Food and Food Supplements with Hypocholesterolemic Effects

Ruitang Deng*

Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, Rhode Island, USA

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Abstract: Hypercholesterolemia is a predominant risk factor for atherosclerosis and associated coronary and cerebrovascular diseases. Control of cholesterol levels through therapeutic drugs, notably statins, have significantly reduced the risk for developing atherosclerosis and associated cardiovascular diseases. However, adverse effects associated with therapeutic drugs warrant to find other alternative approaches for managing hypercholesterolemia, especially for those with borderline cholesterol levels. Food supplements have increasingly become attractive alternatives to prevent or treat hypercholesterolemia and reduce the risk for cardiovascular diseases. This review summarized current patents on food supplements with claims of hypocholesterolemic effects. They can be mainly divided into four categories based on the active ingredients in the supplements: 1) plant sterols or stanols; 2) fiber or polysaccharides; 3) microorganism-derived; and 4) soy protein and phytoestrogens. The efficacy, mechanisms of action and potential side effects are reviewed for each of the four categories. The hypocholesterolemic effects of plant sterols, fiber, *Monascus* products and soy protein preparations have been consistently demonstrated in clinical trails whereas the efficacy of some probiotic bacteria and phytoestrogens-containing supplements remains to be established. Accumulative clinical data show that plant sterols, fiber, soy protein and phytoestrogen are generally considered safe and cause no obvious side effects. However, additional clinical studies are required to establish the safety profiles of certain probiotic bacteria as food supplements.

Keywords: Cholesterol, low-density lipoproteins (LDL), high-density lipoproteins (HDL), bile acids, plant sterols, plant stanols, fiber, polysaccharides, soy protein, phytoestrogen, isoflavones, probiotic bacteria.

INTRODUCTION

Maintenance of cholesterol homeostasis is vital for healthy status and achieved through a regulatory network consisting of genes involved in cholesterol synthesis, absorption, metabolism and elimination. Imbalance of cholesterol level as a result of environmental and genetic factors leads to hypercholesterolemia, a predominant risk factor for atherosclerosis and associated coronary and cerebrovascular diseases [1-4]. Cardiovascular diseases including coronary heart disease and stroke are the leading cause of mortality, accounting for nearly 50% of all deaths in the Western developed world [5-7]. If the current trend continues, the future will be even bleaker considering the fact that globalization and wide spread of Western diet to the developing world has encountered with explosive increase in the rates of obesity and hypercholesterolemia in those regions. Therefore, hypercholesterolemia and its associated cardiovascular diseases represent one of the greatest worldwide economic, social and medical challenges that we are facing now [7, 8].

In blood plasma, cholesterol is transported by lipo-roteins, which can be mainly categorized into four classes, based on the size of cholesterol-lipoprotein complexes: the very-low-density lipoproteins (VLDL), the intermediate-density lipoproteins (IDL), the low-density lipoproteins (LDL), and the high-density lipoproteins (HDL) [1, 3]. Experimental and clinical studies have shown that the amount of cholesterol transported in the VLDL, IDL and LDL classes of lipoproteins, known as pro-atherogenic cholesterol, is a risk factor for the occurrence of cardio-vascular disease [3, 9, 10]. In contrast, cholesterol transported in HDL particles, known as anti-atherogenic cholesterol, has protective effect on cardiovascular disease [3, 9, 10].

Control of cholesterol levels through therapeutic drugs have significantly reduced the risk for developing atherosclerosis and associated cardiovascular diseases [11-14]. Notably, statins, a class of cholesterol-lowering drugs inhibiting cholesterol synthesis, have been most widely prescribed for treating hypercholesterolemia and reducing cardiovascular diseases [12-14]. However, adverse effects associated with therapeutic drugs, such as myopathy, liver damages and potential drug-drug interaction, have been reported [15-19]. Therefore, development of additional therapies for controlling cholesterol levels is warranted, especially for those with better safety profile.

