Plasma antioxidant capacity in response to diets high in soy or animal protein with or without isoflavones¹-⁴

Sonia Vega-López, Kyung-Jin Yeum, Jaime L Lecker, Lynne M Ausman, Elizabeth J Johnson, Sridevi Devaraj, Ishwarlal Jialal, and Alice H Lichtenstein

ABSTRACT

Background: Several clinical trials have suggested that soy intake decreases oxidative stress. Soy isoflavones have antioxidant properties in vitro, but results of supplementation in clinical trials are inconclusive.

Objective: The objective was to evaluate the independent effects of soy protein and soy-derived isoflavones on plasma antioxidant capacity and biomarkers of oxidative stress.

Design: Forty-two hypercholesterolemic (LDL cholesterol > 3.36 mmol/L) subjects aged ≥50 y were provided with each of 4 diets in random order in a crossover design. Diets varied in protein source (10% of energy, soy or animal) and isoflavone content (trace or 50 mg/1000 kcal) and were consumed for 42 d each. Plasma antioxidants, protein carbonyls, malondialdehyde, total antioxidant performance, LDL oxidizability, and urinary F₂-isoprostanes were measured at the end of each dietary phase.

Results: Plasma antioxidant concentrations were not significantly different, regardless of dietary treatment, except for isoflavones, which were higher after isoflavone supplementation (P = 0.0001). Although plasma total antioxidant performance was 10% higher with soy protein intake, regardless of dietary isoflavones (P = 0.0003), soy protein did not significantly affect most individual markers of oxidative stress (LDL oxidizability, urinary F₂-isoprostanes, malondialdehyde, or protein carbonyls in native plasma). However, soy protein was associated with modestly lower concentrations of protein carbonyls in oxidized plasma. There was no significant effect of isoflavones on LDL oxidation, urinary F₂-isoprostanes, or protein carbonyl groups, although, paradoxically, the plasma malondialdehyde concentration was significantly higher after the isoflavone-rich diets (P = 0.04).

Conclusions: Diets relatively high in soy protein or soy-derived isoflavones have little effect on plasma antioxidant capacity and biomarkers of oxidative stress.


KEY WORDS Animal protein, antioxidants, antioxidant capacity, cardiovascular disease, isoflavones, oxidative stress, soy protein

INTRODUCTION

Some epidemiologic data suggest that diets relatively high in soy protein are associated with a decreased relative risk of cardiovascular disease (CVD) and nonfatal myocardial infarction (1) and with lower total and LDL-cholesterol concentrations (2, 3). Early work has suggested a hypocholesterolemic effect of soy products (4), which in 1999 led to the authorization of a health claim for the cholesterol-lowering potential of soy products (5, 6). However, recent studies evaluating the lipid responses to soy consumption have only shown a modest (7–13) or no hypocholesterolemic (14–16) effect.

Several studies have suggested that soy intake aids against oxidative stress, as indicated on the basis of measurements of conjugated dienes in the LDL fraction (17, 18), lag time for copper-induced LDL oxidation (19–21), and plasma concentrations of F₂-isoprostanes (19). However, results from other studies failed to support the antioxidant effects of soy (16, 22). The potential decrease in oxidative stress has been ascribed to the isoflavone component of the soybeans. Soybeans and products derived from soybeans represent the major source of dietary isoflavones (23). Compared with the major antioxidants in plasma (ascorbic acid, uric acid, α-tocopherol, β-carotene, and other carotenoids) (24, 25), the concentration of isoflavones in plasma is relatively low and can reach concentrations comparable with those of carotenoids [1 μmol/L (26), only after the consumption of meals containing soy products high in isoflavones or isoflavone supplements (8, 19, 26–30).

The 2 major isoflavones in soybeans are genistein and daidzein. Kerry and Abbey (31) reported that genistein inhibits copper- and perox radical–mediated LDL oxidation when added to a cell-free oxidation system but not when incorporated into LDL particles before the oxidation reaction. Kapiotis et al...
(32) also reported that genistein, but not daidzein, inhibits copper-mediated LDL oxidation, as shown by the inhibition of thiobarbituric acid–reactive substances and the formation of hydroperoxides in cell-free systems. In contrast with in vitro studies, data from interventional studies have failed to show an antioxidant effect from isoflavone supplements (33–35). One possibility for the discordant observations to date is that there is a potential independent effect of soy protein, soy-derived isoflavones, or the synergistic effect of both. This possibility has not been adequately addressed.

