Soy Protein Concentrate and Isolated Soy Protein Similarly Lower Blood Serum Cholesterol but Differently Affect Thyroid Hormones in Hamsters$^{1,2}$

SUSAN M. POTTER,*$^3$ JOY PERTILE* AND M. D. BERBER-JIMENEZ*$^*$

*Department of Food Science and Human Nutrition and 1Division of Nutritional Sciences, University of Illinois at Urbana/Champaign, Urbana, IL 61801

ABSTRACT There is a wide variation in the hypocholesterolemic response to ingestion of soy protein in humans. One possible explanation is that the different soy protein preparations used contain different spectra of biologically active components. This could affect a number of indices including thyroid hormone status. An increased level of thyroxine has been proposed as an underlying mechanism of the hypocholesterolemic effect of soy protein. The objective of this study was to determine if serum cholesterol and thyroid hormone concentrations differed because of feeding soy protein from different sources. Twenty-nine male weanling golden Syrian hamsters were fed rations containing 25 g/100 g protein from either isolated soy protein (ISP), soy protein concentrate (SPC) or casein for 35 d. Serum total cholesterol concentrations were lower in hamsters fed ISP and SPC compared with those fed casein ($P < 0.05$). No differences in cholesterol concentrations were observed in lipoprotein fractions. Serum thyroxine and free thyroxine were greater only in hamsters fed ISP than in those fed casein ($P < 0.05$), whereas triiodothyronine concentrations were higher in casein-fed than in SPC-fed hamsters ($P < 0.05$). Results indicate that protein from ISP and SPC are both effective in lowering blood cholesterol concentrations, whereas only ISP increases thyroxine concentrations. Therefore, it appears unlikely that modulation of thyroid hormone status is responsible for the cholesterol-lowering effect of soy protein. J. Nutr. 126: 2007–2011, 1996.

INDEXING KEY WORDS:
• soybean protein • cholesterol • thyroid hormones • hamsters

There is a large body of literature indicating that protein from various soy products lowers blood cholesterol in experimental animals and humans with preexisting elevated blood lipids [Anderson et al. 1995, Bhattacharyya et al. 1994, Carroll 1991, Potter et al. 1993]. When evaluating the data from humans, a large variation in responsiveness exists. This could be due to a number of factors. However, variation in soy products may be responsible because of the large number of biologically active compounds present in different soy products including isoflavones, saponins, phytic acid, protease inhibitors, etc. All of these compounds have been implicated in the cholesterol-lowering effect of soy protein.

Although the mechanism by which soy protein lowers cholesterol is unknown, it was recently postulated that soy protein, or a component associated with soy protein, enhances plasma thyroid hormone concentrations [Forsythe 1986, Sholz-Ahrens 1990]. The animal data supporting this hypothesis are intriguing; however, human studies have not shown any clear relationship between thyroid hormone concentrations and soy protein ingestion [Balbir et al. 1993, Ham et al. 1993]. The animal experiments that have shown elevations in thyroid hormones to date have utilized isolated soy protein (ISP)$^4$ products [Forsythe 1986, Sholz-Ahrens 1990]. Although some human investigations on soy protein-induced cholesterol lowering used isolated soy protein products, many others use texturized soy protein, which is largely made by extrusion of soy protein.

$^1$ Funded in part by U.S. Department of Agriculture HATCH Project #60-0346 and a Jonathan Baldwin Turner Undergraduate Research Grant.

$^2$ The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

$^3$ To whom reprint requests should be addressed at 463 Bevier Hall, 905 S. Goodwin Ave., Urbana, IL 61801.

$^4$ Abbreviations used: FT₃, free thyroxine, ISP, isolated soy protein, SPC, soy protein concentrate; T₃, triiodothyronine, T₄, thyroxine, T₄U, triiodothyronine uptake.
TABLE 1

<table>
<thead>
<tr>
<th>Component</th>
<th>SPC</th>
<th>ISP</th>
<th>Casein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein1</td>
<td>35.7</td>
<td>27.8</td>
<td>27.8</td>
</tr>
<tr>
<td>Rice flour2</td>
<td>30.4</td>
<td>38.3</td>
<td>38.3</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Cellulose3</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Choline chloride4</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Potassium carbonate5</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Vitamin mix6</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral mix7</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Cholesterol8</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

1 All three diets were formulated to contain 25 g/100 g protein.
   Protein from either isolated soy protein [ISP, 90.5% protein, Supro 670, Protein Technologies International, St. Louis, MO], soy protein concentrate [SPC, 70% protein, Promax 70, Central Soya, Ft. Wayne, IN], or casein [90.5% protein, Vitamin-Free Test Casein, Harlan/Teklad, Madison, WI].