Despite the wide use of therapeutic drugs for controlling blood cholesterol, the fact remains that it is estimated that greater than 50% of the population of the United States has cholesterol levels at the borderline levels (ranging from 200 to 239 mg/dL). Herbal remedies or food supplements have increasingly become attractive alternatives to prevent or treat hypercholesterolemia, especially for those with cholesterol at the borderline levels. Excellent safety profile, cost-effectiveness and multiple beneficial effects on improving well being all contribute to the emerging trend of

*Address correspondence to this author at the Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, 41 Lower College Road, Kingston, RI 02881, USA;
Tel: 401-874-4950; Fax: 401-874-5787; E-mail: DengR@mail.uri.edu

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increasingly usage of dietary or herbal supplement in United States and around the world.

This review summarized current patents on food supplements with claims of hypocholesterolemic effects. Those patents can be mainly divided into four categories based on the active ingredients in the supplements: 1) plant sterol or stanol; 2) fiber or polysaccharides; 3) microorganism-derived; and 4) soy protein and phytoestrogens. Distinct from early patents that mainly focus on single active component in their claims, it becomes a current trend that more than one active ingredient are claimed in the most of the recent patents or patent applications. Such combination of active ingredients certainly further enhances the therapeutic or preventive value of the food supplements [20], which provide patients with a relatively cheap, safe, and effective way to manage their cholesterol levels and subsequently reduce the cardiovascular risk.

I. PLANT STEROLS OR STANOLS-ENRICHED FOOD OR FOOD SUPPLEMENTS

Plant sterols are found in most plant foods and are particularly abundant in vegetable oils and whole grains. Plant sterols, also known as phytosterols, are structurally and chemically similar to cholesterol and are unsaturated in their sterol ring group. Plant stanols are saturated plant sterols and typically less abundant in nature than plant sterols [21]. The most common phytosterols include β-sitosterol, campesterol, and stigmasterol [22]. An ordinary diet normally contains plant sterols 100-300 mg/day. Most of the plant sterol in the diet is β-sitosterol, and approximately one-third is campesterol. Small amounts of saturated 5α-sitostanols are also present in food. Due to the proven effect on lowering cholesterol level, the use of plant sterol-enriched products is recommended by the International Atherosclerosis Society [23] and the National Heart Foundation of Australia [24]. A number of recent patents or patent applications on food supplements claimed the hypocholesterolemic benefits of their compositions, which consist of at least one of the plant sterols, stanols or their esters [25-35].

Efficacy studies: Clinical trials to evaluate the efficacy of the compositions were carried out in majority of those patents or patent applications. The results from those clinical studies are summarized in Table **1**. Consumption of plant sterols-enriched food or food supplements for a period of 3.5 to 12 weeks result in significant decreases in both total serum cholesterol and LDL-cholesterol with minimal effects on HDL-cholesterol. The reductions range from 2.9% to 12.0% for total serum cholesterol and 2.3% to 15.2% for LDL-cholesterol, respectively Table **1**. It should be noted that the efficacy obtained from most of the studies are not solely due to the activity of plant sterols rather a combined effects of all the active ingredients in the claimed functional food or food supplements. The efficacy data reported in those patents are consistent with the results obtained from other clinical trials [36, 37]. Normen *et al* summarized 57 human trials using plant sterols and found that addition of plant sterol and stanol esters into foods such as margarine results in a mean reduction of LDL-cholesterol level of 9.9% $\pm$ 3.9% [36]. Therefore, it can be concluded that consumption of food supplements enriched with phytosterols have hypocholesterolemic effects.

Mechanisms of action: It has been well established that plant sterols exert their hypocholesterolemic effect by inhibiting intestinal absorption of dietary as well as endogenous cholesterol [38, 39]. However, the molecular mechanisms for such inhibition are still not fully understood. It is generally accepted that the presence of increased quantities of plant sterols compete with cholesterol for micellar solubility, therefore lowering the amount of cholesterol available for absorption by intestinal mucosal cells [40-42].

