

# Soy Protein, Isoflavones, and Cardiovascular Health

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## Abstract:

**Soy protein and isoflavones (phytoestrogens) have gained considerable attention for their potential role in improving risk factors for cardiovascular disease. This scientific advisory assesses the more recent work published on soy protein and its component isoflavones. In the majority of 22 randomized trials, isolated soy protein with isoflavones, as compared with milk or other proteins, decreased LDL cholesterol concentrations; the average effect was &3%. This reduction is very small relative to the large amount of soy protein tested in these studies, averaging 50 g, about half the usual total daily protein intake. No significant effects on HDL cholesterol, triglycerides, lipoprotein(a), or blood pressure were evident. Among 19 studies of soy isoflavones, the average effect on LDL cholesterol and other lipid risk factors was nil. Soy protein and isoflavones have not been shown to lessen vasomotor symptoms of menopause, and results are mixed with regard to soy's ability to slow postmenopausal bone loss. The efficacy and safety of soy isoflavones for preventing or treating cancer of the breast, endometrium, and prostate are not established; evidence from clinical trials is meager and cautionary with regard to a possible adverse effect. For this reason, use of isoflavone supplements in food or pills is not recommended. Thus, earlier research indicating that soy protein has clinically important favorable effects as compared with other proteins has not been confirmed. In contrast, many soy products should be beneficial to cardiovascular and overall health because of their high content of polyunsaturated fats, fiber, vitamins, and minerals and low content of saturated fat.**

**Keywords: AHA Scientific Statements, cardiovascular diseases soybean proteins, isoflavones, cholesterol.**

## INTRODUCTION

Soy protein has gained considerable attention for its potential role in improving risk factors for cardiovascular disease (CVD). In October 1999, the US Food and Drug Administration (FDA) approved labeling for foods containing soy protein as protective against coronary heart disease. The FDA based this decision on clinical studies showing that at least 25 g of soy protein per day lowered total and LDL cholesterol. The FDA requires for the claim that a serving contain at least 6.25 g of soy protein, 25% of the necessary daily amount (25 g), with the expectation that foods containing soy protein would be eaten at least 4 times per day. The FDA also stated that “the evidence did not support a significant role for soy isoflavones in cholesterol-lowering effects of soy protein.”

In 2000, the American Heart Association (AHA) Nutrition Committee released a scientific advisory on soy protein and CVD. At that time, the conclusion was that “it is prudent to recommend including soy protein foods in a diet low in saturated fat and cholesterol.” Since then, many well-controlled studies on soy protein and soy-derived isoflavones substantially added to the knowledge base. For this reason, the AHA Nutrition Committee decided to reevaluate the evidence on soy protein and CVD and update its scientific advisory. Thus, this scientific advisory assesses the more recent work published on soy protein and its component isoflavones. The focus is on blood LDL cholesterol because it is by far the most studied risk factor for CVD, is the primary criterion on which the National Cholesterol Education Program estimates risk and recommends therapy, and forms the basis for the FDA-approved health claim. In this advisory, we also consider the effects of soy protein and isoflavones on several other CVD risk factors: HDL cholesterol, triglycerides, lipoprotein(a), and blood pressure. The medical literature was searched comprehensively for original research publications on the effects of soy protein or isoflavones on CVD risk factors, and all controlled trials that separately listed soy protein and isoflavone content were used. In addition, this advisory reviews the evidence on soy products in other health conditions, including menopausal symptoms, osteoporosis, and cancer.

Soy protein, like any other dietary protein, contains calories and could be used in the diet to replace animal protein or other vegetable proteins. Soy protein also could replace other sources of calories such as carbohydrate or fat, raising the total amount of protein eaten and reducing carbohydrate or fat intake. Most studies exchanged soy protein for other dietary proteins, and this evidence is evaluated in the present advisory. Much less is known about the potential impact on risk factors for CVD of increasing total protein intake by adding soy or other plant protein in place of carbohydrate or fat; this important dietary change is currently being studied.

**The Soy Protein Hypothesis on LDL Cholesterol An Overview.** Animal proteins raise blood cholesterol concentrations in several animal species fed cholesterol-free semisynthetic diets. Casein, the most prevalent protein in milk, has been used most often, although other animal proteins such as pork and beef protein do the same. This is a useful established nutritional model for studying diet-induced hypercholesterolemia and atherosclerosis and an alternative to feeding animals large amounts of cholesterol. In contrast, when soy protein is substituted for the animal protein, hypercholesterolemia does not occur. Thus, either some animal proteins have a direct hypercholesterolemic action, or soy protein has a cholesterol-lowering action. This latter possibility led to intensive work in the late 1970s and 1980s to test the hypothesis that soy protein

can be a nutritional approach to reducing blood cholesterol. This concept gained support from epidemiological observations on diet and CVD in Japan and other Asian countries where large amounts of soy products were eaten and blood cholesterol concentration and CVD incidence were low. However, many differences in diet and lifestyle between Asian and Western countries could explain the differences in the prevalence of CVD.

