Effects of soy isoflavones on atherosclerosis: potential mechanisms\textsuperscript{1–3}

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ABSTRACT It has long been recognized that coronary heart disease rates are lower in Japan, where soy consumption is common, than in Western countries. In experimental studies, atherosclerosis was reduced in animals fed diets containing soy protein compared with those fed diets with animal protein. Recently, several lines of evidence have suggested that the components of soy protein that lower lipid concentrations are extractable by alcohol (eg, the isoflavones genistein and daidzein). We recently evaluated the relative effect of the soy protein versus the alcohol-extractable components of soy on cardiovascular disease and its risk factors. Young male and female cynomolgus monkeys were fed diets that contained either 1) casein-lactalbumin as the source of protein (casein), 2) soy protein isolate from which the isoflavones were alcohol extracted (SPI–), or 3) isoflavone-intact soy protein (SPI+). The SPI+ group had significant improvements in LDL cholesterol and HDL cholesterol. Only HDL cholesterol was significantly improved in the SPI– group males compared with the casein group. The casein group had the most atherosclerosis, the SPI+ group had the least, and the SPI– group was intermediate but did not differ significantly from the casein group. Potential mechanisms by which soy isoflavones might prevent atherosclerosis include a beneficial effect on plasma lipid concentrations, antioxidant effects, antiproliferative and antimigratory effects on smooth muscle cells, effects on thrombus formation, and maintenance of normal vascular reactivity. 

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KEY WORDS Atherosclerosis, coronary heart disease, cynomolgus monkeys, daidzein, genistein, isoflavones, lipoproteins, soybeans, vasomotion, casein

INTRODUCTION Cross-cultural comparisons of coronary heart disease (CHD) mortality rates between the United States and Japan have shown striking differences (1). Age-adjusted CHD mortality rates are 6-fold lower in Japan than in the United States in men aged 40–69 y. Similarly, the death rates from CHD are \textasciitilde8-fold lower in Japan than in the United States in women aged 40–69 y. There are unquestionably many differences between US and Japanese diets other than soy products. There are also lifestyle differences that likely contribute to the differences in CHD rates. However, the data suggest the possibility that soy protein plays a role in reducing atherosclerosis.

Studies in animal models have provided evidence for a role of soy in the prevention of atherosclerosis development and subsequent CHD. As an example, the results of a study published by Huff et al (2) are shown in Figure 1. In this study, rabbits were fed a diet containing either soy protein or casein (an animal protein) for 10 mo. In both the aortic arch and thoracic aorta, the amount of atherosclerosis was significantly less in the group fed soy protein. Other studies of rabbits have confirmed that diets containing soy protein prevented the development of atherosclerosis (3, 4).

EVIDENCE THAT SOY ISOFLAVONES ARE THE ACTIVE COMPONENTS OF SOY Several studies have suggested that some components in soy other than the amino acid composition contribute to its antithrombotic effects. The results of another study done by Huff et al (5) are shown in Figure 2. Rabbits were fed diets that contained either casein or a mixture of amino acids that duplicated the amino acid composition of casein; plasma cholesterol concentrations of these 2 groups of rabbits were nearly the same. A different group of rabbits was fed intact soy protein, and a fourth group was fed a mixture of amino acids identical to the composition of the intact soy protein. The intact soy protein resulted in plasma cholesterol concentrations that were lower than those in rabbits fed the amino acid mixture. This result provided evidence that constituents of intact soy protein other than the amino acids further lowered plasma cholesterol. However, the evidence at this point does not eliminate the possibility that the cholesterol-lowering agent in soy could be a soy peptide acting alone or in combination with the isoflavones.

More recent studies also suggest that an alcohol-extractable component of soy protein lowers plasma cholesterol. Balmir et al (6) fed 1 group of rats an alcohol extract of soy protein in a casein-based diet and fed another group the identical diet with...
casein as the source of protein, but without the extract added. LDL cholesterol concentrations were significantly lower in the group fed the diet with the alcohol-extractable soy components added. Sugano and Koba (7) found that a methanol-extracted soy fraction was not as effective as the unextracted fraction in maintaining low plasma cholesterol concentrations in rats.

Our own work further supports the contention that soy isoflavones play a primary role in modulating plasma lipid and lipoprotein concentrations (8). In a crossover study in young rhesus monkeys, we fed diets containing soy protein isolate from which the isoflavones had been extracted with ethanol (SPI) or with the isoflavones intact (SPI+) (soy protein isolates were from Protein Technologies International, St Louis). We found that plasma LDL- and VLDL-cholesterol concentrations were significantly lower with the SPI+ diet in both males and females. Additionally, females fed the SPI+ diet had significantly higher HDL-cholesterol concentrations relative to concentrations observed when they were fed the SPI− diet.