Potential side effects: Considering the fact that plant sterols are present in most of dietary vegetable, fruits and nuts, and are poorly adsorbed by human body [43], plant

Table 1. Efficacies of Phytosterol-Containing Food Supplements Reported in Recent Patents or Patent Applications

Sample Size	Doses	Duration	% Decrease in Total Cholesterol	% Decrease in LDL-Cholesterol	Statistic Analysis	Reference
22	1.3g/day sterols	5 weeks	3.3	5.6	p<0.05*	[25]
100	3.31g/day, 65% esterified sterols	3.5 weeks	7.4	11.8	N/D**	[27]
100	3.3g/day, 85% esterified sterols	3.5 weeks	6.3	9.0	N/D	[27]
100	0.85g/day	3.5 weeks	3.8	6.1	N/D	[27]
100	0.85g/day, 85% esterified sterols	3.5 weeks	2.9	2.3	N/D	[27]
150	2.6g/day sterols	6 weeks	8.7	13.5	p<0.05	[29]
150	2.6g/day sterols	12 weeks	10.7	15.2	p<0.05	[29]
24	A diet with 12.5% β-sitostanol	5 weeks	9.0	13.0	p<0.05	[29]
34	11g/day, blend	Unknown	6.8	8.3	p<0.001	[30]
24	1.3g/day sterols	4 weeks	12.0	11.9	p<0.004	[31]

* The p value applied to the decrease in both total and LDL-cholesterol.

** N/D, statistic significance was not determined.

sterols are generally considered safe for consumption with no obvious side effects [36, 37]. However, concerns have been raised for high dose intake of plant sterols. First, concomitant with cholesterol reduction, consumption of phytosterols decrease plasma concentrations of some fat soluble vitamins, such as β-carotene, α-carotene, and vitamin E due to the inhibitory effect of phytosterols on their absorption in the intestine [44-49]. Such reduced absorption becomes more pronounced when higher levels of plant sterol intake occur. On the other hand, studies also show that consumption of phytosterols does not significantly affect serum vitamin A (retinol), vitamins D and K [47, 49-52]. The second concern is raised regarding the potential atherogenicity of elevated serum phytosterols in certain individuals. Supporting such concern is the presence of coronary atherosclerosis in sitosterolemic patients, characterized by severely elevated serum phytosterols without increase in cholesterol levels [53-55]. In addition, other epidemiological studies report a potential linkage between phytosterols and atherosclerosis [56-59]. However, such possible association has not been confirmed in studies with animal model and human subjects [60-62]. Mice with inactivation of ATP-binding cassette (ABC) transporters G5 and G8 (ABCG5/8) exhibit over 20-fold increase in serum phytosterols but no significant differences in aortic lesions are found when compared with wt control mice [60]. Also, studies with human subjects found no association between phytosterols and atherosclerosis or cardiovascular diseases [60-62]. Taken together, it appears that the role plant sterols plays in the development of atherosclerosis is not conclusive and additional studies are warranted.

II. FIBER OR POLYSACCHARIDE-ENRICHED FOOD SUPPLEMENTS

It has been well established that dietary fiber has many health benefits including decrease in serum LDL-cholesterol, improving triglycerides, and weight management [63-69]. Compared with low-fiber diet, a high-fiber diet is associated with a lower risk of atherosclerosis [66-69]. Whole grain food made of wheat, oat, barley and rye or food made of bran, such as oat bran, rice bran, soy bran and wheat bran contain high fiber. Fiber can be categorized into water soluble and insoluble. The four major water soluble fibers are β-glucan, psyllium, pectin and guar gum and have been used widely as food additive to increase fiber content. The most common insoluble fiber includes cellulose and lignin. Several recent patents have claims for using fiber or polysaccharides to manage hypercholesterolemia. In addition to β-glucan, psyllium and fiber from oats, peas, beans, soy and grain bran [25, 28, 30, 70-74], polysaccharides from bacterium such as lactobacillus strain [70], and insoluble fiber from carob pulp were also claimed [73, 74].