The aim of this study was to evaluate the effects of soy protein and soy-derived isoflavones, alone or in combination, on plasma antioxidant capacity and a wide range of biomarkers of oxidative stress in older adults with moderately elevated LDL-cholesterol concentrations.

SUBJECTS AND METHODS

Study protocol

Eighteen men and 24 postmenopausal women aged ≥50 y with LDL-cholesterol concentrations >3.36 mmol/L, but otherwise apparently healthy, were recruited from the greater Boston area. Participants were assigned to a sequence of four 42-d dietary periods: soy protein depleted of isoflavones (soy/−), soy protein enriched in isoflavones (soy/+), animal protein with no added isoflavones (animal/−), or animal protein with added isoflavones (animal/+ ) in a crossover design. All participants received each of the 4 diets in random order. The study protocol and diets were described in detail elsewhere (13). Baseline characteristics of the subjects are shown in Table 1. Written consent was obtained from all study volunteers. The study protocol was approved by the Human Investigation Review Committee of New England Medical Center and Tufts University.

All foods and beverages were provided to the study participants and caloric intakes were adjusted to maintain a stable body weight throughout the study. Diets were designed to have similar fatty acid profiles and contents of total fat, carbohydrate, protein, fiber, and cholesterol, which was confirmed by chemical analysis (Table 2). In diets containing isolated soy protein, 25 g/1000 kcal replaced common sources of animal protein from the diet. Diets containing isoflavones had 50 mg/1000 kcal, derived from the isolated soy protein preparation or added to the diet high in animal protein. The diet content of carotenoids, tocopherols, and retinol was calculated by using the Nutrition Data System for Research (NDS-R) software (version 4.04/32), which was developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis (36).

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Soy/−</th>
<th>Soy/+</th>
<th>Animal/−</th>
<th>Animal/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>45</td>
<td>46</td>
<td>47</td>
<td>48</td>
</tr>
<tr>
<td>Total isoflavones (mg/1000 kcal)</td>
<td>4.99</td>
<td>4.99</td>
<td>3.91</td>
<td>3.91</td>
</tr>
<tr>
<td>Retinol (µg/1000 kcal)</td>
<td>172</td>
<td>172</td>
<td>202</td>
<td>202</td>
</tr>
<tr>
<td>總olesterol (mg/1000 kcal)</td>
<td>125</td>
<td>46.21</td>
<td>ND</td>
<td>51.71</td>
</tr>
<tr>
<td>Genistein (mg/1000 kcal)</td>
<td>0.37</td>
<td>27.17</td>
<td>ND</td>
<td>26.80</td>
</tr>
<tr>
<td>Daidzein (mg/1000 kcal)</td>
<td>0.84</td>
<td>13.89</td>
<td>ND</td>
<td>20.68</td>
</tr>
<tr>
<td>Glycitein (mg/1000 kcal)</td>
<td>ND</td>
<td>5.16</td>
<td>ND</td>
<td>4.23</td>
</tr>
</tbody>
</table>

*Table 1 Composition of the experimental diets*

<table>
<thead>
<tr>
<th>Constituent</th>
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</tr>
</tbody>
</table>

*Table 2 Composition of the experimental diets*
SOY PROTEIN, ISOFLAVONES, AND ANTIOXIDANT CAPACITY

Wilmington, NC), and a Waters Millennium 32 data station. The mobile phase was methanol:methyl-terbutyl ether:water (85:12:3, by vol, with 1% ammonium acetate in the water; solvent A) and methanol:methyl-terbutyl ether:water (8:90:2, by vol, with 1% ammonium acetate in the water; solvent B). The gradient procedure was described elsewhere (26). The results were adjusted by an internal standard containing echinenone and retinyl acetate. Plasma genistein and daidzein were measured by time-resolved fluoroimmunoassay with the use of a validated method in the laboratory of Adlercreutz (37).

Markers of oxidative stress

Plasma total antioxidant performance (TAP) was determined fluorometrically with a 1420-multilabel counter (Wallac Victor 2; Perkin-Elmer Life Sciences, Boston) as described by Aldini et al (25) with minor modifications. This method measures the rate of oxidation of 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid (BODIPY 581/591), a lipid-soluble fluorescent probe, and uses the lipid-soluble radical initiator 2,2'-azobis(4-methoxy-2,4-dimethylvaleronitrile) (MeO-AMVN). Oxidation was determined by monitoring the appearance of green fluorescence of the oxidation product of BODIPY (λₜₜ = 500 nm, λₑₜ = 520 nm). The results are expressed as butyl hydroxytoluene equivalents.