2 Amount of rice flour added varied dependent on amount of protein source added.

3 Solka Floc [James Rivers, Berlin, NH].
4 Choline chloride [Harlan/Teklad, Madison, WI].
5 Potassium carbonate [EM SPScience, Gibbstown, NJ].
6 Vitamin Mix #40060 [Harlan/Teklad, Madison, WI, see Terpstra et al. 1991].
7 Mineral Mix #170910 [Harlan/Teklad, Madison, WI, see Terpstra et al. 1991].
8 Cholesterol [Harlan/Teklad, Madison, WI].

concentrate (SPC). The composition of these is quite different. Isolated soy protein contains ~90% protein, whereas SPC contains ~70% protein. Thus, a number of other, potentially biologically active compounds may be present in larger quantities in SPC than in ISP, thereby resulting in differential responses of blood lipids and hormones. Therefore, the current experiment was designed to evaluate the effects of soybean protein from either ISP or SPC on blood cholesterol and thyroid hormone concentrations.

MATERIALS AND METHODS

Experimental design. Male, weanling golden Syrian hamsters [Sasco, Omaha, NE] were fed diets similar in all respects with the exception of dietary protein source being SPC, ISP or casein [Table 1]. Use of animals was approved by the Laboratory Animal Care Advisory Committee at the University of Illinois.

General procedures. Hamsters weighing ~65 g were individually housed in an environmentally controlled room [23°C] with an alternating 12-h light and 12-h dark cycle. Upon arrival, all animals were fed a pulverized commercial pellet diet [Purina Rat Chow, Ralston Purina, St Louis, MO] for 1 wk. Hamsters were then randomly assigned to dietary treatment groups (10 hamsters per group). Composition of the powdered basal diet is provided in Table 1 and was patterned after Terpstra et al. [1991]. Protein concentration was set at 25 g/100 g and was derived from either ISP [Supro, Protein Technologies International, St. Louis, MO], SPC [water extracted, Promax 70, Central Soya, Ft. Wayne, IN] or casein [Vitamin-Free Test Casein, Harlan/Teklad, Madison, WI]. Because the protein concentration of the concentrate was different from the isolate and casein, rice flour was added to each diet according to Terpstra et al. [1994]. Feed and water were consumed ad libitum, and feed intake and weight gain were monitored throughout the study.

After a 35-d experimental period, hamsters were sedated with ketamine/xylazine [10 mg/100 g body weight] and killed by decapitation. Trunk blood was collected and serum was separated. The LDL/VLDL were separated from HDL by precipitation with dextran sulfate-MgCl2 [Warnick et al. 1982] [Sigma Diagnostics, St. Louis, MO]. Remaining serum and HDL-containing supernatants were frozen at -70°C for subsequent analysis.

Serum cholesterol and thyroid hormone. Total and HDL cholesterol were measured enzymatically by a modification of the method described by Allain et al. [1974] using a commercially available reagent [Sigma Diagnostics, St. Louis, MO]. β-Lipoprotein [VLDL + LDL] cholesterol was calculated by subtraction of HDL cholesterol from total cholesterol. Total thyroxine [T₄] triiodothyronine [T₃] and % T₃ uptake [T₃U] were measured by solid-phase radioimmunoassay using kits developed for canine T₃ and T₄ [Britton et al. 1975, Mar-dell and Gerson 1978, Prince and Ramsden 1977] [Diagnostic Products, Los Angeles, CA]. Free T₄ [FT₄] was estimated using the product of total T₄ concentration and T₃U.

Statistical analysis. Data were analyzed using the Statistical Analysis System [SAS, Cary, NC] by a one-way analysis of variance. Differences between treatments were determined by Tukey’s least significant difference test [Steel and Torrie, 1980]. An alpha level of 0.05 was used to indicate statistically significant differences.

RESULTS

One hamster in the SPC group died during the study as a result of respiratory infection, not related to procedures or dietary treatments.

Weight gain and feed efficiency ratios were lower in SPC-fed hamsters compared to those fed casein [P < 0.05] [Table 2]. There were no differences in feed intake among treatment groups.