Preclinical and clinical studies: In a mouse study using reuteran, a branched CC-1, 4/1, 6-glucan produced by some Lactobacillus reuteri strains, it was showed that feeding a "Western diet" with 10% reuteran for 15 days resulted in a decrease in total cholesterol levels by 36.8% [70]. The lipid-lowering effect of a carob pulp preparation rich in insoluble dietary fiber was evaluated in 50 patients with moderate hypercholesterolemia (total cholesterol 232-302 mg/dL) [75]. Consumption of 15 g carob fiber per day as supplement for 4 weeks led to reductions of 7.1% and 10.6% in mean total cholesterol and LDL-cholesterol, respectively. Such reductions were increased to 7.8% and 12.2% after 6 week consumption. On the other hand, HDL-cholesterol and triglyceride levels remain unchanged [75]. Similar results were reported from a randomized, double-blind, placebo-controlled and parallel arm clinical study with 58 volunteers [76]. Intervention with daily intake of 15 g carob fiber for 6 weeks reduced LDL cholesterol by 10.5 % [76]. In another clinical study with 16 subjects, consumption of fiber-enriched food supplement (46.2% fiber: 16.5% soluble and 29.7% insoluble) for 21 days decreased total cholesterol levels by 5 to 7.5% in 7 subjects and 8 to 11% in 6 participants with no significant change in 3 subjects [71]. In a long term 51 week study with 59 subjects with moderate hypercholesterolemia, daily intake of 20 g of a mixture of dietary fibers (guar gum, pectin, soy, pea, corn bran) as food supplement reduced total cholesterol by 5% and LDL-cholesterol by 9% [77]. The reductions were apparent after 3 weeks of treatment. There were no significant effects on the levels of either triglycerides or HDL-cholesterol [77]. It has been reported that oat bran reduces the cholesterol level primarily because of its β-glucan content. Daily uptake of 7.2 g of oat gum for 4 weeks with 20 hypercholesterolemic patients decreased total cholesterol levels by 9% with no significant changes in HDL-cholesterol and triglyceride levels [78]. Taken together, the hypocholesterolemic effects of various fibers have been demonstrated in both preclinical and clinical studies. The magnitudes of reduction depend on the type of fiber, dose, intervention duration and the hypercholesterolemic status of the subjects.

Mechanisms of action: Currently, the mechanisms for cholesterol-lowering effect of water soluble fiber are not fully understood. One favorite hypothesis is that water-soluble fiber binds to bile acids and decrease bile acid reabsorption in the intestine [79-82]. Conversion of cholesterol into bile acids is one of major pathways for removing cholesterol from the body and is negatively regulated by bile acids through repressing cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme for bile acid synthesis [83-87]. Decreased reabsorption of bile acids thus favors cholesterol conversion into bile acids. On the other hand, as biological detergents, bile acids are required for cholesterol solubilization and absorption in the intestine. Binding of bile acids to fiber decreases free bile acids for solublizing cholesterol, thus decreasing cholesterol absorption. The mechanism for insoluble fiber to exert hypocholesterolemic effect is largely unknown. A recent study shows that intake of insoluble fiber derived from cell wall significantly increases fecal excretion of both bile acids and neutral sterols [88]. Such increased fecal excretion of bile acids may lead to hypocholesterolemic effect through similar mechanism as water soluble fiber.

Potential side effects: Results from clinical studies show that fiber supplementations are generally well tolerated. No obvious side effects have been reported from consuming fiber-enriched diet or fiber supplements. In one study using insoluble carob fiber, it was reported that 3 volunteers (6%) felt a sensation of fullness, which led to 2 volunteers dropout the study [71]. The excellent safety profile of fiber may be related to the fact that fiber, particular insoluble fiber, is poorly adsorbed in the intestine, avoiding systemic entering into the body [89].