LDL oxidizability was measured by monitoring the formation of conjugated dienes catalyzed by incubation with Cu²⁺ after the isolation of LDL from plasma by sequential ultracentrifugation. The kinetics of conjugated dienes formation was assessed by continuous absorbance monitoring at 234 nm as described earlier (38).

F₂-Isoprostanes were measured in urine with the use of a previously described enzyme immunoassay (EIA) method (Cayman Chemicals, Ann Arbor, MI) (39). This EIA method was validated against a gas chromatography–mass spectrometry method (n = 68, R = 0.80) (39). F₂-Isoprostanes were standardized by urinary creatinine measured by standard techniques.

Plasma protein carbonyls were measured with a modification of the enzyme-linked immunosorbent assay method as described by Marangon et al (40) before and after a 4-h incubation at 37 °C with 100 mmol/L of the aqueous free radical initiator 2,2'-azobis(2-amidinopropane) hydrochloride (AAPH). Lipid peroxidation was assessed by measuring malondialdehyde with an HPLC system with a fluorescence detector, as previously described (41).

In vitro assessment of antioxidant capacity of purified isoflavones

The antioxidant capacity of the purified isoflavones added to the animal protein–based diet (Archer Daniels Midland Company, Decatur, IL) was tested in vitro with the TAP method described above. Plasma was incubated with isoflavones (0, 0.1, and 1 μmol/L) in the presence and absence of α-tocopherol (1 and 10 μmol/L).

Statistical analyses

Before the statistical analysis was conducted, variables with a skewed distribution (trans β-carotene, lutein, lycopene, and α-tocopherol) were log transformed to achieve normality before analysis. Soy/−, soy protein depleted of isoflavones; soy/+., soy protein enriched in isoflavones; animal/−, animal protein with no added isoflavones; animal/+, animal protein with added isoflavones.

A repeated-measures ANOVA was used to detect differences.

#p value

<table>
<thead>
<tr>
<th>Variable</th>
<th>Soy/−</th>
<th>Soy/+</th>
<th>Animal/−</th>
<th>Animal/+</th>
<th>Protein</th>
<th>Isoflavones</th>
<th>Protein × isoflavones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genistein (nmol/L)</td>
<td>50 ± 37¹</td>
<td>617 ± 498</td>
<td>7 ± 12</td>
<td>695 ± 443</td>
<td>0.5269</td>
<td>0.0001</td>
<td>0.2736</td>
</tr>
<tr>
<td>Daidzein (nmol/L)</td>
<td>15 ± 9</td>
<td>139 ± 91</td>
<td>3 ± 5</td>
<td>275 ± 211</td>
<td>0.0050</td>
<td>0.0001</td>
<td>0.0030</td>
</tr>
<tr>
<td>trans β-Carotene (µmol/L)</td>
<td>1.04 ± 0.89</td>
<td>1.12 ± 0.77</td>
<td>1.19 ± 1.02</td>
<td>1.13 ± 1.03</td>
<td>0.6910</td>
<td>0.9529</td>
<td>0.0130</td>
</tr>
<tr>
<td>Lutein (µmol/L)</td>
<td>0.30 ± 0.14</td>
<td>0.24 ± 0.11</td>
<td>0.29 ± 0.16</td>
<td>0.30 ± 0.17</td>
<td>0.1157</td>
<td>0.0004</td>
<td>0.0118</td>
</tr>
<tr>
<td>Lycopene (µmol/L)</td>
<td>1.60 ± 0.74</td>
<td>1.45 ± 0.66</td>
<td>1.62 ± 0.71</td>
<td>1.50 ± 0.61</td>
<td>0.4150</td>
<td>0.0475</td>
<td>0.8904</td>
</tr>
<tr>
<td>Retinol (µmol/L)</td>
<td>2.17 ± 0.53</td>
<td>2.19 ± 0.51</td>
<td>2.20 ± 0.46</td>
<td>2.21 ± 0.59</td>
<td>0.6074</td>
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<td>0.9502</td>
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<tr>
<td>α-Tocopherol (µmol/L)</td>
<td>35.5 ± 10.3</td>
<td>34.3 ± 11.2</td>
<td>36.7 ± 10.3</td>
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<tr>
<td>γ-Tocopherol (µmol/L)</td>
<td>5.30 ± 2.08</td>
<td>4.91 ± 1.76</td>
<td>5.06 ± 2.14</td>
<td>4.89 ± 1.41</td>
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¹ n = 42. Variables with a skewed distribution (trans β-carotene, lutein, lycopene, and α-tocopherol) were log transformed to achieve normality before analysis. Soy/−, soy protein depleted of isoflavones; soy/+., soy protein enriched in isoflavones; animal/−, animal protein with no added isoflavones; animal/+, animal protein with added isoflavones.

