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Effects of soybean isoflavones, probiotics, and their interactions on lipid metabolism and endocrine system in an animal model of obesity and diabetes

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Abstract

The effects of soybean isoflavones with or without probiotics on tissue fat deposition, plasma cholesterol, and steroid and thyroid hormones were studied in SHR/N-cp rats, an animal model of obesity, and were compared to lean phenotype. We tested the hypothesis that probiotics by promoting the conversion of isoflavone glycosides to their metabolically active aglycone form will have a synergistic effect on body fat, cholesterol metabolism, and the endocrine system. Obese and lean SHR/N-cp rats were fed AIN-93 diets containing 0.1% soy isoflavone mixture, 0.1% probiotic mixture, or both together. Different fat tissues were teased and weighed. Plasma was analyzed for cholesterol and steroid and thyroid hormones. In both phenotypes, isoflavones lowered fat deposition in several fat depots. Probiotics alone had no significant effect on fat depots. Isoflavones lowered total, LDL, and HDL cholesterol in lean rats, but in obese rats isoflavones lowered only total and LDL cholesterol. Isoflavones also lowered many of the steroid hormones involved in lipid metabolism but had no significant effect on thyroid hormones. Probiotics had no significant effect on cholesterol or hormones. Thus, our data show that soy isoflavones also lower plasma cholesterol and that this hypocholesterolemic effect appears to be due in part to the modulation of steroid hormones. Probiotics do not seem to enhance the effect of isoflavones. Published by Elsevier Inc. All rights reserved.

Keywords: Soy isoflavones; Probiotics; Plasma cholesterol; Steroid hormones; Thyroid hormones

1. Introduction

Several studies in animals and humans have shown that the consumption of soybean have beneficial effects in variety of disorders including hypocholesterolemia as well as protection against cardiovascular disease, renal disease, bone resorption, certain forms of cancer, and menopausal symptoms [1–7]. Increasing evidence suggests that soybean may also have beneficial effect against obesity and diabetes [8]. Soy protein has been thought to be responsible for the cholesterol lowering effect of soybean [9–11]. However, isoflavones present in soybean having both weak estrogenic and antiestrogenic activity [12,13] may also partly be responsible for the cholesterol lowering and cardioprotective effects. How soy protein and other components of soybean act to lower cholesterol and lipids is not known; it is possible that effect on endocrine system may be partly responsible since hormones do control lipid metabolism. We therefore studied the effects of soy isoflavones on lipid parameters and the endocrine system in obesity and diabetes.

Probiotics have been used for centuries in the manufacture of cultured dairy products. Probiotics are now widely consumed in the form of fermented milk products such as yogurt or as freeze-dried culture. Probiotics are defined as selected viable microorganisms used as dietary supplements for their potential benefits on human health or disease prevention [14]. The primary probiotic bacteria associated with dairy products have been *Lactobacillus acidophilus, Lactobacillus casei,* and Bifidobacteria. The reduction of total cholesterol or low-density lipoproteins found in plasma is reported to lower the risk of coronary heart disease. There are a few studies that suggest

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that probiotics may also reduce LDL cholesterol, although the research to date is equivocal [15]. Studies on *Lactobacillus acidophilus* NCFM [16,17] have shown that it is able to remove cholesterol from a laboratory growth medium. There are also reports claiming that probiotics may have beneficial effect in obesity, but the evidence to support such an effect is still lacking.

The Lactobacillus strain has been shown to cleave β-glycosidic isoflavones during fermentation of milk supplemented with soygerm powder. The interactions between the Lactobacillus strain and soygerm powder suggest that combining both can exhibit advantageous probiotic effect [18].

The spontaneously hypertensive/NIH-corpulent (SHR/N-cp) rat is a recognized model for the study of obesity and diabetes [19–23]. Obese rats exhibit abdominal (central) obesity and metabolic features of type II diabetes mellitus. Lean phenotypes are hypertensive whereas obese phenotypes are less hypertensive and show insulin resistence [21–23]. Recent studies in our laboratory have shown that dietary supplementation with either soy protein or flaxseed protein lower plasma cholesterol in SHR/N-cp rats. The active constituents present in soybean or flaxseed for hypocholesterolemic effect have not been definitely identified. Since soy protein isolate contains high amount of isoflavones, it is possible that isoflavones may be partly responsible for the hypocholesterolemic effect. In the present study we therefore sought to determine whether combining of soy isoflavones and probiotics have additive effects on body fat, plasma lipid parameters, and hormones in this animal model.