FIGURE 1. Mean (± SE) effects of casein and soy protein on aortic atherosclerosis extent in rabbits. Adapted from reference 2.

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The finding described above prompted us to examine the relative contributions of the soy protein amino acids and the soy isoflavones and to extend the findings in the previous study to include atherosclerosis (9). We randomly assigned 85 male and 75 female cynomolgus monkeys (Macaca fascicularis) to 1 of 3 treatment groups: casein and lactalbumin as the source of protein (casein), the SPI− diet described above, or the SPI+ diet described above. All monkeys were fed the diets for 14 mo, during which time cardiovascular disease risk factors, including plasma lipids, were measured. A subset of 11 males from each of the 3 groups was then necropsied and evaluated for atherosclerosis.

FIGURE 2. Mean (± SE) effects of intact proteins and amino acid mixtures on total plasma cholesterol (TPC) in rabbits. Adapted from reference 5.

The diets were identical in the percentages of energy from protein (18.5%), fat (40.6%), and carbohydrate (40.9%), and they had the same amount of cholesterol (0.074 μg/J or 0.31 mg/kcal). There were no isoflavones in the casein diet, low amounts (equivalent to 16 mg per person per day) in the SPI− diet, and ~10-fold higher amounts of isoflavones (equivalent to 143 mg per person per day) in the SPI+ diet. These human equivalencies for isoflavone consumption were calculated assuming an average daily energy intake of 8368 kJ (2000 kcal) based on averages for Western populations.

Plasma lipoprotein concentrations are shown in Table 1. Because monkeys have relatively low plasma triacylglycerol concentrations, the LDL + VLDL cholesterol is almost entirely LDL. For both males and females, the group fed the SPI− diet had only slightly lower LDL + VLDL cholesterol concentrations compared with the casein group, but the group fed the SPI+ diet had LDL + VLDL cholesterol concentrations significantly lower than those in both the SPI− and casein groups. There were also beneficial effects on HDL cholesterol; the SPI+ group had higher HDL-cholesterol concentrations than the casein group and the SPI+ group had the highest concentrations.

Plasma lipoprotein results are shown schematically in Figures 3 and 4. SPI− and SPI+ group means are expressed as the percentage difference from the casein group means to evaluate the relative effects of the amino acid composition compared with alcohol-extractable components (eg, isoflavones). For both males and females, the LDL + VLDL cholesterol concentrations in the SPI− group were ~8% lower than in the casein group (Figure 3). In the SPI+ group, the males had LDL + VLDL concentrations ~40% lower than those in the casein group, and the females’ concentrations were ~30% lower (Figure 3). For both males and females,

### Table 1

<table>
<thead>
<tr>
<th>LDL + VLDL cholesterol (mmol/L)</th>
<th>Casein</th>
<th>SPI−</th>
<th>SPI+</th>
<th>p²</th>
<th>Casein vs SPI−</th>
<th>Casein vs SPI+</th>
<th>SPI− vs SPI+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>11.30 ± 0.62</td>
<td>10.42 ± 0.57</td>
<td>7.84 ± 0.59</td>
<td>0.67</td>
<td>0.0006</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>10.78 ± 0.62</td>
<td>10.01 ± 0.57</td>
<td>6.47 ± 0.57</td>
<td>0.74</td>
<td>&lt;0.0003</td>
<td>&lt;0.0003</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>1.09 ± 0.10</td>
<td>1.29 ± 0.08</td>
<td>1.60 ± 0.08</td>
<td>0.27</td>
<td>0.0003</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>0.98 ± 0.08</td>
<td>1.19 ± 0.08</td>
<td>1.50 ± 0.08</td>
<td>0.09</td>
<td>&lt;0.0003</td>
<td>0.006</td>
<td></td>
</tr>
</tbody>
</table>

¹± SEM. Means are adjusted for baseline variable. Data for the males are from reference 9.

² p values adjusted for multiple comparisons by Bonferroni method.
Effects of diets containing soy protein with some residual isoflavones (SPI−) or isoflavone-intact soy protein (SPI+) on LDL + VLDL cholesterol concentrations in female and male cynomolgus monkeys. The bars represent the average percentage increase (±SEM) in LDL+VLDL cholesterol concentrations compared with the group fed casein.