Serum total cholesterol concentrations were lower
TABLE 2

<table>
<thead>
<tr>
<th>n</th>
<th>Weight gain</th>
<th>Feed intake</th>
<th>Feed efficiency ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/35 d</td>
<td>g gain/g feed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPC 9</td>
<td>35.6 ± 10.2b</td>
<td>297 ± 61</td>
<td>0.13 ± 0.05b</td>
</tr>
<tr>
<td>ISP 10</td>
<td>44.3 ± 10.5b</td>
<td>266 ± 55</td>
<td>0.17 ± 0.04b</td>
</tr>
<tr>
<td>Casein 10</td>
<td>50.0 ± 6.7a</td>
<td>286 ± 41</td>
<td>0.18 ± 0.03a</td>
</tr>
</tbody>
</table>

1 Values are means ± SD. Means within columns with different superscript letters are different (P < 0.05).

TABLE 3

<table>
<thead>
<tr>
<th>n</th>
<th>Total</th>
<th>HDL</th>
<th>LDL</th>
<th>HDL/LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>mmol/L</td>
<td>mol/mol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPC 9</td>
<td>6.62 ± 0.8b</td>
<td>3.37 ± 1.1</td>
<td>3.25 ± 1.1</td>
<td>1.27 ± 0.9</td>
</tr>
<tr>
<td>ISP 10</td>
<td>7.03 ± 0.5b</td>
<td>3.40 ± 0.6</td>
<td>3.63 ± 0.8</td>
<td>1.06 ± 0.7</td>
</tr>
<tr>
<td>Casein 10</td>
<td>8.28 ± 1.2a</td>
<td>4.09 ± 1.0</td>
<td>3.99 ± 1.2</td>
<td>1.16 ± 0.6</td>
</tr>
</tbody>
</table>

1 Values are means ± SD. Means within columns with different superscript letters are different (P < 0.05).

TABLE 4

<table>
<thead>
<tr>
<th>n</th>
<th>T4²</th>
<th>FT4</th>
<th>T3</th>
<th>T3U</th>
</tr>
</thead>
<tbody>
<tr>
<td>nmol/L</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPC 9</td>
<td>55.1 ± 12.9b</td>
<td>17.5 ± 0.6b</td>
<td>2.81 ± 0.78b</td>
<td>31.7 ± 4.7</td>
</tr>
<tr>
<td>ISP 10</td>
<td>74.5 ± 16.7a</td>
<td>22.7 ± 0.2a</td>
<td>3.15 ± 0.58ab</td>
<td>30.5 ± 1.0</td>
</tr>
<tr>
<td>Casein 10</td>
<td>60.4 ± 6.4b</td>
<td>16.7 ± 0.6b</td>
<td>3.75 ± 0.98a</td>
<td>27.7 ± 8.6</td>
</tr>
</tbody>
</table>

1 Values are means ± SD. Means within columns with different letters are different (P < 0.05).
2 T4 = thyroxine; FT4 = free thyroxine; T3 = triiodothyronine; T3U = triiodothyronine uptake.

SOYBEAN PROTEIN, SERUM CHOLESTEROL, BLOOD LIPIDS AND HORMONES 2009

concentrations in hamsters compared with feeding casein. We did not observe any differences in the lipoprotein fractions. However, Terpstra et al. (1994) reported that the β-lipoprotein component (LDL + VLDL) was responsible for the depression in total cholesterol that they observed in hamsters fed a cholesterol-free purified diet containing ISP compared with those fed casein. When cholesterol was added [0.1 g/100 g] to these diets, there was no significant difference in β-lipoprotein concentration due to wide between animal variation, despite a 56% lower value in ISP-fed animals. In a second study by this group comparing casein with ISP and SPC, significant differences in total and LDL + VLDL cholesterol were achieved only in hamsters fed a semipurified diet containing 40 g/100 g protein (Terpstra et al. 1994). When hamsters were fed diets containing 20 g/100 g protein, differences in lipemia were not present. Earlier, Beynen and Schouten (1983) reported reductions in total cholesterol concentrations in hamsters fed soybean protein compared with those fed casein. Pooled lipoprotein fractions also had lower VLDL, LDL, LDL, and HDL cholesterol concentrations. However, these diets contained 1 g/100 g cholesterol, and because samples were pooled from all animals for lipoprotein analysis, statistical evaluation was not possible.

