotrienyl acetates from palm oil per day for 8 wk in addition to an AHA step I diet. Serum lipid and lipoproteins were not affected.

In a review of animal and human studies using different supplements containing variable compositions of the tocotrienol-rich fraction from palm oil, Qureshi et al. (119) tried to explain the controversial effects of tocotrienols from palm oil by suggesting that the most effective supplements provided 15–20% α-tocopherol and ~60% γ- (and δ-) tocotrienol, whereas the less effective supplements consisted of ≥ 30% α-tocopherol and 45% γ- (and δ-) tocotrienol. They postulated that the cholesterol-lowering action of the tocotrienols might be attenuated by α-tocopherol, as shown in chickens (119). In contrast, other studies with HepG2 cells (120) or rats (121) demonstrated that α-tocopherol did not decrease the activity of γ-tocotrienol.

Like palm oil, rice bran oil is a rich source of tocotrienols, especially γ-tocotrienol, which is claimed to be the most hypocholesterolemic agent (122). In 1997, Qureshi et al. (123) suggested that the cholesterol-lowering effect of tocotrienols from rice bran oil were even more encouraging than the effects seen in their previous studies with tocotrienols from palm oil (113,114). They postulated that these promising effects could be attributed to two new types of tocotrienols, which were isolated from rice bran oil and identified as desmethyl (P_{21}) and didesmethyl (P_{25}) tocotrienols (104,123). Daily consumption of ~200 mg of a novel tocotrienol-rich fraction from rice bran oil, which contained 12 mg of α-tocopherol, 25 mg of α-tocotrienol, 42 mg of γ-tocotrienol, 20 mg of δ-tocotrienol, 9 mg of *d*-tocotrienol, 34 mg of P_{25}-tocotrienol, 36 mg of unidentified tocopherols and tocotrienols, and 20 mg of sterols and triacylglycerol for 4 wk reduced serum LDL cholesterol concentrations by 23% in hypercholesterolemic men and women who were consuming a NCEP step I diet. Serum HDL cholesterol and triacylglycerol concentrations were stable throughout the study (123).

In summary, despite the positive results of Qureshi et al. (113,114,123), the claimed hypocholesterolemic effect of tocotrienols from either palm oil or rice bran oil is highly controversial. In fact, except for Tan et al. (112) who conducted a study that was not well controlled, no other research groups have been able to confirm the findings of Qureshi and his colleagues. It is therefore not very likely that tocotrienols have a cholesterol-lowering effect for the general population.

CONCLUSION

Diet plays a major role in reducing the risk of CHD. This has led to the search for specific foods and food components that may help to improve the serum lipoprotein profile. In this respect, we have focused this review on the effects of different food components that have received much attention for the last 10 y.

The studies about the effects of β-glucan from oats on the lipoprotein profile showed positive as well as no effects on LDL cholesterol. Not only the viscosity in the gastrointestinal tract, but also the food matrix and/or food processing may influence the cholesterol-lowering potential of β-glucan from oats. Like oats, yeast and barley are also rich in β-glucan, but the chemical structure and characteristics of yeast-derived β-glucan differ from β-glucan from oats and barley. It is not clear yet whether β-glucan from yeast acts in a similar way as β-glucan from oats or barley. Those issues raise the question whether each food enriched with a proven bioactive compound, or each food of which the bioactive compound or mode of administration is not certain, such as for soy and garlic, needs to be tested before being put on the market. Discrepant and unexpected results, however, may be better explained if we gain more insight into mechanisms of action. In fact, such knowledge may even be helpful in predicting the cholesterol-lowering potential of a food.

Furthermore, it is important to bear in mind that some people may be more responsive to a dietary component and

may benefit more from dietary changes than other people. The focus of this study was on effects of dietary components on serum lipoproteins in groups of subjects. Genetic and environmental factors that render groups or individuals more or less responsive should be identified.

It is also important to monitor not only the effects of food components on the lipoprotein profile but also on other aspects of health because animal studies suggest that garlic preparations may inhibit atherosclerotic plaque formation, despite a lack of effect on plasma lipids (124). The cholesterol-lowering effects of soy isoflavones, for instance, which are structurally related to estradiol, are explained by their weak estrogenic activity through binding of isoflavones to estrogen receptors. Postmenopausal women who use estrogens generally have lower rates of cardiovascular disease than those who are not supplemented. However, estrogens at high doses can have adverse effects, such as thrombotic complications, but may still have beneficial effects on serum LDL cholesterol concentrations. To our knowledge, however, such adverse effects have never been shown for phytoestrogens. Another example is that the plant sterols and stanols not only lower serum lipoprotein levels but also plasma levels of the fat-soluble carotenoids. These effects, however, may not be clinically important.

In conclusion, it is important to document the cholesterol-lowering effect of a functional food ingredient, and its mechanism, under various conditions, such as in combination with cholesterol-lowering diets or drugs, and in different population groups. Attention should be paid not only to beneficial but also to potentially adverse health effects.

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