Early indications that soy protein had much less effect in humans than in animals came from direct application of the animal model to humans. Diets similar to those eaten by humans, based on either soy protein or casein, were fed to rabbits, and, as expected, casein produced hypercholesterolemia. However, when the same diets were fed to healthy people, the protein source did not affect blood cholesterol. Others studied the effect of casein in strict vegetarians who ate no dairy or animal proteins to provide a human counterpart to the mainly vegetarian animal (eg, rabbit) models. Compared with soy protein, no effect of casein on blood cholesterol was found.

In the late 1970s and early 1980s, the soy protein hypothesis was greatly strengthened as a result of studies by Sirtori et al and Descovich et al, who found that diets high in soy protein, replacing nearly all the animal protein, substantially reduced blood LDL cholesterol by 20% to 30% in severe hypercholesterolemia. Because the soy protein diets were also reduced in saturated fat and cholesterol and increased in polyunsaturated fat and because the patients also often lost weight on the dietary protocols, the results were often confounded. The authors reported that textured soy protein (50% soy flour, 50% soy protein concentrate) but not soy protein isolate (90% soy protein) was effective. This raised the possibilities that, rather than the soy protein itself, the nonprotein components of the soy protein preparation or the effect of soy displacing cholesterol-raising fats in the diet could have had a blood cholesterol-lowering action. Results of other early studies of soy protein in hypercholesterolemic subjects showed either cholesterol reduction or no effect.

A meta-analysis published in 1995 attempted to reconcile the many divergent findings among studies of soy protein. In 29 controlled studies, a trend emerged that soy protein selectively reduced blood cholesterol in direct proportion to the degree of hypercholesterolemia. For example, in those with severely elevated blood cholesterol (>335 mg/dL), soy protein reduced blood cholesterol by 20%. Only a 7% reduction occurred in those with cholesterol levels between 259 and 333 mg/dL; if the initial blood cholesterol was <255 mg/dL, there was no significant effect. Thus, the response to soy protein was determined more by the initial blood cholesterol level and, surprisingly, not by the amount of soy protein eaten, which ranged widely from 18 to 124 g/d. When the control group was not included in the statistical analysis, there was a significant correlation between the dose of soy protein and the degree of cholesterol reduction. However, an analysis without a control group introduces the effects of confounding and drift in serum cholesterol that often occur in experimental situations. This meta-analysis also was limited by the quality of the studies; studies were less well controlled in people with hypercholesterolemia than in those with average cholesterol levels. It is difficult to determine how much effect this had on the overall results of the meta-analysis. Thus, the available literature provided some support, albeit with limitations, for the concept that soy protein is an effective treatment for severe hypercholesterolemia, that it produces a mild benefit in people with moderate elevations of cholesterol, but that it has no effect in those with mildly elevated or average cholesterol levels. The soy protein hypothesis culminated in FDA approval of a health claim for soy protein in foods.

**Soy Isoflavones.** Subsequent to the meta-analysis by Anderson et al, many well-controlled studies explored the soy protein hypothesis with greater specificity. In addition, recognition that soy protein products contain bioactive molecules called phytoestrogens or isoflavones added a fascinating new aspect to the soy protein hypothesis. Isoflavones remain in soy protein preparations that are not extracted with alcohol. During the preparation of soy protein isolate, the soy is washed with alcohol, removing a substantial amount of the isoflavones. The soy isoflavones have strong biological properties in animals, causing arterial vasodilation, lowering serum cholesterol, and inhibiting atherosclerosis in postmenopausal monkeys. This led to the intriguing idea that the presence and amount of isoflavones explain the variable results of soy studies; only those that used high-isoflavone preparations produced favorable results. Isoflavone content was not known in many of the earlier studies. Several subsequent studies tested the effects of soy protein and isoflavones separately.

