Dietary Isoflavones Reduce Plasma Cholesterol and Atherosclerosis in C57BL/6 Mice but not LDL Receptor–Deficient Mice\textsuperscript{1,2}

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ABSTRACT Susceptibility to atherosclerosis is determined by a combination of genetic and environmental factors, including diet. Consumption of diets rich in soy protein has been claimed to protect against the development of atherosclerosis. Potential mechanisms include cholesterol lowering, inhibition of lipoprotein oxidation and inhibition of cell proliferation by soy proteins or isoavones, such as genistein, that are present in soy. This study was designed to determine whether soy isoavones confer protection against atherosclerosis in mice and whether they reduce serum cholesterol levels and lipoprotein oxidation. C57BL/6 and LDL receptor–deficient (LDLr-null) mice were fed soy protein–based, high fat diets with isoavones present (IF+, 20.85 g/100 g protein, 0.027 g/100 g genistein, 0.009 g/100 g daidzein) or diets from which isoavones, and possibly other components, had been extracted (IF−, 20.0 g/100 g protein, 0.002 g/100 g genistein, 0.001 g/100 g daidzein). Because LDLr-null mice develop extensive atherosclerosis and hypercholesterolemia after minimal time on a high fat diet, they were fed the diets for 6 wk, whereas C57BL/6 mice were fed the diets for 10 wk. Plasma cholesterol levels did not differ between LDLr-null mice fed IF− and those fed IF+, but were 30% lower in C57BL/6 mice fed the IF+ diet than in those fed the IF− diet. Susceptibility of LDL to oxidative modification, measured as the lag phase of conjugated diene formation in LDLr-null mice, was not altered by isoavone consumption. All LDLr-null mice developed atherosclerosis, and the presence or deficiency of dietary isoavones did not influence atherosclerotic lesion area. In contrast, atherosclerotic lesion area was significantly reduced in C57BL/6 mice fed IF+ compared with those fed IF−. Thus, this study demonstrates that although the isoavone-containing diet resulted in a reduction in cholesterol levels in C57BL/6 mice, it had no effect on cholesterol levels or on susceptibility of LDL to oxidative modification in LDLr-null mice. Further, dietary isoavones did not protect against the development of atherosclerosis in LDLr-null mice but did decrease atherosclerosis in C57BL/6 mice. These findings suggest that soy isoavones might lower cholesterol levels by increasing LDL receptor activity, and the reduction in cholesterol may offer some protection against atherosclerosis. J. Nutr. 128: 954–959, 1998.

KEY WORDS: • genistein • isoavone • mice • atherosclerosis

Soy has been postulated to decrease risk of atherosclerosis in several ways. Soy protein–based diets lower plasma cholesterol levels in animals and humans compared with casein-based diets (Anderson et al. 1995, Khosla et al. 1991). Soy has also been reported to have antioxidant properties (Kanazawa et al. 1993 and 1995, Wei et al. 1993 and 1996) that could protect against atherosclerosis by reducing LDL oxidation, believed to play a primary role in atherogenesis (Witzum and Steinberg 1991). Finally, soy protein consumption may exert numerous antiatherogenic effects at the level of the arterial wall, including inhibition of cytokine expression by macrophages, thought to contribute to both lesion initiation and progression (Geng et al. 1993).

