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EFFECT OF SOY ISOFLAVONES ON THE SERUM LIPID PROFILE AND VASCULAR FUNCTION

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ABSTRACT

Soybeans are unique among the legumes because they are a concentrated source of isoflavones. A number of components of soy have been studied to investigate their potential effect on CVD. Soy proteins have been shown to lower LDL-cholesterol and to have neutral or slightly positive effect on HDL-cholesterol. However, to what extend actually is the role of the isoflavone in this action remained contradictive. This review describes the characteristics and the nature of the soy isoflavones as well as covering the results from several studies to determine the effect of soy isoflavones on the serum lipid and vascular functions.

Key words: Soy, soy isoflavones, vascular function

INTRODUCTION

Soybeans are exceptional foods because of their rich nutrient content. These legumes contain complex carbohydrates, vegetable proteins, dietary fibre, oligosaccharides, phytochemicals, vitamins, and minerals (Anderson *et al*., 1999). What is known that all foods including soybean are complex collections of chemicals that can be beneficial or unfavourable for many people in many situations.

Soy by itself is not a magic food, but it is rather an example of different kinds of food that together in a complete diet can have a positive effect on health. As consumers have pursued healthier lifestyles in recent years, consumption of soy foods has risen steadily, encouraged by scientific studies showing from this products. Soybeans are inexpensive sources of plant protein with potential to be used worldwide as substitutes for animal-protein sources.

Soybeans are also unique among the legumes because they are concentrated source of isoflavones. These plant compound are structurally very similar to human hormone estrogen, hence the name used for them is phytoestrogens (Lampe, 2003). There is a keen interest in soy isoflavones because they are biologically active in the human body and may have the potential to reduce the risks of certain diseases.

Research suggests that soy isoflavones maybe beneficial in menopausal health (Knight *et al*., 1996), bone health (Anderson & Carner, 1997), cardiovascular health (Anthony *et al*., 1998), cognitive health and possibly immunity.

Cardiovascular disease (CVD) is disease of heart circulation system (heart, arteries, capillaries, and veins). Heart disease belongs to this CVD, and the risk is influenced by many factors that include blood cholesterol, blood pressure, arterial stiffness, development of atherosclerotic plaque, etc. Soy isoflavones may reduce one or more of these heart disease risk factors.

Lowering elevated serum cholesterol level is widely acknowledged as an important in significantly reducing the risk of CVD. Several evidence have established that the consumption of soy protein where isoflavones are part of it lowers serum cholesterol. In 1999 the U.S. Food and Drug Administration authorized a health claim for soy protein and the reduced risks of heart disease (FDA, 1999).

While the hypocholesterolemic effect of soy protein has been well established, the role of isoflavones in the hypocholesterolemic effect of

soy is inconsistent. A meta-analysis performed with data compiled from 10 clinical trials found soy isoflavones were not associated with significant changes in Low Density Lipoprotein (LDL) cholesterol or High Density Lipoprotein (HDL) cholesterol (Weggemans & Trautwein, 2003).

Although the data do not support the use of soy isoflavones by itself for serum cholesterol reduction, additional research suggests isoflavones may impact other aspects of CVD that equally important. Soy isoflavones have been shown in isolated human plasma studies to reduce LDL oxidation and improve platelet and clotting mechanisms. The data is still preliminary around, but the area of most promise is the effect of isoflavones on vascular function. Soy isoflavones improve compliance or elasticity of large and medium-sized arteries as well as improve endothelial-dependent dilation of peripheral blood vessels (Squardito *et al*., 2002; Steinberg *et al*., 2003).

Therefore, this review is aimed to discuss the effect of soy isoflavones on the serum lipid profile and vascular function and to see whether they are more effective when used together with or without soy protein.

CHARACTERISTICS OF SOY ISOFLAVONES

Isoflavones are a subclass of the more ever-present flavonoids. The basic structural feature of flavonoid compound is the flavone nucleus which is composed of 2 benzene rings (A and B) linked through a heterocyclic pyrane C ring (Figure 1). The position of the benzenoid B ring is the basis for dividing flavonoid class into flavonoids (2-position) and isoflavonoids (3-position). In soybeans, isoflavones are present as glycosides (bound to a sugar molecule). Fermentation or digestion of soybeans or soy product results in release of the sugar molecule from the isoflavone glycoside, leaving an isoflavone aglycone.