A current theory concerning the cause of the hypocholesterolemic effect of soy protein states that a component of soy protein stimulates the endocrine system, resulting in elevations in thyroid hormone concentrations that in turn result in lowering of blood cholesterol concentrations (Forsythe 1986). Data from the current study show that increases in thyroxine concentrations occurred only when ISP was fed even though similar cholesterol concentrations were found in hamsters fed both soy protein products. Both Forsythe (1986), in gerbils, and Sholz-Ahrens et al. (1990), in minipigs, reported increases in thyroxine concentrations when ISP was compared with casein. In addition, Forsythe (1986) also reported elevations in thyroid stimulating hormone concentrations. In humans, we have not noted differences in any of the thyroid hormones in subjects consuming ISP or soy flour compared with those consuming milk proteins (Balmir et al. 1993, Ham et al. 1993).

The differences observed in thyroxine concentrations between SPC- and ISP-fed hamsters are most likely a result of a component being added or taken away from the ISP-containing diet. Because diets were formulated to contain equal amounts of protein from these two sources, rice flour was the only component other than protein that was varied in the diet. Rice flour is primarily complex carbohydrates (mostly starch) and sucrose, and removal of carbohydrates is not known to alter thyroid hormone concentrations without significant changes in body weight, thus, it is unlikely that the effect was due to this component. Furthermore, T₄ and free T₄ concentrations were similar in the groups
fedsPCandcassein, whereas thecasein diet contained the same amount of rice flour as the ISP diet. It is therefore more likely that variations in the wide assortment of bioactive phytochemicals present in soy products were responsible. Phytochemicals vary in both quantity and availability based on processing of soy (Anderson and Wolf 1994) and include compounds such as saponins, isoflavones, phytic acid and trypsin inhibitors.

Lueprasitsakul et al. (1990) reported that a synthetic plant isoflavone (EMD 21388) displaced thyroxine from its binding protein and increased serum-free thyroxine in rats. The structure of this compound (3-methyl-4',6-dihydroxy-3',5'-dibromo-flavone) is somewhat similar to that of the soybean isoflavone genistein (4',5,7-trihydroxy-isoflavone). Given this, we have expected feeding the SPC to have produced higher levels of serum T₄ and free T₄ due to the higher concentration of isoflavones (Table 4). This was not the case, indicating that another compound inherent to ISP may have been responsible or that a combination of factors are involved.

Enlargement of the thyroid gland has been reported in rats and chicks fed unheated soybeans (Sharpless et al. 1939, Wilgus et al. 1941). Human infants have also been reported to develop goiter when fed soy protein-based formulas in the past (Hydowitz 1960). However, iodine supplementation appears to have eliminated this problem in infant formulas currently in use (Liener 1994). The substance responsible for these observations is not known, but these effects indicate that there are biologically active compounds in various soy products that influence thyroid function.

Some have postulated that a low-molecular-weight oligopeptide affects the thyroid gland (Konijn et al. 1973), whereas others believe it could be a result of saponins reducing reabsorption of thyroxine in the gut [Suwa and Kimura 1981]. The soy products used in the current study have different profiles of isoflavones and saponins as well as different levels of other potentially bioactive compounds (Liener 1994). Thus, it is a possibility that the hamsters consuming SPC had an elevated fecal loss of thyroxine, because of the higher saponin content, thereby lowering serum concentrations of this hormone compared with hamsters fed ISP. It is also possible that the different isoflavone quantities and proportions may affect binding sites of thyroxine on transthyretin leading to the differences observed. However, we would have expected elevations in thyroxine in hamsters fed SPC, which was water extracted and thus contained much higher levels of isoflavones than the ISP. Another possibility that cannot be ignored is that a peptide or other compound present or absent in one of these protein preparations could have been involved.

In conclusion, hamsters fed protein from either soy protein concentrate or isolated soy protein had cholesterol concentrations that were lower than in those fed casein; however, these effects were not modulated by the thyroid hormones. The reason for the differential response in serum thyroid hormone concentrations between hamsters fed SPC and ISP is not known but is likely due to a variation in phytochemical, or possibly soluble protein/aminio acid, composition of these two soy products.

ACKNOWLEDGMENTS

The authors thank Protein Technologies International [St. Louis, MO] for provision of the isolated soy protein and Central Soya [Ft. Wayne, IN] for provision of the soy concentrate used in this study.

LITERATURE CITED


SOYBEAN PROTEIN, SERUM CHOLESTEROL, BLOOD LIPIDS AND HORMONES