III. MICROORGANISM-DERIVED FOOD SUPPLEMENTS

Probiotic agents are referred to live organisms, such as bacteria, which confer not only traditional nutrition values but also health benefits when they are consumed. Probiotic products have a long history of being used as food supplements, especially in Asia and Europe, and are now becoming a new popular category of food supplements in the United States. Two probiotic agents, including *Bacillus coagulans* and *Rhodospirillum rubrum,* and fungi *Monascus,* are claimed in the recent patent applications for managing hypercholesterolemia [90-92]. *Bacillus coagulans* is a non-pathogenic lactic acid-producing bacterium and has been used for production of lactic acid [93], fermented food [94] and animal feeds additives [95, 96]. *Rhodospirillum rubrum* is a member of *Rhodospirillum* genus, characterized by being phototrophic using light as an energy source. The bacterium is found in natural water and mud, and is used in sewage purification, biomass production of animal foodstuff and plant fertilizer [97]. Fungi *Monascus* are used for production of fermented red rice, red rice wine and other food products [98, 99]. Due to their ability to produce different color pigments, *Monascus* strains are also widely used by food and cosmetic industries for coloring agents.

Preclinical and Clinical studies: In a study with 17 hyperlipidemic patients, intake of 360 million spores of *Bacillus* daily for 3 months significantly reduced total cholesterol by 31.5% and LDL-cholesterol by 35.2% with a marginal increase in HDL-cholesterol and no change in triglyceride level [100]. In a controlled study with 20 patients, consumption of two tablets (0.5g per capsule of *Bacillus coagulans* dry powder) daily for 60 days decreased LDL cholesterol by 31-43% and serum triglyceride levels by 11-16% while increased HDL cholesterol level by 7-15%. In addition, the intervention significantly reduced the carotid artery blockage in 3 of the patients from greater than 85% to about 40% [90].

In a preclinical study with male Wistar rats, animals receiving a chow diet containing additional 10% (v/v) freeze-dried *Rhodospirillum rubrum* for 8 weeks exhibited a significant reduction in serum total cholesterol by 25% compared with the animals receiving chow diet. Further analysis of the cholesterol fractions revealed that the decrease in total cholesterol level was due to a decrease in the plasma LDL fraction, while the HDL fraction remained unchanged in the *Rhodospirillum rubrum*-fed animals [91]. Similar results were achieved in a short-term study with C57Black/6 mice. Feeding mice with 10% *Rhodospirillum rubrum*-containing diet for a week reduced total serum cholesterol level by 20% in comparison with mice receiving regular chow diet [91].

Extensive preclinical and clinical trials have been carried out to evaluate the cholesterol-lowering effect of *Monascus*-derived preparations. In preclinical studies, the hypocholesterolemic activity of *Monascus* preparations was demonstrated in rat [101, 102], hamster [103-104], rabbit [105, 106], quail [107], and chicken [108]. Consistent with the results from the preclinical studies, significant reduction in total and LDL-cholesterol levels were achieved following intake of various *Monascus* preparations in hypercholesterolemic patients or healthy volunteers [92, 109-113]. In a small pilot study, 4 out of 6 volunteers consuming 65 mg *Monascus* composition daily for 2 weeks decreased total cholesterol by 19.8% and LDL-cholesterol by 22.6% [92]. In a clinical study with 131 moderately hypercholesterolemic patients, the hypocholesterolemic effect of *Monascus purpureus* was evaluated in comparison with cholesterol-lowering drug pravastatin [109]. One hundred and eleven subjects received *Monascus purpureus* extract whereas 20 participants took pravastatin. At the end of study, total cholesterol was significantly reduced by about 20% in both groups, suggesting similar efficacy between *Monascus purpureus* extract and pravastatin [109]. In a clinical study with 72 patients to assess the efficacy and safety of *Monascus purpureus* Went rice in the management of nephrotic dyslipidemia, oral administration of *Monascus purpureus* Went rice in a dose of 600 mg twice per day for one month and then once daily for 11 months significantly reduced cholesterol levels in 6 and 12 months [110]. In another clinical trail with 79 patients, consumption of *Monascus purpureus* Went rice at a dose of 600 mg daily for 8 weeks reduced LDL-cholesterol by 27.7% and total cholesterol by 21.5% [111]. Similar efficacy was also obtained in a study (15 hypercholesterolemic patients) with *Monascus* garlic fermented extract. Intake of 450mg of extract daily for 4 weeks significantly reduced total and LDL-cholesterol levels [112]. Liu *et al.* summarized 93 randomized clinical trials with a total of 9625 subjects and found that consumption of Chinese red yeast rice extract, a traditional dietary seasoning of *Monascus purpureus*, resulted in significant reduction in both total and LDL-cholesterol levels [113]. In addition, the hypocholesterolemic activities of the extract appeared to be comparable to the therapeutic statin drugs [113]. Taken together, the hypocholesterolemic effect of *Monascus* preparations have been consistently demonstrated in preclinical and clinical studies.