2. Methods and materials

2.1. Animals

A total of 32 male lean and 32 male obese SHR/N-cp rats were obtained from the National Institutes of Health at approximately 5–6 weeks of age. At this age, obesity is already evident in SHR/N-corpulent (cp/cp) rats as indicated by higher body weight (average 125 g) than their lean littermates (average 96 g) and increased abdominal girth. The experimental protocol was approved by the Institutional Animal Care and Use Committees of the Agricultural Research Service, United States Department of Agriculture (Beltsville, MD), and by the George Washington University (Washington, DC). All animals were housed individually in stainless steel wire cages with controlled temperature (21–25°C) and relative humidity (40–50%) and maintained on a reverse 12-hour dark (9 AM to 9 PM) and light (9 PM to 9 AM) cycle.

2.2. Diets and experimental protocol

All animals were provided with a Purina rat chow and were maintained on this diet for 2 weeks until 7–8 weeks of age. Food and water were consumed ad libitum. The rats were then randomly divided into four groups of eight lean rats and four groups of eight obese rats and fed AIN-93 diet [24]. Group 1 rats were fed 20% casein; group 2 rats were fed 20% casein with 0.1% soybean isoflavone mixture containing genistein, daidzein, and glycetin; group 3 rats were fed 20% casein with 0.1% probiotic mixture (10^{10} colony forming units [cfu] containing *Lb. acidophilus* (LA 140), *Lb. casei subsp. casei* (LC 107), and *Bifidobacterium bifidum* (BBL 730); and group 4 rats were fed 20% casein with 0.1% isoflavone mixture and 0.1% probiotic mixture. All diets were identical and contained similar amounts of protein, fat, carbohydrates, minerals, and vitamins. All diets contained the following (in g/kg): dextrinized cornstarch, 155; sucrose, 100; soybean oil, 40; cellulose, 50; mineral mix (AIN-93M-MX), 35; vitamin mix (AIN-93-VX), 10; L-cystine, 1.8; choline bitartrate, 2.5; and tert-butylhydroquinone, 0.008. Casein and L-cysteine were purchased from Sigma Chemicals (St. Louis, MO). Soy isoflavone mixture was obtained gratis from Protein Specialties Division, Archer Daniels Midland (Decatur, IL). Tert-butylhydroquinone was purchased from Aldrich Chemical Co. (Milwaukee, WI). All other ingredients were purchased from Dyets Inc. (Bethlehem, PA).

The diet was supplemented with the probiotics mixture to obtain enhanced intestinal milieu. When probiotics mixture was added to diets, Lactobacilli and Bifidobacterium strains showed growth of 131×10^8 and 35×10^8 respectively. In view of these results, we attempted to supplement the rats fed with mixture of probiotics concentration to meet nutritional requirements.

All animals were fed the experimental diets for 20 weeks and were weighed biweekly throughout the study. Food intake was measured biweekly over 2-day period. At the end of the feeding period, after an overnight fast, animals were anesthetized under carbon dioxide and blood was drawn by cardiac puncture. Blood was collected in tubes containing ethylenediaminetetraacetate (EDTA) (1.4 g/L) and Trasylol (100 kU/L), and plasma was separated for subsequent biochemical analyses and stored at −70°C. Immediately after sacrifice, the abdomen was opened by a midline incision; the liver, heart, and kidney were then quickly removed and weighed, frozen in liquid nitrogen, and stored at −70°C. Perirenal fat, ileal fat, subdiaphragmatic fat, and epididymal fat were teased and weighed.