Soy isoflavone glycosides are called genistin, daidzin, and glycitin, while the aglycones are called genistein, daidzein, and glycitein, subsequently. These aglycones are the compunds that are commonly reffered to as isoflavone compounds present in soy products. Structure of these three isoflavones can be seen in Figure 1.

Isoflavones are polyphenolic compound that are capable of exerting estrogen-like effects. For this reason, they are classified as phytoestrogens, i.e. compounds with estrogenic activity derived from plants (Lampe, 2003). Soybeans are the richest source of soy isoflavones in human diets. In soybeans, isoflavones exist in the entire plant including the seeds, leaves, stems, seedling and roots. In seeds which are mostly consumed, isoflavones have a high concentration around hypocotyls (Kudou *et al*., 1991). The biological effect of soy isoflavones is strongly affected by their metabolism, which is dependent on the activity of bacteria that colonize human intestine (Rowland *et al*., 2003). Although glycosides of isoflavones are readily hydrolyzed by intestinal bacteria enzymes, Setchell (1998) stated that little is known about the biological activity of individual isoflavones. It seems that glycoside isoflavones are hydrolyzed by intestinal bacteria to aglycones prior to being absorbed. However, not everyone has the same intestinal microflora ability to hydrolyze the glycosides. Therefore, it is important to analyse the individual forms of isoflavones instead of total isoflavones in foods, because foods vary widely in aglycone contents.

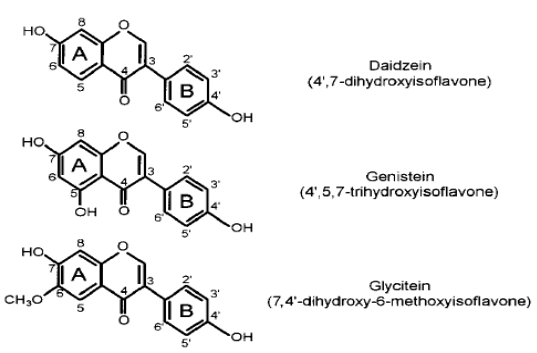

Figure 1. Structure of the primary isoflavones in soybeans (Messina, 1999).

Soy isoflavones are known to have weak estrogenic activity. Estrogens are signalling molecules that exert their effects by binding to estrogen receptors within cells. The estrogen-receptor complex interacts with DNA to change the expression of estrogen responsive genes. Estrogen receptors are present in numerous tissues other than those associated with reproductive system including bone, liver, heart and brain. Soy isoflavones can bind to estrogen receptors, taking

off the effects of estrogen in some tissues and blocking the effects of estrogens in others.

Soy isoflavones and their metabolites also have biological activities that are not related to their interactions with estrogen receptor (Barnes *et al*., 2000). By inhibiting the synthesis and activity of certain enzymes involved in estrogen metabolism, soy isoflavones may alter the biological activity of endogenous estrogens. In addition, soy isoflavones have been found to inhibit tyrosine kinases, enzymes that play critical roles in the signalling pathways that stimulate cell proliferation. One of the importan roles of estrogen is that it influences the CVD. The role of estrogen deficiency in CVD was further indicated that the risk of CVD in women taking this hormone was half reduced. Therefore, the isoflavones act as phytoestrogens can have a beneficial on CVD. Many authors have suggested that soy isoflavones may affect plasma lipid concentrations through their estrogenic action or affect the artery walls directly (i.e., maintaining normal vascular function or preventing smooth muscle cell migration and proliferation). However, several studies still showed inconsistent result of the effect of soy isoflavones on reduced CVD risks. First of all, it is important to determine the impact, if any, of soy isoflavones on plasma lipid parameters.

EFFECT OF ISOFLAVONES ON SERUM LIPID LEVELS

There are several studies conducted on animals as well as in humans to determine the effects of soy isoflavones by which they may modify the lipid profile. Balmir *et al*. (1996) carried out a study with casein-based diet to which isoflavones were added in rats. The protein sources were alcohol-washed soy protein isolate, intact protein isolate, casein, or casein to which a soy protein extracts rich in isoflavones was added. The result showed that rats fed with soy protein isolate and casein with isoflavones exhibited a decrease in plasma LDL-Cholesterol concentration in comparison with rats fed with alcohol washed soy protein isolate. This indicated that a minimal quantity of isoflavones may be necessary to obtained reduced LDL concentration. However, the extract obtained was composed of only 79% of isoflavones. The remaining 21% were unknown composition. Therefore, the beneficial effects of the extract can not be attributed with certainty to its isoflavone content because some other components present in intact soy protein isolate that may have an effect on plasma lipids. In addition, the isoflavones present in intact soybean protein isolate may increase the execration of biliary acids and promote the expression of the LDL receptor gene.