It remains unclear what components of soy contribute to its protective affects. Several studies suggest that a component of soy proteins lowers LDL cholesterol levels by increasing the expression of LDL receptors (Khosla et al. 1991, Lovati et al. 1992, Sirtori et al. 1984). Other studies suggest that the cholesterol-lowering effects of soy can be attributed to soy isoavones, the most abundant of which are genistein, daidzein and glycitein. Soy isoavones have been shown to decrease total, VLDL and LDL cholesterol levels while increasing HDL.
cholesterol levels in peripubertal thessus monkeys fed soy protein–based diets with or without the isoflavone components (Anthony et al. 1996). Genistein alone has been shown to increase LDL receptor gene expression in Hep-G2 cells in the presence of hepatic growth factor (Kanuck and Ellsworth 1995). Genistein also is a potent antioxidant, scavenging both hydrogen peroxide and superoxide anion (Wei et al. 1993 and 1996). In addition, genistein has been shown to inhibit platelet activation (Nakashima et al. 1991, Ozaki et al. 1993) and growth factor activity (Hill et al. 1990). Inhibition of these processes may be attributable in part to the fact that genistein is a protein tyrosine kinase inhibitor (Akiyama et al. 1987). Tyrosine kinase activity is required for intracellular growth factor signaling, and growth factors such as platelet-derived growth factor are believed to play a role in cell migration and proliferation in atherosclerosis (Ross 1993). Thus, soy isoflavones, including genistein, may confer protection from increased susceptibility to atherosclerosis through a variety of mechanisms.

The purpose of this study was to determine whether soy isoflavones protect against the development of lesions in atherosclerosis-prone mice. The effects of soy isoflavones on atherosclerotic risk factors, plasma cholesterol levels and LDL oxidation were also determined. Specifically, we examined the effect of soy proteins with or without the presence of isoflavones extracted, on the development of fatty streak lesions in fat-fed C57BL/6 mice, a model for high LDL cholesterol. In preparing the diets, selenium and \( \alpha \)-tocopherol were omitted to maximize any potential antioxidant effects of genistein and daidzein. Because the two mouse strains differ only at the LDL receptor locus, this study tested directly the role of the LDL receptor in isoflavone-mediated antiatherosclerotic effects. We hypothesized that consumption of soy isoflavones would reduce plasma cholesterol and atherosclerosis in C57BL/6 mice and would reduce LDL oxidation in LDLr-null mice.

**MATERIALS AND METHODS**

**Animals.** Female C57BL/6 (C57BL/6) and female LDLr-null mice on a C57BL/6 background were obtained from the Jackson Laboratory, Bar Harbor, ME. Mice, age 7 to 9 wk, were fed a pelleted rodent nonpurified diet (Wayne Rodent BLOX 8604, Harlan Teklad, Madison, WI) for 2 wk before initiation of the diet studies. Mice were weighed weekly upon introduction of the experimental diets. Mice were maintained in a temperature controlled (25°C) facility with a strict 12-h light:dark cycle and were given free access to food and water. Food was removed from the mice 4 h before the collection of blood from the retroorbital sinus into tubes containing 4% EDTA. Plasma was stored at \(-70°C\) before analysis. Mice were killed by cervical dislocation. This project was approved by the Animal Care and Use Committee of the University of Washington (Protocol #2140-07).

**Diets and feeding.** Experimental diets were based on a diet developed by Nishina et al. (1990) (Table 1). The diet contained, by weight, 15% fat, 1.0% cholesterol and 0.5% sodium cholate. Soy protein isolates Supro 670 and Supro 670-IF were as follows: protein, 870.0 and 907.0 g/kg; fat, 40.0 and 70.0 g/kg; carbohydrate, 1.0 and 1.0 g/kg; moisture, 50.0 and 49.0 g/kg; and ash, 39.0 and 36.0 g/kg, respectively. Cocoa butter was obtained from Harlan Teklad (Madison, WI). Selenium-free mineral mix, \( \alpha \)-tocopherol–free corn oil, and \( \alpha \)-tocopherol–free vitamin mix were obtained from Harlan Teklad.