The 3 major isoflavones found in soybeans are genistin, daidzin, and glycitin. Their abundance in soy protein preparations varies widely and depends on the processing techniques used during production. These compounds have both estrogenic and antiestrogenic activity and effects that are unrelated to estrogen activity. Dehulling, flaking, and defatting soybeans produces a relatively pure preparation of protein that is low in isoflavones, whereas methods used to produce textured soy protein result in a preparation that retains the isoflavones. Isoflavone concentrations range from &2 mg/g protein in textured soy protein, soy flour, and soy granules to 0.6 to 1.0 mg/g protein in isolated soy protein. Intakes of 45 g soy flour have resulted in a 20- to 40-fold increase and a 50- to 100-fold increase in blood and urinary isoflavones, respectively, and there is a dose-dependent relationship at more moderate intakes.

## **Effect of Soy Protein on LDL Cholesterol and Other Lipoproteins**

**Soy Protein With Isoflavones.** First, we summarize studies that tested soy protein that contained a substantial amount of isoflavones. Because it was recognized that isoflavones could be the bioactive component attributed to soy protein, studies published in the late 1990s and beyond generally stated the amount and type of isoflavones in the soy protein. In 22 randomized trials, isolated soy protein with isoflavones was compared with casein or milk protein, wheat protein, or mixed animal proteins. The range of soy protein was 25 to 135 g/d; the range for isoflavones was 40 to 318 mg. LDL or non-HDL cholesterol concentrations decreased in most studies, statistically significantly in 8, with an overall effect of &3% (weighted average). A recent meta-analysis that included 10 studies published from 1995 to 2002 found a similar percentage reduction in LDL cholesterol with no dose effect. Over all studies in Table 1, there is no apparent dose effect; the 8 studies with 50 g of soy protein showed a drop in LDL cholesterol concentration similar to those using a smaller amount of soy, &3% overall (Table 1). This cutpoint for daily soy protein intake, 50 g, defines a large amount, half or more of the daily average total protein intake in the United States. No significant effects were evident for HDL cholesterol or triglycerides in most of the studies; the weighted average effects were very small: 1.5% for HDL cholesterol and -5% for triglycerides.

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## **TABLE 1. Soy Protein and Blood Lipid Risk Factors: Effects of Soy Protein With Isoflavones.**

**Soy Protein Without Isoflavones.** In 7 trials, soy protein, washed with alcohol to remove isoflavones, was compared with casein or milk protein or various animal proteins (Table 2). Two studies showed small significant decreases in LDL cholesterol. These studies were very carefully controlled feeding studies, with all meals formulated according to strict nutritional specifications, and complete meals were provided to the participants. Specifically designed to sort out the effects of the protein from the effects of the isoflavones, the studies showed an effect of protein but not isoflavones on LDL cholesterol. The declines in LDL cholesterol were small, 2% to 7%, relative to the large amounts of soy protein eaten daily, 50 to 55 g. However, other well-controlled studies did not find significant effects of soy protein on LDL cholesterol, and the average change across all 7 studies was only a 1% to 2% decrease. Changes in HDL cholesterol and triglycerides were generally small and were nonsignificant in 6 of the 7 trials. No dose effect was evident.

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## **TABLE 2. Effects of Soy Protein With Low or No Isoflavones**

**Effect of Isoflavones.** Some studies compared soy protein that did or did not contain isoflavones (Table 3), whereas other studies tested isoflavones in pill form as compared with placebo. A wide range of isoflavone amounts was studied. One study compared the effect of isoflavones provided with either soy or animal proteins. Among these 19 studies, only 3 showed significant reductions in LDL cholesterol concentration, and the effect among all studies (weighted average) was nil, 0%. Changes in HDL cholesterol and triglycerides were not significant and showed no trend toward an effect of isoflavones. Despite large increases in blood isoflavone concentrations, there is no indication of a dose effect on blood lipids. A recent meta-analysis concluded that isoflavones do not affect blood lipid concentrations.

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## **TABLE 3. Effects of Isoflavones**

### ***Influence of Initial Blood LDL Cholesterol Level***

In the Anderson et al meta-analysis, a strong gradient of LDL cholesterol reduction was found among studies according to initial cholesterol level. Lichtenstein et al and Crouse et al found slightly more LDL cholesterol reduction in people with LDL cholesterol >160 to 164 mg/dL than in those with lower levels, although Dent et al did not find an effect in women with hypercholesterolemia as compared with women with average cholesterol levels. However, a larger percentage reduction in LDL cholesterol in hypercholesterolemia is not evident among the 22 recent trials (Table 1). Among studies of isoflavones, no relation is evident between initial cholesterol and cholesterol lowering (Table 3).