Yamakoshi *et al*. (2000) showed in rabbits, soy isoflavones may decrease atherosclerosis (variable combination of changes of the inside layer of the arterial wall) development by another mechanisms. Diet containing 1 g/100 g isoflavones aglycones had no effect on serum lipid profile. However, the atherosclerotic area of the aorta was lower in the high isoflavone group diet. The authors suggested that antioxidant action of isoflavones and their antioxidant metabolites soothed atherosclerotic development by inhibiting the oxidation of LDL. In another study performed in female hamster, alcohol washed soy protein isolate would prevent the total and non HDL. However, the protein source has no effect on total cholestrolemia, HDL, and non HDL, or on hepatic cholesterol concentration, suggesting that isoflavones or other non-protein components of soy may take part in function for cholesterol metabolism (Lucas *et al*., 2001).

A soy protein extract rich in isoflavones and alcohol washed soy protein isolates was used in the above studies to examine the effects of isoflavones on the lipid profile in animals. It is still impossible to conclude with confidence the effects observed are due only to isoflavones.

There were several studies also conducted in primates because they have similarities in lipid metabolism with that of humans. These studies revealed the role of protein component in hypocholesterolemic effect of soy protein isolate. Indeed, soy protein isolate had beneficial effects on plasma cholesterol concentrations even when compared with casein to which an alcohol extract of soy protein was added. It then can not be concluded that isoflavones do or do not have any independent contribution to the cholesterol lowering effect of soy (Wagner *et al*., 1997; Greaves *et al*., 1999). In addition, in monkeys large LDL are more atherogenic than small LDL. In humans, however, an increase in small, dense LDL concentrations is associated with higher risk of CVD.

Controlled clinical studies have been conducted to make clear the role of isoflavones in

the preclusion of CVD and above all their effect on serum lipid profile. It has been studied mostly in post-menopausal women. In the early studies, a soy protein isolates was often used as the source of isoflavones. Crouse *et al.* (1999) tried to see the effect of isolated soy protein with naturally occurring isoflavones on lipids and lipoprotein whether the cholesterol lowering effect of soy is due to the soy protein alone or to soy isoflavones in soy protein. The subjects 156 people with LDL-cholesterol ≥ 140 mg/dl serum were subjected to five diet intervention group: 25 g of casein, 25 g of isolated soy protein containing either 3, 27, 37, or 62 mg isoflavones. The results showed that isolated soy protein with 62 mg of naturally occurring isoflavones reduces total cholesterol and LDL-cholesterol levels more than isolated soy protein with trace amounts of isoflavones. Across the range of 3 to 62 mg isoflavones, the isolated soy protein with higher levels of naturally occurring isoflavones resulted in even greater cholesterol lowering. Isolated soy protein with naturally occurring isoflavones had a greater cholesterol lowering effect in individuals with higher baseline cholesterol levels.

Studies in Asian postmenopausal women with normal baseline cholesterol levels showed no significant effect of isoflavones on total, LDL, and HDL cholesterol concentrations at the end of the study in comparison with placebo or baseline values (Uesugi *et al*., 2002). It is possible that usual elevated consumption of isoflavones may have attenuated the ultimate effect of isoflavones on serum lipid in these studies. However, when 40 0r 80 mg of soy isoflavones were supplemented to Australian postmenopausal women in whom initial serum lipid was normal or slightly elevated, no effect was observed on serum lipid profile (Netsel *et al*., 1997). In their study, peri and postmenopausal women were treated with 80 mg isoflavones in a tablet or with a placebo pill and then these groups were switched to receive to other treatment. They found that the isoflavones pills had no effects on plasma lipid concentrations, although they did find a beneficial effect on systemic arterial compliance (a measure of elasticity of arteries).

On the contrary, it can be put forward that the impact of isoflavones on serum lipid profile maybe asserted in hypercholesterolemic than in normocholesterolemic subjects, as in the case for the effect for soy protein (Anderson *et al*., 1995). They showed that hypocholesterolemic effect of soy protein was more asserted in subjects with high baseline cholesterol concentrations. In the study of Lichtenstein *et al*. (2002), the hypolipidemic effect of soy protein in hypercholesterolemic subjects was independent from a possible effect of isoflavones. It can be put forward that similar to the effect of soy protein, the possible impact of isoflavones on serum lipids may be observed only in subjects with high baseline plasma cholesterol concentrations.