² A repeated-measures ANOVA was used to detect differences.

³ x ± SD (all such values).

RESULTS

Plasma antioxidants

As by design, plasma genistein and daidzein were significantly higher during the isoflavone-supplemented periods (Table 3), which reflects compliance with the intervention regimen. Likewise, by design, plasma concentrations of trans β-carotene, lutein, lycopene, retinol, α-tocopherol, and γ-tocopherol were relatively high and, although statistically significant differences were identified between the study phases, the magnitude of the

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Table 3

Fasting plasma antioxidant concentrations at the end of each dietary phase. The kinetics of conjugated dienes formation was assessed by isolation of LDL from plasma by sequential ultracentrifugation.

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² A repeated-measures ANOVA was used to detect differences.

³ x ± SD (all such values).
differences would not be predicted to have a physiologically significant effect. Standardization of plasma antioxidants on the basis of total cholesterol concentrations did not significantly alter the results (data not shown).

Plasma total antioxidant performance

As shown in Figure 1, plasma TAP was modestly higher (10%) at the end of the soy-protein phases than during the animal protein phases, regardless of the isoflavone content of the diet.

Biomarkers of oxidative stress

The different biomarkers of oxidative stress at the end of each dietary phase are depicted in Table 4. There were no significant effects of dietary protein or isoflavones on the susceptibility of LDL to oxidation measured by detection of conjugated diene formation (lag time and oxidation rate) or on in vivo lipid peroxidation, assessed by measuring urinary F2-isoprostanes. There was no significant effect of protein source or isoflavone intake on native protein carbonyl groups. However, animal protein intake was associated with a significantly higher concentration of protein carbonyl groups after incubation of plasma with the radical initiator AAPH. Plasma malondialdehyde concentration, a marker of lipid peroxidation, was 10% higher after the isoflavone-supplemented periods.

**In vitro assessment of TAP of isolated isoflavones**

Incubation of plasma with isoflavones before the TAP analysis at concentrations of 0.1, 0.5, 1.0, and 10 μmol/L resulted in an increase in TAP of 7%, 8%, 10%, and 11%, respectively (P > 0.05 for all). Incubation of plasma with α-tocopherol at 1.0 and 10 μmol/L resulted in an increase in TAP of 10% and 17%, respectively (P > 0.05 for both). Further addition of isoflavones (0.1 and 1 μmol/L) to the 1 μmol/L α-tocopherol system provided an additional increase in TAP of 14% (P = 0.03) and 24% (P = 0.01), respectively. In the presence of 10 μmol α-tocopherol/L, TAP was further increased by 21% (P = 0.0002) and 26% (P = 0.001) when coincubated with 0.1 and 1 μmol isoflavones/L, respectively.

**DISCUSSION**

This study assessed the independent and combined effects of soy protein and soy-derived isoflavones on antioxidant capacity and biomarkers of oxidative stress in adults with moderately elevated LDL-cholesterol concentrations. The current data suggest a modest difference in plasma TAP between the 2 types of protein investigated, independent of isoflavones intake. Similar to the current data, a multiple regression analysis showed that after 12 wk of treatment, plasma total antioxidant status was positively affected by soy protein but not by soy isoflavones (22). These results are supported by reports suggesting that soy-protein isolate and a soy peptide were effective in reducing paraquat-induced oxidative stress in rats (42, 43). The antioxidant activity of soy protein has also been attributed to the iron-chelating properties of its phytic acid. Previously, Swain et al (22) reported that supplementation with 40 g protein/d provided as isoflavone-rich soy-protein isolate, isoflavone-poor soy protein isolate, or whey protein did not significantly affect plasma total antioxidant status in a double-blind parallel trial. The design of the current study did not allow the determination of which protein type induced the differences observed.