2.3. Analytical measurements

Plasma was analyzed enzymatically for cholesterol (total, HDL, and LDL) using Alcyon analyzer, ATAC 8000 (Abbott Laboratories) and kits from Elan Diagnostics (catalog no. 541-235) while total protein was measured using the same equipment and kits from Biochem Lab Systems (catalog no. 582-008). LDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol. Steroid and thyroid hormones were measured by radioimmunoassays as previously described [25]. Lactobacilli from feces of

Table 1
Effects of soy isoflavones and probiotics on gained weight and tissue weights in a genetic model of obesity and diabetes

Phenotype	Diet	Weight gain (g)	Liver (g)	Heart (g)	Kidney (g)	Spleen (g)	Adrenal gland (mg)
Lean	Casein	252.7 ± 9.0	14.26 ± 1.60	1.7 ± 0.10	3.26 ± 0.07	0.78 ± 0.03	40.03 ± 3.18
	Isoflavones (I)	213.3 ± 17.8	13.15 ± 1.50	1.7 ± 0.09	3.13 ± 0.07	0.69 ± 0.03	36.51 ± 2.98
	Probiotics (P)	279.4 ± 17.8	15.79 ± 1.50	1.7 ± 0.09	3.24 ± 0.07	0.73 ± 0.03	34.96 ± 2.98
	I + P	206.0 ± 17.8	13.38 ± 1.50	1.5 ± 0.09	3.34 ± 0.07	0.64 ± 0.03	33.83 ± 2.98
Obese	Casein	371.8 ± 9.7	35.01 ± 1.73	1.7 ± 0.10	3.78 ± 0.08	0.76 ± 0.04	41.80 ± 3.43
	Isoflavones (I)	345.6 ± 9.0	28.87 ± 1.60	1.5 ± 0.10	3.20 ± 0.07	0.68 ± 0.03	46.17 ± 3.18
	Probiotics (P)	393.3 ± 18.9	31.53 ± 1.60	1.7 ± 0.10	3.67 ± 0.07	0.70 ± 0.03	42.40 ± 3.18
	I + P	349.6 ± 18.9	26.50 ± 1.60	1.6 ± 0.10	3.33 ± 0.07	0.69 ± 0.03	47.14 ± 3.18
ANOVA	Phenotype (Ph)	$P < 0.0001$	$P < 0.0001$	NS	$P < 0.016$	NS	$P < 0.001$
	Diet (D)	$P < 0.002$	$P < 0.015$	NS	$P < 0.011$	$P < 0.025$	NS
	Ph × D	NS	NS	NS	NS	NS	NS

Values are means ± SEM of 8 rats.
ANOVA = analysis of variance; NS = not significant.

rats fed the mixture of probiotics were enumerated anaerobically as described by Gilliland et al. [26]. Bifidobacterium counts were determined anaerobically according to the method of Doleyres et al. [27].

2.4. Statistical analysis

Results are expressed as mean ± standard error of the mean. Comparisons between groups for the data in Tables 1 and 2 were made using one-way analysis of variance (ANOVA) and, for the data in Table 3, by two-way ANOVA. When an effect was statistically significant ($P < 0.05$) mean comparisons were done. A Sidak adjusted significance level was used for the pair-wise comparisons of the means, with the overall significance level set at 0.05.

3. Results

Table 1 shows the effects of isoflavones and probiotics on body weight and tissue weights. Obese rats had significantly higher body weight, liver, kidney, and adrenal gland weights. Dietary isoflavones, with or without probiotics, significantly reduced body weight and weights of liver, kidney, and spleen. Probiotics given alone had no significant effect on body weight or on any tissues. No significant effect of any of the diets was observed on food intake (data not shown).

Table 2 summarizes data on the effect of isoflavones and probiotics on various fat depots. There was no significant difference in epididymal fat pad weights between lean and obese rats. However, obese rats had significantly higher tissue weight for perirenal, subdiaphragmatic, and ileal fat pads. Dietary isoflavones significantly reduced tissue weight of epididymal, perirenal, and subdiaphragmatic fat depots. Similarly, isoflavones reduced ileal fat pad weight in obese rats but not in lean rats. Probiotics given alone had no significant effect on any of the fat tissue weights.