**TABLE 1**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>IF–</th>
<th>IF+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supro 670</td>
<td>0.0</td>
<td>208.5</td>
</tr>
<tr>
<td>Supro 670-IF</td>
<td>200.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Protein</td>
<td>181.4</td>
<td>181.4</td>
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<tr>
<td>Fat</td>
<td>1.4</td>
<td>8.4</td>
</tr>
<tr>
<td>Carbohydrate</td>
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<td>0.2</td>
</tr>
<tr>
<td>Moisture</td>
<td>9.8</td>
<td>10.4</td>
</tr>
<tr>
<td>Ash</td>
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<td>8.1</td>
</tr>
<tr>
<td>Sucrose</td>
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</tr>
<tr>
<td>Cocoa butter</td>
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<td>150.0</td>
</tr>
<tr>
<td>Corn oil</td>
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<td>10.0</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>6.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Mineral mix</td>
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<td>50.0</td>
</tr>
<tr>
<td>Vitamin mix</td>
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<td>10.0</td>
</tr>
<tr>
<td>Sodium cholate</td>
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<td>5.0</td>
</tr>
<tr>
<td>Choline chloride</td>
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<td>10.0</td>
</tr>
<tr>
<td>Alphacel</td>
<td>42.0</td>
<td>40.6</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>10.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

1 Soy protein isolates Supro 670 and Supro 670-IF were supplied by Protein Technologies International (St. Louis, MO). Compositions of Supro 670 and Supro 670-IF were as follows: protein, 870.0 and 907.0 g/kg; fat, 40.0 and 70.0 g/kg; carbohydrate, 1.0 and 1.0 g/kg; moisture, 50.0 and 49.0 g/kg; and ash, 39.0 and 36.0 g/kg, respectively.

2 Cocoa butter was obtained from Harlan Teklad (Madison, WI).

3 Selenium-free mineral mix, \( \alpha \)-tocopherol–free corn oil, and \( \alpha \)-tocopherol–free vitamin mix were obtained from Harlan Teklad.

plasma of LDLr-null mice by density gradient ultracentrifugation by single vertical spin (Chung et al. 1980). LDLr-null mouse plasma was weighed weekly upon introduction of the experimental diets. stored in air-tight containers at 70°C before analysis. Mice were killed by cervical dislocation. This project was approved by the Animal Care and Use Committee of the University of Washington (Protocol #2140-07).

**Experimental diets were based on a diet developed by Nishina et al. (1990) (Table 1). The diet contained, by weight, 15% fat, 1.0% cholesterol and 0.5% sodium cholate. Soy protein isolates Supro 670 and Supro 670-IF were a kind gift from E. C. Henley (Protein Technologies International, St. Louis, MO). The isoflavone-containing diet (IF+) was prepared using Supro 670 Isolated Soy Protein (Supro 670, Protein Technologies International), which contains 1.27 mg/g and 0.42 mg/g of genistein and daidzein, respectively. The lower isoflavone diet (IF–) was prepared using Supro 670-Isolavone-Free (Supro 670-IF) in which the concentration of genistein (0.121 mg/g) and daidzein (0.052 mg/g) were reduced after ethanol extraction. Other components potentially extracted with genistein and daidzein were not quantified. To correct for the resulting differences in protein content after extraction, 20.85 g/100 g of Supro 670 and 20.0 g/100 g of Supro 670-IF were used in diet preparation. These concentrations resulted in genistein and daidzein concentrations of 0.027 and 0.009 g/100 g in the IF+ diet, respectively, and 0.002 and 0.001 g/100 g in the IF– diet, respectively. Changes in fat content were corrected by using 1.7 g/100 g corn oil in the IF+ diet compared with 1.0 g/100 g in the IF– diet. To maximize the potential antioxidant effects of genistein and daidzein, other dietary antioxidants were omitted from the diets by the use of selenium-free mineral mix, vitamin mix without \( \alpha \)-tocopherol, and \( \alpha \)-tocopherol–strapped corn oil (Harlan Teklad). Diets were stored in air-tight containers at 20°C and fresh diet was provided daily. Animal acceptance of purified diets was improved by gradual introduction of the diet over a 4-d period as follows: 2 d of 50:50 nonpurified diet to purified diet, 2 d of 25:75 nonpurified diet to purified diet, followed by 100% purified diet as described (LeBoeuf et al. 1993).

Because LDLr-null mice develop extensive atherosclerosis and hypercholesterolemia after minimal time consuming a high fat diet (Ishibashi et al. 1994), they were fed the diets for 6 wk, whereas C57BL/6 mice were fed the diets for 10 wk. Plasma was collected before initiation of the diets and at 3 and 6 wk (LDLr-null mice) or 3, 6 and 10 wk (C57BL/6 mice).

