Fourth International Symposium on the Role of Soy in Preventing and Treating Chronic Disease

Soy, Soy Phytoestrogens and Cardiovascular Disease

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ABSTRACT Dietary soy protein has been shown to have several beneficial effects on cardiovascular health. The best-documented effect is on plasma lipid and lipoprotein concentrations, with reductions of ~10% in LDL cholesterol concentrations (somewhat greater for individuals with high pretreatment LDL cholesterol concentrations) and small increases in HDL cholesterol concentrations. Dietary soy protein improves flow-mediated arterial dilation of postmenopausal women but worsens that of men. Soy isoflavone extracts improve systemic arterial compliance, an indicator of atherosclerosis extent. Complete soy protein but not alcohol-washed soy protein reduces atherosclerosis of postmenopausal monkeys. No definite experimental evidence exists currently to establish that the cardiovascular benefits of soy protein are accounted for by its isoflavones. J. Nutr. 132: 566S–569S, 2002.

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It has been known for ~60 y that replacement of animal protein in the diet with soy protein reduces hyperlipoproteinemia and atherosclerosis (1). The increased level of research intensity over the past 10–12 y has resulted from evidence that soy consumption might not only improve several aspects of postmenopausal health but also improve cardiovascular health. The urgency about increasing understanding of soy and cardiovascular health of women is because coronary heart disease was then and remains the leading cause of both morbidity and mortality of postmenopausal women. By the early 1980s there was convincing observational data that postmenopausal replacement with mammalian estrogens inhibited the early progression of coronary artery atherosclerosis.

Research on the potential health benefits of soy and soy phytoestrogens for postmenopausal women progressed rapidly during the 1990s. We like to believe that members of our research group at Wake Forest University School of Medicine (then the Bowman Gray School of Medicine) were pioneers in stimulating that research effort. During the period from 1988 to 1990, we became convinced that the primary public health issue in postmenopausal hormone replacement therapy was poor acceptance of existing therapies (mammalian estrogens), which resulted in 80–85% of the at-risk population going untreated. Those issues led us in 1995 to change the direction of our long-standing National Institutes of Health, National Heart, Lung and Blood Institute Program Project entitled Coronary Heart Disease of Females. We proposed to the National Heart, Lung and Blood Institute to launch a program of research and in the application stated the following: “The rationale for choosing soybean estrogens as a potential nutritional alternative to the current standard conjugated equine estrogens therapy is based on the protective effect of those compounds against the development of breast cancer, the likely lack of a harmful effect on the uterus, and an experimental basis for assuming probable favorable effects on coronary artery atherosclerosis and osteoporosis.” The research has been productive and the results have been the basis of two recent chapters (2,3).

This brief overview is not comprehensive but rather reflects my view of the status of research on soy and the cardiovascular system.

Plasma lipids and lipoproteins

Dietary soy protein has well-documented beneficial effects on plasma lipid and lipoprotein concentrations. The effects in human subjects are reductions in LDL cholesterol of ~13%; reductions in plasma triglycerides of ~10%; and increases in HDL cholesterol, greater in some subjects than others, with average increases of ~2% (4). These beneficial effects of soy protein on plasma lipoprotein concentrations culminated recently in the U. S. Food and Drug Administration’s approval

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of 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" (5). Recent research has focused primarily on efforts to identify the components of soy protein responsible for the beneficial lipoprotein changes.

During the 1970s and early 1980s, the amino acid composition of soy was investigated for its effect on plasma lipid and lipoprotein metabolism and its role in atherogenesis inhibition. Huff et al. (6) reported one of the more clarifying experiments. In that experiment groups of rabbits were fed diets that contained either casein or a mixture of amino acids that duplicated the amino acid composition of casein. The plasma cholesterol concentrations of the two groups were found to be essentially the same. The same procedure was followed with soy protein, with one group fed the intact protein and another group fed a mixture of amino acids that was identical to the composition of intact soy protein. The soy protein amino acid mixture did not have the same hypocholesterolemic effect as the intact protein, providing evidence that there were components of the intact soy protein other than the amino acids that independently lowered plasma cholesterol concentrations or interacted with the protein moiety to affect lipoprotein metabolism favorably.