Mechanisms of action: It is currently unknown how *Bacillus* and *Rhodospirillum* bacteria exert their observed hypocholesterolemic effect. As probiotic bacteria, it is generally believed that those bacteria confer a healthy benefit by colonizing in the gut and inhibiting the growth of other pathogenic organisms in the same environment [114-117]. Another hypothesis is that probiotic bacteria produce specific enzymes or metabolites beneficial for improving the pathological conditions, such as hypercholesterolemia. However, no direct evidence has been demonstrated. It has been well established that fungi *Monascus* naturally produce bioactive compounds monacolins, which inhibit the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Among those monacolins, monacolin K is identical to the potent cholesterol-lowering drug lovastatin [118, 119]. Although the yields of monacolins produced vary by different *Monascus* strains under various incubation conditions, the hypocholesterolemic effects of those *Monascus* preparations are believed to be due to the presence of monacolins. However, such notion was challenged by the finding that potent hypocholesterolemic activity were detected in *Monascus* preparations lacking monacolins [92], indicating the presence of other bioactive compounds responsible for the observed activity.

Potential side effects: As a widely used probiotic bacterium, *Bacillus* is generally considered safe. Such view is supported by clinical and epidemiological studies [120]. Currently, no clinical data are available for assessing the safety of *Rhodospirillum* preparations as food supplement. In a preclinical study with rats, feeding of a 10% freeze-dried *Rhodospirillum*-containing diet for 8 weeks resulted in no significant changes in various clinical and toxicological parameters, including plasma glucose, uric acid, urea, creatinine, glutamyl oxaloacetic transaminase, glutamyl pyruvic transaminase, haematocrit, and haemoglobin, and urinary glucose and protein [91]. Such safety profile is consistent with the current practice that *Rhodospirillum* strains are used as animal feed additives [97]. *Monascus* has a long history being used as food supplements in Asia and is considered safe [98, 99]. However, concern has been raised regarding some Monascus strains producing a second metabolite mycotoxin citrinin under certain conditions. Citrinin is found hepatotoxic and nephratoxic, adversely affecting the function of both liver and kidney [98]. Therefore, minizing citrinin content is a priority to improve the safety of Monascus-containing food supplement. In addition, the active ingredients monakolins in *Monascus* preparations may have similar side effects exhibited by therapeutic statin drugs. Finally, it should be emphasized that as living organisms, probiotics carry theoretical safety risks as food supplements. Such risks include acquiring virulence factors or antibiotic resistance from environments or pathogenic organisms [121]. Although such incidence has not been reported, surveillance measurements should be implemented to ensure the safety of those products.

IV. SOY PROTEIN AND PHYTOESTROGEN AS FOOD OR FOOD SUPPLEMENTS

It has been a long history that as a high quality protein, soy protein is widely used as an excellent alternative source for animal protein, especially in Asian countries. A variety of soy protein products are currently available on the food market, such as tofu, soy butter, soy nuts, soy milk and some soy burgers. Consumption of soy protein products in the United States has grown significantly since a healthy benefit claim of soy protein was issued by FDA in 1999 [122]. Soy protein preparations are enriched with isoflavones, known as phytoestrogens. The major isoflavones in soy protein preparations include genistein, daidzein, glycitein, their naturally occurring glycosides (genistin, daidzin and glycitin), and malonyl glycosides. Their abundance in soy protein preparations varies widely and depends on the processing techniques used during production [123]. In addition to soy protein, other plants such as red clover also contain high amount of phytoestrogens. Several current patent applications have claims using soy protein or phytoestrogens as at least one of the ingredients in their compositions to manage hypercholesterolemia [25, 28, 33 71, 124, 125].