Plasma antioxidants other than isoflavones also contribute to the total antioxidant capacity and, in absolute concentration, variations among studies would not be predicted to have a physiologically significant effect. Standardization of plasma antioxidants on the basis of total cholesterol concentrations did not significantly alter the results (data not shown).

**TABLE 4**

Biomarkers of oxidative stress at the end of each dietary phase

<table>
<thead>
<tr>
<th>Variable</th>
<th>Soy/−</th>
<th>Soy/+</th>
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<th>Protein</th>
<th>Isoflavones</th>
<th>Protein × Isoflavones</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL oxidation</td>
<td>71.1 ± 20.6&lt;sup&gt;1&lt;/sup&gt;</td>
<td>69.1 ± 19.9</td>
<td>72.2 ± 18.9</td>
<td>70.1 ± 15.0</td>
<td>0.6303</td>
<td>0.3101</td>
<td>0.9712</td>
</tr>
<tr>
<td>Lag time (min)</td>
<td>3.98 ± 1.31</td>
<td>4.01 ± 1.30</td>
<td>4.05 ± 1.35</td>
<td>3.98 ± 1.51</td>
<td>0.8531</td>
<td>0.8745</td>
<td>0.7517</td>
</tr>
<tr>
<td>Urinary F2-isoprostanes (ng/mg creatinine)</td>
<td>0.76 ± 0.53</td>
<td>0.56 ± 0.26</td>
<td>0.61 ± 0.58</td>
<td>0.67 ± 0.46</td>
<td>0.8151</td>
<td>0.1731</td>
<td>0.0833</td>
</tr>
<tr>
<td>Carboxyl groups (nmol/mg protein)</td>
<td>0.13 ± 0.12</td>
<td>0.13 ± 0.06</td>
<td>0.12 ± 0.06</td>
<td>0.12 ± 0.07</td>
<td>0.0208</td>
<td>0.6244</td>
<td>0.7864</td>
</tr>
<tr>
<td>Oxidized plasma</td>
<td>0.42 ± 0.13</td>
<td>0.45 ± 0.15</td>
<td>0.46 ± 0.16</td>
<td>0.45 ± 0.14</td>
<td>0.0209</td>
<td>0.3691</td>
<td>0.1670</td>
</tr>
<tr>
<td>MDA (μmol/L)</td>
<td>0.81 ± 0.17</td>
<td>0.83 ± 0.27</td>
<td>0.75 ± 0.17</td>
<td>0.87 ± 0.32</td>
<td>0.7940</td>
<td>0.0447</td>
<td>0.2633</td>
</tr>
</tbody>
</table>

<sup>1</sup> n = 42. MDA, malondialdehyde; soy−, soy protein depleted of isoflavones; soy+, soy protein enriched in isoflavones; animal−, animal protein with no added isoflavones; animal+, animal protein with added isoflavones.

<sup>2</sup> A repeated-measures ANOVA was used to detect differences.

<sup>3</sup> ± SD (all such values).
actually exceed that of the isoflavones. In the current study, all diets were formulated to be rich in fruit and vegetables and, therefore, relatively rich in antioxidants. Despite the somewhat higher content of lycopene, α-carotene, β-carotene, α-tocopherol, and γ-tocopherol in the soy protein–containing diets, differences observed in plasma trans β-carotene, lutein, and lycopene concentrations were small and not significantly associated with type of protein. Lycopene and trans β-carotene concentrations were above the 90th percentile reported for subjects aged 50–70 y in the third National Health and Nutrition Examination Survey (NHANES III) during all dietary periods. Similarly, plasma tocopherol concentrations were above the 75th percentile throughout the study.

In the current study, soy-protein intake was only associated with a lower concentration of protein carbonyl groups after incubation of plasma with a water-soluble radical initiator (AAPH), compared with animal protein, whereas no other effects on biomarkers of oxidative stress were observed. This observation extends the results reported by Steinberg et al (16). After the provision of soy or milk supplements, with and without isoflavones, no significant effect on copper-mediated LDL susceptibility to oxidation was observed. In contrast, Ashton et al (21) reported a longer lag phase of copper-induced LDL oxidation after meat was substituted with tofu. In a small study, Tikkanen et al (20) documented that the consumption of 3 soy bars per day (21 g protein, 36 mg genistein, and 21 mg daidzein) for 2 wk resulted in a longer lag phase of copper-induced LDL oxidation than that at baseline. Jenkins et al (17, 18) reported a decrease in LDL conjugated dienes after consumption of a minimum of 36 g soy protein/d, regardless of the isoflavone content of the foods. Of these reports, only the report of Steinberg et al (16) included information on dietary antioxidant intake and reported a high intake of dietary antioxidants, similar to that in the current study. It is possible that the lack of effect of soy consumption on biomarkers of oxidative stress in both studies was masked by the relatively high concentration of other plasma antioxidants.