Several studies provided evidence that the component of soy protein responsible for a large part of its cardioprotective effects was alcohol extractable (7–10). In the study by Sugano and Koba (8), various fractions of soy protein were isolated and then were or were not extracted with methanol. One of the fractions (the undigested fraction after treatment with pepsin) was hypocholesterolemic, but when that fraction was extracted with methanol, it was less effective in preventing hypercholesterolemia.

In the study by Balmir et al. (7), rats were fed diets containing ethanol-extracted soy protein (soy’), unextracted soy protein (soy”), casein, or casein with an alcohol extract of soy flour added (casein”). The alcohol extract was treated with acetone to precipitate sugars, saponins, and proteins; thus, it was principally a phytoestrogen-containing extract. These investigators found that rats fed the Casein”, soy”, and soy’ diets had significantly lower LDL cholesterol concentrations than did the casein group and these latter three groups did not significantly differ from each other. There was also a trend toward higher HDL cholesterol concentrations in the two groups fed the diets containing isoflavones (phytoestrogens) compared with the isoflavone-devoid groups.

A number of studies with monkeys have sought to determine how much the plasma lipid-lowering properties of soy protein relate to the presence of the isoflavones and how much they relate to effects of the soy peptides. The approach has been to compare soy’ with soy” (alcohol washed to remove the isoflavones) (9–11). The HDL cholesterol concentrations of monkeys fed soy” have generally been higher than those of monkeys fed soy”, and the LDL plus VLDL cholesterol concentrations are much lower in monkeys fed soy” compared with those fed soy”.

We and others interpreted those observations as evidence that the plasma lipid-lowering properties of soy protein were related primarily to the presence of the isoflavones. That interpretation may have been incorrect in light of evolving findings. Greaves et al. (12) were unable to achieve the plasma lipid-lowering effects of soy protein when the exact amount of soy isoflavones contained in soy protein were added to a diet containing casein + lactalbumin. A more recent monkey study further contradicted the notion that the isoflavones are responsible for the lipid-lowering effect of soy (13). The plasma lipid-lowering effect seen with soy” could not be restored by adding back the whole alcohol extract or its isoflavones to soy”, dispelling the expectation that isoflavone extracts in concert with dietary soy peptide that had been alcohol extracted previously would have beneficial plasma lipid effects. It is important to note, however, that one cannot rule out the possibility that alcohol extraction of soy protein alters in some way attributes of the protein that are important in cholesterol metabolism or properties of the protein that are important in its interactions with isoflavones in modulating cholesterol metabolism.

Uncertainty continues about the importance of isoflavones as a part of the soy protein matrix. Three studies in human subjects suggest that soy protein with higher levels of isoflavones might have more robust effects on lowering LDL cholesterol concentrations than soy protein with lower isoflavone amounts (14–16). Crouse et al. (14) noted an increasing reduction in LDL cholesterol concentrations with increasing isoflavone content (from 3 to 62 mg) in 25 mg soy protein. In a study in normocholesterolemic premenopausal women, soy protein (53 g/d) with the highest isoflavone content (129 mg) had a more robust effect than did the same amount of soy protein with approximately one-half the isoflavone content (65 mg) on lowering LDL cholesterol concentrations (15). In postmenopausal women, consumption of soy protein (63 mg/d) with 132 mg isoflavones lowered LDL cholesterol concentration more than did the same amount of soy protein with ~65 mg isoflavones (16).

Clinical studies have tended to support the more recent monkey studies finding little or no cardiovascular benefits of administering soy isoflavone extracts. In a study with peri- and postmenopausal women, treatment for 5 wk with 80 mg/d of soy isoflavone extract improved systemic arterial compliance, an indicator of vascular stiffness (17), but there was no effect on endothelium-dependent flow-mediated dilation or on plasma cholesterol concentrations of HDL or LDL. The lack of effect of soy isoflavones extracts on flow-mediated dilation and plasma lipoprotein concentrations was confirmed in a more recent study (18).