**Plasma lipid and lipoprotein determination.** Plasma total and HDL cholesterol concentrations were determined using a colorimetric kit (Diagnostic kit no. 236691, Boehringer Mannheim, Indianapolis, IN) with cholesterol standards (Preciser #125512, Boehringer Mannheim). HDL cholesterol concentrations were determined after the selective precipitation of VLDL/LDL by 20% polyethylene glycol (Izzo et al. 1978).

**Conjugated diene formation.** The lag phase of conjugated diene formation was determined after isolation of LDL particles from the plasma of LDLr-null mice by density gradient ultracentrifugation by single vertical spin (Chung et al. 1980). LDLr-null mouse plasma (200 µL) was brought to a volume of 1.5 mL with 1.006 g/L NaCl and adjusted to a density of 1.21 kg/L with solid KBr. A density

**Abbreviations used:** IF+, isoflavone-containing diet; IF–, isoflavone-free diet; LDLr-null, LDL receptor–deficient.
gradient was formed in a Beckman OptiSeal centrifuge tube (Beckman Instruments, Fullerton, CA) by underlaying the adjusted plasma beneath 3.5 mL of a 1.006 kg/L NaCl solution. Samples were centrifuged at 4.16 × 10^5 g for 80 min at 7°C in a Beckman NVT 65.2 rotor. The top 1.0 mL was sliced from the tube, and LDL were collected in the next 1.5 mL. LDL were then passed over S300 Sephadex columns with PBS to remove EDTA and to desalt. LDL oxidation was initiated by the addition of CuSO_4 (5 μmol/L final concentration) to a spectrophotometer cuvette containing 100 g cholesterol/L in PBS. The appearance of conjugated dienes was monitored at 234 nm at 37°C every 5 min for 8 h. Three characteristic phases (lag, propagation and decomposition) were observed from which the lag time was calculated (Esterbauer et al. 1989) as described previously (Chait et al. 1993).

**Aortic sinus lesion area.** Atherosclerotic fatty streak lesions were quantified by evaluation of lesion size in the aortic sinus as described (Paigen et al. 1987), with modifications (Kunjathoor et al. 1996). Briefly, the heart and proximal aorta were removed from the mice, cleaned of peripheral fat under a dissecting microscope and sectioned directly under and parallel to the atrial leaflets. The upper section was embedded in OCT (optimal cutting temperature) medium (Miles, Elkhart, IN) and frozen. Every other section (10-μm thick) throughout the aortic sinus (400 μm) was taken for analysis. The distal portion of the aortic sinus is recognized by the three valve cusps that are the junctions of the aorta to the heart. Sections were stained with oil red O (Sigma, St. Louis, MO) and counterstained with Harris' hematoxylin (Sigma) according to a modification of the method of Kruth (1984). Briefly, sections were rinsed in 13.1 mol/L propylene glycol for 4 min before immersion (1 h) in propylene glycol containing 0.01 mol/L oil red O. Sections were then washed sequentially in 11.2 mol/L propylene glycol, 6.6 mol/L propylene glycol and water before immersion in Harris' hematoxylin (1 min). Sections were then rinsed in 0.14 mol/L ammonium hydroxide followed by a final rinse in tap water.

Lesion areas per section were counted using a Compaq 386 computer (Compaq Computers, Houston, TX) equipped with an FG-100 image acquisition board, a high resolution video camera and a Sony video monitor. Area measurements were done with the use of the Optimas Image Analysis software package (Bioscan Optimas, Version 5.2, Bioscan, Edmonds, WA).

**Statistical analysis.** Data are reported as means ± SEM. Statistical differences were determined by ANOVA using SYSTAT for the Macintosh (Version 5.2, SYSTAT, Evanston, IL). Within strain differences were detected using two-way ANOVA (diet × time). Three-way ANOVA was used to analyze total cholesterol data (diet × time × strain) up to the 6-wk time point. Post-hoc analyses of significance were made using Tukey's test for additivity. Student’s t test was used to compare independent means. P < 0.05 was accepted as significant.