The effects of isoflavones and probiotics on plasma cholesterol is shown in Table 3. Total cholesterol as well as HDL and LDL cholesterol levels were higher in obese rats than in lean rats. Isoflavones reduced total as well as HDL

Table 2
Effects of soy isoflavones and probiotics on fat tissues in a genetic model of obesity and diabetes

Phenotype	Diet	Epididymal fat (g)	Perirenal fat (g)	Subdiaphragmatic fat (g)	Ileal fat (g)
Lean	Casein	8.43 ± 0.45	11.25 ± 2.42	0.54 ± 0.03	0.33 ± 0.13
	Isoflavones (I)	5.94 ± 0.38	5.98 ± 1.79	0.26 ± 0.09	0.33 ± 0.08
	Probiotics (P)	7.35 ± 0.88	7.13 ± 1.96	0.44 ± 0.24	0.64 ± 0.03
	I + P	6.50 ± 0.35	5.42 ± 1.69	0.26 ± 0.03	0.32 ± 0.03
Obese	Casein	8.10 ± 0.50	38.81 ± 3.66	1.32 ± 0.21	3.53 ± 0.80
	Isoflavones (I)	6.12 ± 0.78	36.21 ± 3.59	1.04 ± 0.24	3.02 ± 0.93
	Probiotics (P)	7.68 ± 0.45	39.19 ± 3.67	1.94 ± 0.24	4.84 ± 1.14
	I + P	5.90 ± 0.78	34.94 ± 3.55	1.20 ± 0.24	2.31 ± 0.93
ANOVA	Phenotype (Ph)	NS	$P < 0.0001$	$P < 0.0001$	$P < 0.0004$
	Diet (D)	$P < 0.001$	$P < 0.0001$	$P < 0.05$	NS
	Ph × D	NS	$P < 0.003$	NS	NS

Values are means ± SEM of 8 rats.
Abbreviations as in Table 1.

Table 3
Effects of soy isoflavones and probiotics on plasma glucose, cholesterol, and triglycerides in a genetic model of obesity and diabetes

Phenotype	Diet	Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	LDL cholesterol (mmol/L)
Lean	Casein	2.15 ± 0.25	0.63 ± 0.07	1.47 ± 0.002
	Isoflavones (I)	1.61 ± 0.23	0.47 ± 0.06	1.09 ± 0.002
	Probiotics (P)	1.92 ± 0.28	0.50 ± 0.06	1.13 ± 0.001
	I + P	1.49 ± 0.23	0.43 ± 0.06	1.04 ± 0.001
Obese	Casein	4.85 ± 0.32	0.69 ± 0.07	3.82 ± 0.002
	Isoflavones (I)	3.78 ± 0.29	0.82 ± 0.07	2.91 ± 0.001
	Probiotics (P)	4.84 ± 0.25	1.10 ± 0.07	3.71 ± 0.001
	I + P	3.25 ± 0.25	0.55 ± 0.07	2.63 ± 0.002
ANOVA	Phenotype (Ph)	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
	Diet (D)	$P < 0.0001$	$P < 0.0001$	$P < 0.004$
	Ph × D	NS	$P < 0.06$	NS

Values are means ± SEM of 8 rats.
Abbreviations as in Table 1.

and LDL cholesterol in both lean and obese rats. Probiotics had small and nonsignificant effect on lowering HDL and LDL cholesterol in lean but not in obese rats. However, the effect was not statistically significant. No significant changes were observed in total/HDL and total/LDL cholesterol ratios among any groups.

Table 4 summarizes the effects of isoflavones and probiotics on steroid hormones. Plasma androstenedione and testosterone levels were significantly lower in obese rats than in lean phenotypes, whereas corticosterone was significantly higher in obese rats than in lean phenotypes. Aldosterone was also higher in obese rats but the difference did not reach statistical significance. Isoflavones given alone decreased plasma testosterone levels in lean and obese rats, but probiotics alone were effective in lowering plasma testosterone levels only in lean rats. There was a significant interaction between the effects of isoflavones and probiotics on plasma testosterone, inasmuch as both given together reduced levels in lean rats but increased the testosterone in obese rats.