**LDL oxidation**

Interest is increasing in the role of LDL particle oxidation on both atherogenesis and vascular function. Tikkanen et al. (19) gave soy protein supplements (containing 60 mg isoflavones) to healthy volunteers. Soy treatment significantly prolonged LDL oxidation by ~20 min. Reduced oxidation potential could not be related to incorporation of the isoflavones or their metabolites into the LDL particles because they were present in only very small amounts. Based on their finding that estradiol fatty acid esters were incorporated into LDL, Helisten et al. (20) described a very exciting new observation that fatty acid esterification of soy isoflavones permits their incorporation into LDL particles that results in much greater oxidation resistance (21).

Our group compared arterial lipid peroxidation concentrations in monkeys fed casein + lactalbumin with those fed soy”. The concentrations were ~17% less in those fed soy”. Similar to interest in lipids and lipoproteins, there is interest in determining the importance of isoflavones in mediating the effects of soy protein on LDL oxidation. Wiseman et al. (22) explored the question after administering soy” or soy” to healthy humans. The oxidation resistance of those given soy” was significantly greater than those given soy”. Complicating that clear finding are the findings of Hodgson et al. (23) and Samman et al. (24), who could find no effect of soy isoflavone extracts in LDL oxidation.
Arterial function

Normal arterial function is critical in the prevention of ischemic changes in the end organs served by particular arteries. There is considerable interest in better understanding arterial functions that relate to the occurrence of ischemic heart disease. The arterial functions that have been evaluated fall into two broad categories: endothelium-mediated arterial dilation and measurements of arterial stiffness, also called arterial compliance. Both of these will be summarized briefly and separately.

Endothelium-mediated vasodilation

Two approaches are used to evaluate endothelium-mediated vasodilation. One determines the response of arteries to the perfusion of acetylcholine. The normal endothelium response to acetylcholine is to release diluting substances, such as nitric oxide, that result in dilation of the artery in question. The other approach is flow-mediated dilation whereby flow is restricted; the response of the normal endothelium again is to release dilating substances that prompt arterial dilation when flow is reestablished. These techniques are usually applied to the brachial artery after reduction of flow with an inflatable cuff.

Several years ago our group (25) reported that administering soy to premenopausal cynomolgus monkeys for 6 mo inhibited coronary artery vascular constriction in response to acetylcholine by \( \sim 12\% \) compared with another group that was administered soyoil and showed constriction in response to the intracoronary artery perfusion of acetylcholine. Subsequently, we were unable to demonstrate coronary artery dilation to acetylcholine in surgically postmenopausal monkeys [plasma estradiol concentrations usually \(< 5 \text{ pg/mL (18.36 pmol/L)}\) given soy\(^{+}\)]. That unexpected finding led Williams et al. (26) to study the relationship between estradiol and the response of postmenopausal monkeys to soy\(^{+}\). In that more recent study, it was found that surgically postmenopausal monkeys with very low plasma estradiol concentrations responded to acetylcholine with coronary artery constriction, whereas those given both estradiol and soy\(^{+}\) responded with a greater degree of coronary artery dilation than those given the equivalent amount of estradiol. A statistically significant interaction was demonstrated between the effects on vasodilation of estradiol and soy diet.

Teede et al. (27) reported recently on a comprehensive study to evaluate the effect of a soy supplement comparable with soy\(^{+}\) on arterial function of 213 humans (108 men and 105 postmenopausal women, 50–75 y old). The 3-mo treatment was 40 g soy protein isolate that contained 118 mg isoflavones and the placebo was casein. The soy treatment worsened brachial artery dilation of the men and had no significant effect on the flow-mediated dilation in the brachial artery of the postmenopausal women. It is unclear whether the lack of effect in the postmenopausal women related to a large number of women having very low plasma estradiol concentrations. Similarly, an isoflavone extract was shown to have no effect on flow-mediated dilation of postmenopausal women (17).