HDL cholesterol concentrations were ≈20% higher in the SPI− group than in the casein group; in the SPI+ group, HDL concentrations were ≈50% higher in both males and females compared with the casein group (Figure 4). These data suggest that alcohol-extractable components such as soy isoflavones contribute in a major way to the regulation of plasma lipid concentrations. We cannot be certain whether the effects seen in the SPI− group were due to the residual isoflavonoids in the isolate or the amino acids.

The effects of these 3 diets on the prevalence of coronary artery atherosclerosis (percentage of the groups that had atherosclerotic lesions) were evaluated. “Affected” cases were defined as those with atherosclerotic plaques (ie, lesions with plaque thickness greater than half the thickness of the media), whereas “protected” cases had no atherosclerotic plaques. Overall, 73% of the casein group, 64% of the SPI− group, and 45% of the SPI+ group were affected. We saw the same stepwise relation among groups for the extent of atherosclerosis (measured as plaque size in affected cases). The casein group had the most atherosclerosis (average plaque area = 0.13 mm2), the SPI+ group had the least (plaque area = 0.02 mm2), and the SPI− group was intermediate (plaque area = 0.06 mm2). The SPI+ group had significantly smaller lesions than either the casein group (P = 0.003) or the SPI− group (P = 0.05). Lesions in the SPI− group were about half the size of those in the casein group, but the difference was not significant (P = 0.16).

Atherosclerotic plaque sizes (expressed as the percentage difference compared with the casein group) are shown schematically in Figure 5. The SPI− group had plaque sizes ≈50% smaller than those of the casein group, and the SPI+ group had plaque sizes ≈85% smaller. Thus, these soy protein isolates with 2 different concentrations of isoflavones affected both the prevalence of atherosclerosis and plaque size. It is noteworthy that the soy protein isolate fed to the SPI− group was not devoid of isoflavones. Again, we are uncertain how much of the difference between the casein and SPI− groups was due to the residual isoflavonoids in the extracted soy protein and how much might have resulted from the different amino acid compositions.

### POTENTIAL MECHANISMS BY WHICH ISOFLAVONES MIGHT INHIBIT ATHEROSCLEROSIS DEVELOPMENT

There are multiple mechanisms by which the isoflavones in soy (primarily genistein and daidzein) might prevent the development of atherosclerosis and subsequent CHD. The isoflavones are structurally similar to estrogen and bind to the estrogen receptor, so it is biologically plausible that they protect against atherosclerosis development as estrogen agonists. Genistein is also a tyrosine kinase inhibitor, and some of the cardiovascular protection might be mediated by this mechanism. Some of the potential mechanisms have thus far only been shown in vitro, whereas others have been seen in vivo.

The first mechanism may be via alterations in liver metabolism (10), resulting in the improvements in plasma lipids. A second potential mechanism is that genistein has antioxidant properties (shown in cell culture in references 11 and 12). Kanazawa et al (13) reported that soy cream reduced the size of LDL particles and that soymilk protected LDL from being peroxidized. If genistein has the same effect in vivo, it may prevent the oxidation of LDL particles, making them less likely to be taken up by the artery wall and thus less atherogenic. A third potential mechanism relates to genistein’s ability to inhibit in vitro the migration and proliferation of smooth muscle cells (14–16), which are important in promotion and progression of the atherosclerotic process. Genistein may also suppress thrombus formation by inhibiting platelet activation (17, 18) and aggregation (19, 20) and reducing platelet serotonin uptake (21). Wilcox and Blumenthal (22) reviewed other potential effects of genistein on thrombotic mechanisms.
Genistein might also affect vascular reactivity (23–25), which is impaired in atherosclerotic arteries. Estrogen restores normal vasomotion rapidly, both in ovariectomized monkeys (26) and postmenopausal women (27). When we studied vascular reactivity in monkeys fed the SPI− diet, lumen diameter constricted by ≈6%. In contrast, monkeys fed the SPI+ diet had a 6% dilation in lumen diameter. Additionally, acute administration of genistein, injected 20 min before testing, restored vasodilation in the SPI− group (28). Genistein’s effect on vascular reactivity might affect atherosclerosis development and also might have implications for angina.

It remains to be seen how much of genistein’s effect on atherosclerosis is mediated through effects on plasma lipids and lipoproteins compared with direct effects on artery walls (i.e., maintaining normal vascular function or preventing smooth muscle cell migration and proliferation). If progress in this field continues at the rate seen in the past 2 y, remarkable strides should be made in understanding the mechanisms by which soy and its isoflavones benefit cardiovascular health. Research must also continue so that we can better understand the roles of genistein and daidzein in this process.

REFERENCES