**RESULTS**

**Weight gain of mice.** Initial and final weights did not differ between mice fed IF− and IF+ in either strain. However, final weights were significantly greater than initial weights in both strains. Initial and final weights for LDLr-null mice were 19.9 ± 0.3 and 24.0 ± 0.6 g, respectively (P < 0.001). Initial and final weights for C57BL/6 mice were 20.0 ± 0.2 and 23.5 ± 0.4 g, respectively (P < 0.001).

**Genetic and dietary regulation of plasma lipids and lipoproteins.** Total cholesterol levels for C57BL/6 mice fed either the IF− or the IF+ diet were similar initially and after 3 wk of consuming the diets (Fig. 1). After 3 wk, total cholesterol levels decreased markedly for mice fed the IF+ diet and were significantly lower at both 6 and 10 wk (P < 0.001 and P

![FIGURE 1 Plasma cholesterol concentrations in response to isoflavone-free (IF−) or isoflavone-containing (IF+) diets of C57BL/6 mice fed the diets for 10 wk. Plasma total cholesterol (TC), VLDL/LDL cholesterol (VLDL/LDL), and HDL cholesterol (HDL) were determined at 0, 3, 6 and 10 wk as described in Materials and Methods. Each point represents the mean ± SEM, n = 20. In comparing diet effects, * indicates a significance level of P < 0.001; † indicates a significance level of P < 0.0001. Time effects are described in the text.](https://academic.oup.com/jn/article-abstract/128/6/954/4722385)
TABLE 2

Plasma total cholesterol concentrations of LDL receptor-deficient (LDLr-null) mice fed isoflavone-free (IF−) and isoflavone-containing (IF+) diets for 0, 3 and 6 weeks

<table>
<thead>
<tr>
<th>Time</th>
<th>IF−</th>
<th>IF+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol/L</td>
<td></td>
</tr>
<tr>
<td>0 wk</td>
<td>6.5 ± 0.7</td>
<td>6.3 ± 1.0</td>
</tr>
<tr>
<td>3 wk</td>
<td>75.4 ± 4.9</td>
<td>75.6 ± 4.4</td>
</tr>
<tr>
<td>6 wk</td>
<td>105.1 ± 7.2</td>
<td>93.4 ± 7.6</td>
</tr>
</tbody>
</table>

< 0.0001, respectively) than at 3 wk. This decrease was mirrored by the VLDL/LDL cholesterol levels (Fig. 1). HDL cholesterol levels remained relatively constant and were not different between the mice fed IF− and IF+ for the 10 wk (Fig. 1). Thus, the decrease in total cholesterol levels in C57BL/6 mice fed the IF+ diet was attributable primarily to a decrease in the VLDL/LDL fraction.

Plasma cholesterol levels of LDLr-null mice were significantly greater than those of C57BL/6 mice at times 0, 3 and 6 wk (P < 0.0001), consistent with the known role of the LDL receptor in clearance of plasma cholesterol. VLDL/LDL and HDL cholesterol levels were not determined for LDLr-null mice because of the limited volume of plasma available. Total cholesterol levels of LDLr-null mice fed the IF− and IF+ diets were not significantly different at any time (Table 2). After 3 wk, total cholesterol levels increased almost 12-fold; after 6 wk, the increase was ~15-fold compared with time 0 levels in mice fed either diet. Thus, the lack of LDL receptor-mediated clearance of lipoproteins in LDLr-null mice in combination with the diets resulted in profound increases in total cholesterol.

Oxidative susceptibility of LDL. Lag phase of formation of conjugated dienes by isolated LDL particles was measured only in LDLr-null mice because not enough substrate for the lag phase determination was obtained from C57BL/6 mice. After mice had consumed the diets for 3 wk, the lag phase of conjugated diene formation did not differ in LDLr-null mice fed either IF− or IF+ (Table 3). After 6 wk, the lag phase decreased significantly from the 3-wk rates (P < 0.0001) to similar rates in LDL from mice fed either diet. Thus, isoflavone consumption did not alter oxidative susceptibility of LDL particles in LDLr-null mice.