The effects of soy isoflavones on brachial artery dilation are not understood completely at this time. Walker et al. (28) compared the effects of genistein or daidzein given via direct injection into the brachial artery with estradiol given in the same way. The subjects were men 20 to 51 y old and premenopausal women 29 to 39 y old. Genistein infusion resulted in increased brachial artery dilation of both men and women comparable to the effect of estradiol and the effect could be blocked by a nitric oxide synthase inhibitor. There was no effect of daidzein infusion. It should be noted that the women were premenopausal and there may have been interactions with estradiol accounting for some of the dilation as has been described for monkeys. Another uncertainty relative to reported trials with postmenopausal women was the high plasma concentrations attained by the arterial infusions (forearm venous blood concentrations of \( \sim 2 \mu\text{mol/L} \) compared with \( \sim 0.3 \mu\text{mol/L} \) among women given soy supplements).

Arterial compliance

Unlike endothelial-mediated vasodilation (primarily nitric oxide-dependent), arterial compliance relates to the constriction and dilation of arteries associated with systole and diastole. Arterial compliance relates to the components of the artery wall, such as elastin, proteoglycans and smooth muscle cell function. In humans, supplementation with soy protein or the administration of isoflavone extracts seems to improve arterial compliance. In a placebo-controlled, randomized, cross-over study with 21 peri- and postmenopausal women treated for 5 wk with 80 mg/d of purified soy isoflavones, improvement of \( \sim 26\% \) was noted in systemic arterial compliance (17). The study by Teede et al. (27) found that soy significantly improved pulse wave velocity \( (P = 0.02) \). The importance of arterial stiffness or arterial compliance increased after Van Popele et al. (29) found a strong association between arterial stiffness and atherosclerosis extent at various sites in the population-based study known as the Rotterdam Study.

Atherosclerosis

As indicated, there is considerable literature establishing that substitution of animal protein (usually casein) with soy protein results in reduced atherosclerosis resulting from diets containing added cholesterol. Current research focuses primarily on the identification of the components of soy protein that provide the atherosclerosis protection. We (11) reported the results of a randomized, prospective trial using surgically postmenopausal cynomolgus monkeys comparing the effect of soy\(^{+}\) \((n = 56)\) and soy\(^{+}\) \((n = 59)\) on atherosclerosis extent. Over the 3-y trial, the monkeys fed soy\(^{+}\) had a more favorable lipid profile than did those fed soy\(^{-}\), including higher plasma concentrations of HDL cholesterol and lower plasma concentrations of LDL plus VLDL cholesterol. The diet containing the soy\(^{+}\) resulted in a reduced amount of coronary artery atherosclerosis (although not statistically significant with the group sizes studied, \( P = 0.12 \)) compared with the control group fed soy\(^{-}\). The effect of soy\(^{+}\) on common and internal carotid artery atherosclerosis was more robust with reductions reaching statistical significance \( (P = 0.02) \). Because the study did not include a group fed an animal protein such as casein, we cannot speculate about the degree, if any, of the antiatherogenic effect of soy\(^{+}\). Furthermore, we cannot know the components of the soy\(^{+}\) that provided atherosclerosis protection.

One report described the effect of a soy isoflavone extract on atherosclerosis (30). In that study, rabbits were fed an isoflavone glycone-rich extract without soy protein, and atherosclerosis was said to be attenuated. There was no effect of saponin extract.

Research on the cardiovascular effects of soy protein and soy isoflavones has progressed rapidly during the past decade. Several conclusions seem clear. Intact soy protein provides more cardiovascular benefits than does alcohol-washed soy
protein. Whether the difference between intact soy protein and alcohol-washed soy protein relates to the isoflavones is uncertain. The addition of soy isoflavone extracts to diets containing animal protein or alcohol-washed soy protein does not provide plasma lipid concentration benefits. Soy isoflavone extracts given to human subjects do not result in cardiovascular benefits except for improvements in systemic arterial compliance.

LITERATURE CITED