Therefore, it can be suggested that isoflavones might need to be given in the presence of soy protein in order to have their effects on plasma lipids. However, the finding of beneficial effect of the purified isoflvones pills on systemic arterial compliance might suggest that purified isoflavones do have some biologic effects.

EFFECT OF SOY ISOFLAVONES ON THE VASCULAR FUNCTION

The other possible effect of soy isoflavones to reduce the risk of CVD is their effect on the vascular function. There were several studies conducted to confirm this feature. Normal vascular function was maintained in premenopausal monkeys fed soy protein with isoflavones compared to a group fed isoflovone-devoid soy protein and genistein restored normal vascular function in the latter group (Honore *et al*., 1997). They reported that in females fed with low isoflavone diet, arteries constricted in response to acetycholine (an endothelium-dependent vascular response), whereas arteries from female fed a high isoflavone diet dilated. Moreover, genistein administered intravenously to animals fed to the low isoflavone diet resulted in dilation in previously constricted vessels. They concluded that dietary isoflavones enhance dilator response to acetylcholine of arteries in female monkeys.

In placebo-controlled, randomized, cross over study with 21 peri- and post-menopausal women, treatment for 5 weeks with 80 mg/day of purified soy isoflavones (45 mg genistein) improved systemic arterial compliance, and indicator of vascular elasticity by 26%, acetylcholine-mediation dilation in forearm similar in placebo groups, and no changes was observed in arterial pressure (Nestel *et al*., 1997). It then can be suggested that soy isoflavones significantly improved systemic arterial compliance in perimenopausal and menopausal women.

Another important mechanism by which soy isoflavones might improve CVD is the effect they have on platelets. Williams & Clarckson (1998) used 12 female non human primates. The results showed that a group fed soy protein isolate without the isoflavones had a 26% greater reduction in blood flow after collagen-induced platelet activation than did a group fed soy protein isolate with the isoflavones. The precise mechanism for this protection against reduction in blood flow by the isoflavones could not be determined in this study. The isoflavones might inhibit platelet activation and aggregation and reduce the amount of serotonin in the paltelets, all of which could contribute to a reduction in coronary vasopasm and thrombosis.

Anthony *et al*. (1997) tried to see the effect of isolated soy protein with or without isoflavones on atherosclerosis in male non human primates. The results showed that those in soy plus isoflavones with atherosclerotic plaques had smaller lesions, compared with other group (without isoflavones). They then concluded that beneficial effects of isolated soy protein on atherosclerosis appear to be mediated primarily by the isoflavone component and could not determine if the beneficial effects seen in soy without isoflavones were related to the protein itself or to the trace amounts of isoflavones remaining.

CONCLUSION

A number of components of soy have been studied to investigate their potential effect on CVD. Soy proteins have been shown to lower LDL-Cholesterol and to have neutral or slightly positive effect on HDL-Cholesterol. Greater cholesterol lowering effects were observed as the level of naturally occurring isoflavones in soy protein increased; however, extracted isoflavones (without soy protein) do not appear to lower cholesterol levels. Therefore it might be possible to say that isoflavones will be much effective in lowering LDL as applied together with soy protein.

The results obtained on the effects of isoflavones in humans and animals were not consistent. It is likely due to great variability in the design experiment. If isoflavones have any beneficial effects in humans, it is apparently of small amplitude in comparison with the effect of soy protein itself. Consumption of soy protein without isoflavones does not have the same benefits on cholesterol lowering as soy protein with isoflavones. Furthermore, the determination of individual isoflavone plasma concentrations should assist greatly in determination of the true impact of isoflavones on human lipid metabolism. Even though there is presently little evidence that isoflavones may improve the lipid profile on their own, they may have other benefical effects that may prevent CVD. Soy protein with isoflavones and/or soy isoflavones may potentially inhibit atherosclerosis; improve systemic arterial compliance and reactivity in arteries; have actions at steps in coagulation which may inhibit vascular lesions.

While there is quite convincing evidence that soybean with isoflavones have beneficial effects on CVD risk factors and atherosclerosis, there remain unanswered questions. First, uncertainty of what amount of soy protein and isoflavones is necessary to have maximal benefits and minimal risks. Second, what is the optimal combination of isoflavones, i.e. whether genistein the active isoflavone or the mixture of genistein, daidzein, and glycitein is more effective? Third, to what extend is the possibility of the unknown compounds to contribute to this beneficial effect. Still further research is needed to account these features.

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