Fatty streak lesions. LDLr-null mice developed lesions ~100-fold greater than those of C57BL/6 mice regardless of diet (Fig. 2). Dietary isoflavones did not induce a significant reduction in lesion area in LDLr-null mice. However, isoflavone consumption in C57BL/6 mice induced a significant, 50% decrease in atherosclerotic lesion size (Fig. 2B, P < 0.05). In fact, 9 of the 20 mice fed the IF+ diet did not develop any atherosclerosis. Thus, although isoflavone consumption in LDLr-null mice did not alter atherosclerotic lesion size, atherosclerotic lesion size in C57BL/6 fed the IF+ diet was significantly reduced compared with lesion size in C57BL/6 mice fed the IF− diet.

DISCUSSION

This study was designed to determine whether the isoflavones in soy protein isolate confer protection from atherosclerosis, reduce total plasma cholesterol levels and protect against lipoprotein oxidation in atherosclerosis-susceptible mice. Although isoflavone consumption did not alter atherosclerosis, plasma cholesterol levels or oxidative susceptibility in LDLr-null mice, it did lead to a 30% decrease in plasma cholesterol levels and a 50% reduction in atherosclerotic lesion area in C57BL/6 mice. Because the diets were formulated to be different only in isoflavone content, we conclude that these results are attributable to soy isoflavones. Still, we cannot exclude the possibility that other biologically active compounds that were co-extracted with the isoflavones may have contributed to the effects. However, in a similar study feeding soy protein isolates from the same source, Anthony et al. (1996) argued that soy saponins are the only other ethanol-extractable substances that may affect plasma lipid levels, and those effects are negated in the presence of soy protein (Pathirana et al.

FIGURE 2 Aortic sinus lesion areas for LDL receptor-deficient (LDLr-null) and C57BL/6 mice fed for 6 or 10 wk, respectively, soy-based diets with isoflavones (IF+) or with the isoflavones extracted (IF−). Each open circle represents an individual animal. Mean values are indicated by the horizontal lines. For LDLr-null mice (Panel A), the mean values for the mice fed IF− and IF+ were 360 ± 94 and 295 ± 36 mm², respectively. For C57BL/6 mice (Panel B) the mean values for the mice fed IF− and IF+ were 6.9 ± 1.6 and 3.1 ± 1.0 mm², respectively. Note the difference in scale between Panels A and B. Hearts were collected, sectioned and stained with oil red O for quantification of aortic sinus area lesions as described in Materials and Methods.
1981). Furthermore, these authors asserted that the extensive processing of the soy protein isolate before alcohol extraction resulted in the extracted and unextracted proteins being similar for all components other than isoflavone content (Anthony et al. 1996).

Rats consuming selenium- and vitamin E-deficient diets with or without cholesterol added became hypercholesterolemic (Mazur et al. 1996, Stone et al. 1994). In preparing the diets for this study, selenium and α-tocopherol were omitted to maximize any potential antioxidant affects of genistein and daidzein. The cholesterol levels obtained after feeding these diets were within the usual ranges of cholesterol for C57BL/6 (Lusis et al. 1987, Qiao et al. 1994) and LDLr-null mice (Ishibash et al. 1994, Masucci-Magoulas et al. 1996) fed similar diets containing selenium and vitamin E. Any cholesterol-elevating effects resulting from selenium and vitamin E depletion should be the same for all mice studied. Nonetheless, it is possible that different results might have been obtained by using diets that were not deficient in selenium and vitamin E.