Clinical studies: Extensive clinical trials have been carried out to evaluate the hypocholesterolemic benefit of soy protein preparations and the data from most of those clinical studies have been reviewed [126-130]. Although variations exist in sample size, patient hypercholesterolemic status, preparation process, dose and intervention duration, it appears that soy protein consumption decreases total cholesterol levels most significantly in subpopulation with severe hypercholesterolemia with moderate effect in moderately hypercholesterolemic patients and marginal or minimal effect in low or non-hypercholesterolemic subjects [131-133]. Consistent with such trend is the results from a small clinical study with 15 patients in a recent patent application [125]. Daily consumption of 5 g soy protein preparation for 2 months reduced total serum cholesterol levels by16.4%, 7.8% and 2.4% in subjects with high, medium and low pretreatment cholesterol levels, respectively [124].

Efficacy regarding phytoestrogens, such as isoflavones, in reducing cholesterol levels is still questionable. Several clinical studies showed that isoflavones or isoflavones-enriched preparations had significant hypocholesterolemic activity reducing total cholesterol levels from 6.5% to 10.2% [134-137]. However, such hypocholesterolemic claim has been challenged by the results from other clinical trials [138-144]. Those studies found that consumption of isoflavones at various doses failed to significantly reduce cholesterol levels or reduction of cholesterol levels by soy protein preparations was independent of isoflavones. One possible explanation for such discrepancy may be that the expression of dose in total isoflavone is not the adequate measurement as variations in types (such as genistein vs daidzein) and forms (such as aglycone, glycoside, vs malonyl glycoside) of isoflavones may have significant impact on cholesterol-lowering activity. Alternatively, the results suggest that isoflavones are not the active ingredients in soy protein preparations responsible for the hypocholesterolemic activity. In a small clinical trial with 7 healthy volunteers, daily consumption of 100-150 g navy bean (containing 60-100 mg isoflavones) for 3 weeks had no effect on total cholesterol levels. However, daily intake of 5 g red clover extracts (containing 100 mg isoflavones) significantly reduced total serum cholesterol levels by an average of 7.3% [124]. Whether other components in the red clover extracts other than isoflavones produced such benefit remains to be determined. Taken together, most of human clinical trials support the notion that soy protein preparations have hypocholesterolemic effects. However, the efficacy of phytoestrogens, such as isoflavones, has not been fully established.

Mechanisms of action: The molecular mechanisms for soy protein preparations to exert cholesterol-lowering effect are still unknown. Although it was once proposed that isoflavones enriched in soy protein preparations were the active ingredients, more and more evidence suggest that components other than isoflavones might be responsible for the activity. Isoflavones have a spatial structure similar to mammalian estrogens, such as 17β-estrodiol (E2) with weak agonistic activity to estrogen receptors (ERs). Compared with E2, isoflavones have about one hundredth to one thousandth ER agonistic activity exhibited by E2. Studies with various approaches have shown that isoflavones bind to the ligand binding domain of ER in a very similar fashion as E2 except for the interaction with helix 12 of the receptors [145, 146]. Considering the fact that isoflavones exhibit over 20 times higher binding affinity for ERβ than for ERα, it was proposed that isoflavones should not be considered as typical "estrogens" rather as natural selective estrogen receptor modulators (SERM) [147]. As SERMs, isoflavones can exert partial agonist or antagonist actions on ER in a tissue-, pathway- or isoform-specific manner. Estrogen and certain SERMs have been shown to have hypolipidemic properties [148, 149] whereas other SERMs have been reported to inhibit estrogen's cardiovascular protective function [150]. Thus, it remains to be determined: 1) whether isoflavones are the active ingredients for soy protein's hypocholesterolemic effect; and 2) whether isoflavones act as agonists or antagonists of ER to exert their possible hypocholesterolemic activity.