Discrepancies in the effects observed in other studies are hard to interpret because of the use of a wide variety of soy products providing from 15 to 52 g soy protein/d, each containing different amounts of isoflavones.

Although in vitro measures have suggested an antioxidant effect of isolated isoflavones (31, 32), the in vivo evidence is equivocal. No differences in urinary F$_2$-isoprostanes or LDL oxidation (lag time or oxidation rate) were observed in the current study. In contrast, high (56 mg) compared with low (<2 mg) daily isoflavone intakes resulted in lower concentrations of plasma 8-epi-prostaglandin F$_2$α, a biomarker of in vivo lipid peroxidation, and longer lag times for LDL oxidation (19). However, other studies have failed to find an antioxidant effect of soy-derived isoflavones when provided as supplements, using either ex vivo LDL oxidizability (33, 34) or urinary F$_2$-isoprostanes (35) as biomarkers of oxidative stress.

In the current study, malondialdehyde concentrations—an indirect marker of lipid peroxidation—were unexpectedly higher after the isoflavone-supplemented dietary periods. Wiseman et al (19) reported that plasma malondialdehyde did not significantly differ regardless of dietary isoflavones. However, in their study, the malondialdehyde concentration was 6% higher after the high-isoflavone period, albeit not significant. Despite the similar amounts of dietary isoflavones between the 2 studies, participants in the current study were older (mean age of 63 y compared with 30 y), had slightly lower plasma isoflavone concentrations, and had higher plasma malondialdehyde concentrations (almost 4 times those in Wiseman et al’s study), regardless of dietary period. Perhaps more importantly, plasma cholesterol concentrations were higher in the current study, which likely contributed to increased oxidative stress.

In contrast with the human data, in vitro and animal studies have more consistently reported an antioxidant effect of soy-derived isoflavones. Kerry and Abbey (31) reported that the addition of genistein to human LDL resulted in lower malondialdehyde concentrations over an 8-h azo-initiated oxidation incubation. Similarly, animal studies have shown lower malondialdehyde concentrations in plasma (44) and in the aortic arch (45) of animals fed high doses of isoflavones. Noteworthy, the concentrations of isoflavones used in both in vitro and animal studies are higher than what can be achieved in the plasma of subjects consuming diets supplemented with isoflavones, as reported in the current study and in similar studies (19). Although the higher malondialdehyde concentrations observed after the subjects consumed the isoflavone-containing diets in the current study is of interest, it could not be determined whether isoflavone intake led to a greater extent of lipid peroxidation or was secondary to other reactions (46).

The lack of a protective effect of isoflavones against LDL oxidizability might be related to their relative hydrophobicity (47). Meng et al (48) reported that in vitro free genistein and daidzein are only incorporated into LDL to a small extent and do not significantly influence oxidation resistance measured by copper-induced conjugated diene formation and that they require esterification to become more readily incorporated into LDL. Therefore, because of the limited incorporation of isoflavones into LDL, any measures that involve the isolated lipoprotein need to be interpreted cautiously. In addition, because some of the isoflavones in plasma could be in the conjugated form, they may not have been available to function as an antioxidant (49).

This study had several limitations. The possibility cannot be ruled out that the antioxidant effect of α-tocopherol and other antioxidants present in high concentrations in plasma may have masked any potential effects of isoflavones. This possibility is supported by the results from the in vitro assessment of the antioxidant capacity of isoflavones, which suggest that the small additive antioxidant effect of isoflavones is greater when α-tocopherol concentrations are lower (1 μmol/L). Moreover, the possible interaction between different antioxidants in vivo may enhance the overall antioxidant status, making the evaluation of the effect of particular antioxidants hard to interpret.

In conclusion, consumption of diets rich in soy protein and soy-derived isoflavones do not appear to decrease oxidative stress. Despite the modest increase in plasma TAP associated with soy-protein intake, this finding was not reflected in specific effects on measures of oxidative stress as potentially affected by either soy protein or soy-derived isoflavones. The presence of other antioxidants from a nutritionally adequate diet may have mitigated the antioxidant effect of isoflavones previously reported in in vitro systems.

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