### ***Influence of Serum Cholesterol-Lowering Diet***

In their meta-analysis, Anderson et al reported that soy protein tended to have less effect on LDL cholesterol in trials in which the participants were eating a low-fat and low-cholesterol diet as compared with a more usual higher-fat and higher-cholesterol diet. In 11 of the studies listed in Tables 1 through 3, soy protein or isoflavones were tested in combination with a serum

cholesterol-lowering diet. The average reduction in LDL in these studies was 2%, similar to that in the full group. Thus, the effect on LDL of soy protein or isoflavones does not appear to be modulated by the saturated fat and cholesterol content of the diet.

**Effects on Lipoprotein(a).** Lipoprotein(a), an LDL-like lipoprotein that is an independent predictor of CVD, was increased by soy protein in 2 studies and unchanged in 9 others. Meinertz et al found that alcohol-extracted soy protein, lacking isoflavones, did not raise lipoprotein(a) as found in their earlier study of intact soy protein, which suggests an adverse effect of isoflavones. However, isoflavones had no effect on lipoprotein(a) in 6 other studies, nor did soy protein that contained isoflavones.

**Effects on Blood Pressure.** Several studies tested the effect of soy protein with isoflavones, as compared with casein or milk protein, on blood pressure. Blood pressure decreased significantly in 1 study but not in the other 5 studies. The weighted average change is -1 mm Hg systolic blood pressure. Several studies that evaluated the effect of soy isoflavones also did not find a significant effect on blood pressure.

### **Effects on Health Conditions Related to Estrogens**

**Menopausal Vasomotor Symptoms.** Because of their weak estrogenic activity, soy isoflavones have been hypothesized to improve several estrogen-dependent conditions, including perimenopausal vasomotor symptoms (hot flashes) and postmenopausal bone loss. A recent review examined 11 clinical trials of soy protein or isoflavones for treating hot flashes. Only 3 of 8 studies with treatment lasting >6 weeks found modest improvement in hot flashes, and most benefits disappeared after 6 weeks. Five additional studies not included in that review showed no benefit for hot flashes of soy isoflavones. Longer studies showed no benefit of isoflavones at 24 weeks or 2 years. Substantial reduction in hot flashes, often 40% to 60%, occurred in the placebo or control group in these studies, similar to the reduction in the soy group. In contrast, estrogen replacement markedly reduces hot flashes, more so than placebo. Thus, it seems unlikely that soy isoflavones have enough estrogenic activity to have an important impact on vasomotor symptoms of estrogen deficiency in perimenopausal women.

**Osteoporosis.** Another estrogenic effect of soy isoflavones could be to reduce bone loss after menopause; this hypothesis gains strength from population studies and certain animal models of osteoporosis. However, clinical trials so far have had insufficient duration and size to be conclusive, and results have varied. The studies used either direct measurements of bone mineral content and density in the spine and hip or biochemical indices of bone resorption or formation to test the effect of soy isoflavones ranging in amount from 54 to 300 mg, but most studies used 80 to 110 mg. Soy isoflavones lessened bone loss over 6 to 24 months in some studies, whereas other trials did not show a benefit over the same duration. There is also inconsistency in the studies showing favorable effects, with one study showing benefit in the spine but not hip and another showing the opposite, or improvement in bone mineral content but not bone mineral density. Diminution of bone loss, indicated by a reduction in biochemical markers of bone resorption, was found in some studies but not in others. The amounts of isoflavones were similar in studies that

found favorable or no effects. The longest study in any primate species was in postmenopausal monkeys (*Cynomolgus macaques*); after 3 years, soy isoflavones did not slow bone loss, whereas estrogen replacement increased bone mineral content and density, as expected. These varied results of clinical trials suggest the need for investigations of isoflavones and bone health that have substantial sample size and long duration to provide a definitive result.

**Cancer.** The weak estrogenic action of soy isoflavones and other phytoestrogens suggested the possibility that they could lessen the deleterious effects of more potent endogenous estrogens on breast and endometrial cancer. This hypothesis came from the low incidence of breast and endometrial cancers in Asian countries where soy products are prevalent in the diet and from certain animal models of breast and endometrial cancer showing benefit of soy isoflavones. In reality, a host of complexities have emerged that make it impossible to state a clinical recommendation for the use of soy isoflavones. In epidemiological studies, associations varied between intake of soy foods and isoflavones and incidence of breast cancer; some showed protective associations, and others showed no association. Clinical studies suggested that soy phytoestrogens stimulate epithelial cell proliferation in breasts of premenopausal women, a potential precursor of cancer. Animal and cell culture experiments also found a cancer-stimulating effect. Phytoestrogens reduce the activity of enzymes that inactivate endogenous estrogens, potentially leading to increased active estrogen concentrations. Nonlinear dose effects, unique effects of specific types of isoflavones, changes in isoflavone composition and structure during the processing of soy foods, and interperson variation in isoflavone metabolism all could affect cancer initiation and progression and are virtually unexplored in the clinical arena. It has been hypothesized from animal experiments that soy isoflavones could be protective throughout adult life only if eaten in childhood or puberty. Case-control studies in Shanghai and in Asian Americans found that high soy intake in adolescence was associated with low risk for breast cancer in adulthood. Finally, several recent expert reviews and editorials concluded that the research overall remains insufficient to know whether certain phytoestrogens are protective or harmful for breast cancer and at what dose and time period, if any, in a woman's life they are active.