Soy protein consumption is known to reduce cholesterol absorption and thereby decrease plasma cholesterol levels in swine and rats (Beynen et al. 1990, Nagaoka et al. 1997). Although consumption of soy protein may have inhibited cholesterol absorption in this study, C57BL/6 mice that consumed the IF+ diet had reduced plasma cholesterol compared with C57BL/6 mice fed the IF− diet. This suggests that soy isoflavones either compounded the inhibition of cholesterol absorption or reduced cholesterol levels through another mechanism. LDLr-null mice that consumed the IF+ diet showed no reduction in plasma cholesterol levels compared with mice fed the IF− diet. Because the only difference in C57BL/6 mice and the LDLr-null mice was the LDL receptor deficiency, these findings suggest that LDL receptor-mediated cholesterol clearance is one mechanism by which total plasma cholesterol was lowered in the C57BL/6 mice that consumed the IF+ diet. This conclusion is in agreement with studies that have shown an increase in LDL receptor-mediated cholesterol removal in both humans and rabbits fed soy protein–based diets (Khosla et al. 1991, Lovat et al. 1992).

Isoflavones have antioxidant properties including scavenging of hydrogen peroxide and superoxide anion (Wei et al. 1993 and 1996). Our results showed no difference in susceptibility of LDL to oxidative modification in LDLr-null mice fed either the IF− or IF+ diet for 3 and 6 wk. LDLr-null mice fed an atherogenic diet (21 g/100 g fat, 0.15 g/100 g cholesterol) for 6 mo were found to have autoantibody titers against malondialdehyde-lysine, an epitope formed during the oxidative modification of lipoproteins, that increased over the time of diet consumption (Palinski et al. 1995). This is reflective of increasing oxidative modification of LDL with time and helps to explain our results. That is, as the mice became increasingly hypercholesterolemic with time consuming the diet, the LDL became increasingly susceptible to oxidation regardless of the presence or absence of isoflavones in the diet.

Another explanation for the lack of effect of the IF+ diet in modifying susceptibility of LDL to oxidation in LDLr-null mice might be that the isoflavones were either not present in a high enough concentration to provide an antioxidant effect or that they were not physically interacting with LDL to provide antioxidant protection. Although isoflavones are found associated with proteins in soy, there is currently no evidence that isoflavones are associated with lipoproteins in mammals. Because they are present as water-soluble glucuronide conjugates, isoflavones are likely to be removed during isolation of the LDL for the oxidation studies. Thus the lack of effect of isoflavone consumption on the length of the lag phase of conjugated diene formation is perhaps not surprising. Therefore, if isoflavones are exerting an antioxidant effect, they are likely to be doing so in the aqueous milieu of the lipoprotein, in a manner similar to vitamin C and uric acid.

A recent meta-analysis of human studies showed that consumption of soy protein was associated with a reduction in plasma cholesterol levels, although it was not possible to determine whether these effects could be attributed to the isoflavones in the soy protein–rich diets (Anderson et al. 1995). It has been demonstrated that soy protein–based diets that included soy isoflavones decreased total cholesterol in rhesus monkeys compared with soy protein–based diets from which the isoflavones had been extracted (Anthony et al. 1996). Our results in C57BL/6 mice agree with the latter findings. Total cholesterol was reduced 30% in these mice as a result of isoflavone consumption. In contrast, isoflavone-consuming LDLr-null mice showed no reduction in total cholesterol compared with those not consuming isoflavones. These findings are consistent with a cholesterol-lowering effect of isoflavones mediated primarily through increased LDL receptor activity. Further, isoflavone consumption reduced atherosclerotic lesion size by 50% in C57BL/6 mice but did not alter lesion size in LDLr-null mice. The reduced atherosclerosis and cholesterol-lowering effect observed in C57BL/6 mice, coupled with previous studies in other animal models and humans, suggests that increased isoflavone consumption may have long-term benefits in reducing both atherosclerosis and atherosclerotic risk.

ACKNOWLEDGMENT

We are grateful to E. C. Henley of Protein Technologies International, St. Louis, MO, who kindly provided the soy protein isolates.

LITERATURE CITED


DIETARY ISOFLAVONES REDUCE ATHEROSCLEROSIS IN MICE

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