Potential side effects: As estrogenic compounds, concerns have been raised for phytoestrogens to be used as food supplements. One critical question is whether phytoestrogens increase the risk of cancers, especially breast cancer and prostate cancer. Several clinical studies have been conducted to evaluate such possible side effect [151-155]. The results from those studies indicate that phytoestrogens are well tolerated and do not increase the risk for breast and prostate cancer. In a study with 30 healthy postmenopausal women, daily administration of 900 mg soy isoflavones for 84 days had no detectable effects on DNA damage, apoptosis, and changes indicative of estrogenic stimulation. It was concluded that unconjugated soy isoflavones appeared to be safe and well tolerated in healthy postmenopausal women [151]. In a recently reported study, the associations between dietary phytoestrogen (isoflavonoids, lignans, and coumestrol) intake and risk of breast cancer were evaluated. The results revealed that intakes of lignan, isoflavonoid, or coumestrol were not associated with breast cancer risk overall or before or after 50 years of age [152]. In a randomized, double-blind, placebo-controlled pilot trial with 401 healthy women aged 35-70 years with a family history of breast cancer, the safety and tolerability of a standardized 40 mg red clover isoflavone dietary supplement were evaluated for three years [153]. No significant differences in breast density, endometrial thickness, serum cholesterol, follicle stimulating hormone levels and bone mineral density were detected between those taking red clover isoflavones and placebo. The results support the growing body of evidence that treatment with red clover isoflavones is safe and well tolerated in healthy women [154]. Two recent clinical studies investigated the possible side effects of isoflavones on men with or without prostate cancer [155, 156]. Fifty three prostate cancer patients received either purified isoflavones (80 mg daily) or placebo for 12 weeks. At the end of the study, although significant increases in plasma isoflavones were detected, no clinical toxicity was detected [154]. The other study assessed the effect of an isoflavonoid extract from red clover on prostate, liver function, quality of life, and sexual function in men. Twenty men (mean age 65 years) were treated with a daily dose of 60 mg isoflavone extract for 1 year. It was concluded that daily oral administration of 60 mg of the isoflavone extract was well tolerated and caused no side effects [155]. In contrast to the favorable findings from those clinical studies, three cases of adverse reaction from phytoestrogen usage were reported [156]. Abnormal uterine bleeding with endometrial pathology in three women was found to be related to a high intake of soy products. In summary, soy protein preparations and phytoestrogens are generally considered safe and well tolerated with no increase in risk for cancers. However, additional studies with high doses and long-term are required to ascertain their safety.

CURRENT & FUTURE DEVELOPMENTS

Food supplements have increasingly become attractive alternatives to prevent or treat hypercholesterolemia and reduce the risk for cardiovascular diseases. The hypocholesterolemic effects of plant sterols, fiber, *Monascus* products and soy protein preparations have been consistently demonstrated in clinical trails whereas the efficacy of certain probiotic bacteria, such as *Bacillus* and *Rhodospirillum*, and phytoestrogens-containing supplements remains to be fully established. The active ingredients in soy protein preparations responsible for the hypocholesterolemic effects remains to be elucidated as more and more evidence indicates that isoflavones, once believed to be the active ingredients, might not be the mediators of the hypocholesterolemic activity of soy protein products. Currently, our understanding on the molecular mechanisms for most of those active ingredients is incomplete or still lacking, especially for probiotic bacteria and soy protein preparations. Although it is becoming a trend to combine various active ingredients to enhance the efficacy of food supplements in lowering cholesterol, most of such combinations are based on a trial-error approach. Understanding the underlying mechanisms will provide the molecular basis for rational combination of active ingredients to achieve maximal beneficial effects and minimize potential adverse reaction.

Accumulative data from preclinical and clinical studies show that plant sterols, fiber, soy protein and phytoestrogens are generally considered safe and cause no obvious side effects. However, no clinical data are currently available for evaluating the safety of probiotic *Rhodospirillum* preparations. Future clinical studies are required to establish the safety profiles of probiotic *Rhodospirillum* as food supplement. Finally, new approaches, such as genetic modification of *Monascus* strains and alteration of production process, to minimize or totally eliminate the production of mycotoxin citrinin should be taken to ensure the safety of *Monascus* products as food supplements.

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CONFLICT OF INTEREST

The authors has no conflict of interest.

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