Concepts with regard to soy isoflavones and breast cancer are applicable to uterine endometrial cancer, an estrogen-dependent cancer, although data are much less extensive. Soy food or isoflavone intake was associated with low risk for endometrial cancer in case-control studies in Shanghai, Hawaii, and California. This suggests that soy phytoestrogens have antiestrogenic effects on the uterus. However, a single pilot trial of soy isoflavones given together with estrogen to perimenopausal or postmenopausal women found no lessening of estrogen-mediated stimulation of the endometrium. Several clinical trials found that isoflavones did not affect the uterine endometrium of perimenopausal or postmenopausal women. However, these trials may have had insufficient duration (3 to 6 months) or sample size to identify an effect. Recently, a relatively large placebo-controlled trial in postmenopausal women found that isoflavone tablets caused endometrial hyperplasia, a precursor to cancer, after 5 years in 6 of 154 women compared with none on placebo ( $P < 0.05$ ). Another 5 women in the phytoestrogen group had proliferative endometrium compared with none in the placebo group after 5 years. These effects were not found at 2½ years. Thus, some cautionary evidence indicates that soy phytoestrogens have enough estrogenic activity to stimulate the endometrium of postmenopausal women, although the

evidence overall is inadequate to draw conclusions on whether soy protein or isoflavones taken by perimenopausal or postmenopausal women eventually would cause endometrial cancer.

Soy isoflavones have estrogenic, antiandrogenic, and other activities that could prevent prostate cancer or slow its progression. Prostate cancer incidence is relatively low in Asian countries where soy products are commonly eaten, and certain epidemiological studies have shown an inverse association between soy foods, serum phytoestrogen levels, and prostate cancer. However, as pointed out by Messina, the epidemiological findings are inconsistent, and there are important limitations in study design. Soy isoflavones prevent the development and growth of prostate cancer in animal models. In prostate cancer cells, genistein reduced the synthesis of prostate-specific antigen, a marker of prostate cancer development and progression that is in extensive clinical use. However, soy isoflavones did not reduce either prostate-specific antigen or serum testosterone levels in men with early-stage prostate cancer or in healthy middle-aged men. Thus, the effectiveness of soy isoflavones in preventing or treating human prostate cancer is unknown.

## Conclusions

Earlier research indicating that soy protein, as compared with other proteins, has clinically important favorable effects on LDL cholesterol and other CVD risk factors has not been confirmed by many studies reported during the past 10 years. A very large amount of soy protein, more than half the daily protein intake, may lower LDL cholesterol by a few percentage points when it replaces dairy protein or a mixture of animal proteins. The evidence favors soy protein rather than soy isoflavones as the responsible nutrient. However, at this time, the possibility cannot be ruled out that another component in soybeans could be the active factor. No benefit is evident on HDL cholesterol, triglycerides, lipoprotein(a), or blood pressure. Thus, the direct cardiovascular health benefit of soy protein or isoflavone supplements is minimal at best. Soy protein or isoflavones have not been shown to improve vasomotor symptoms of menopause, and results are mixed with regard to the slowing of postmenopausal bone loss. The efficacy and safety of soy isoflavones for preventing or treating cancer of the breast, endometrium, and prostate are not established; evidence from clinical trials is meager and cautionary with regard to a possible adverse effect. For this reason, use of isoflavone supplements in food or pills is not recommended. In contrast, soy products such as tofu, soy butter, soy nuts, or some soy burgers should be beneficial to cardiovascular and overall health because of their high content of polyunsaturated fats, fiber, vitamins, and minerals and low content of saturated fat. Using these and other soy foods to replace foods high in animal protein that contain saturated fat and cholesterol may confer benefits to cardiovascular health. Soy protein also may be used to increase total dietary protein intake and to reduce carbohydrate or fat intake. However, much less is known about the potential impact of high-protein diets on risk factors for CVD. In the meantime, these remain dynamic areas for research. The AHA will continue to monitor the results and modify its advisory statement as needed.

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