As shown in Table 5, there was a significant phenotypic effect on plasma levels of T3 and T4. Plasma T3 was higher in obese rats but T4 was lower in obese rats than lean phenotypes. The plasma levels of TSH were highly variable among different groups. Similarly large differences were observed within groups.

4. Discussion

In the present study we observed significant effect of isoflavones on body weight in lean as well as obese rats. However, there was no effect on food intake. In genetically obese mice and in aromatase knockout (ER-deficient) mice, soybean or isoflavones have been reported to reduce body weight [28,29]. In ovariectomized mice the isoflavone genistein had no significant effect on body weight or food intake [30]. A decrease in body weight was observed only with high intake of genistein [30]. We observed a decrease in liver, kidney, and spleen weights but not in heart or adrenal gland weight. Naaz et al. did not observe any decrease in muscle weight in mice fed genistein [30]. Thus,

Table 4
Effects of soy isoflavones and probiotics on plasma steroid hormones in a genetic model of obesity and diabetes

Phenotype	Diet	Total estrogen (pmol/L)	Androstenedione (nmol/L)	Testosterone (nmol/L)	DHEA (nmol/L)	Corticosterone (nmol/L)	Aldosterone (pmol/L)
Lean	Casein	298.22 ± 37.04	4.82 ± 0.25	4.35 ± 0.79	2.00 ± 0.09	1142.90 ± 219.66	1403.12 ± 303.01
	Isoflavones (I)	274.97 ± 34.65	4.80 ± 0.53	2.60 ± 0.69	1.83 ± 0.05	799.20 ± 219.66	959.77 ± 283.44
	Probiotics (P)	311.79 ± 34.65	4.42 ± 0.24	1.99 ± 0.34	1.89 ± 0.06	795.17 ± 135.49	1469.10 ± 327.29
	I + P	277.04 ± 34.65	5.15 ± 0.49	2.08 ± 0.29	2.01 ± 0.09	752.06 ± 144.84	1009.02 ± 108.31
Obese	Casein	282.06 ± 40.00	3.78 ± 0.25	0.63 ± 0.14	1.97 ± 0.09	1611.31 ± 171.38	1775.23 ± 327.29
	Isoflavones (I)	341.85 ± 37.04	4.11 ± 0.25	0.26 ± 0.10	2.06 ± 0.09	1622.87 ± 219.66	1652.03 ± 303.01
	Probiotics (P)	312.68 ± 40.00	4.08 ± 0.25	0.53 ± 0.16	1.94 ± 0.16	1930.99 ± 219.66	1681.36 ± 303.01
	I + P	230.30 ± 40.00	4.79 ± 0.23	0.80 ± 0.34	2.08 ± 0.12	1800.52 ± 144.84	1210.45 ± 108.31
ANOVA	Phenotype (Ph)	NS	$P < 0.015$	$P < 0.0001$	NS	$P < 0.0001$	NS
	Diet (D)	NS	NS	NS	NS	NS	$P < 0.047$
	Ph × D	NS	NS	$P < 0.05$	NS	NS	NS

Values are means ± SEM of 8 rats.
Abbreviations as in Table 1.

Table 5
Effects of soy isoflavones and probiotics on plasma thyroid hormones in a genetic model of obesity and diabetes

Phenotype	Diet	T3 (nmol/L)	T4 (nmol/L)	TSH (mU/L)
Lean	Casein	1.42 ± 0.11	60.20 ± 2.93	3.86 ± 0.54
	Isoflavones (I)	0.93 ± 0.11	64.49 ± 5.37	3.70 ± 1.08
	Probiotics (P)	0.97 ± 0.11	53.64 ± 5.37	1.35 ± 0.54
	I + P	0.92 ± 0.12	58.42 ± 5.03	1.18 ± 0.54
Obese	Casein	1.30 ± 0.22	9.05 ± 2.93	2.57 ± 0.42
	Isoflavones (I)	1.20 ± 0.21	16.39 ± 2.93	3.41 ± 0.79
	Probiotics (P)	1.42 ± 0.21	14.37 ± 5.08	5.85 ± 2.48
	I + P	1.34 ± 0.22	17.13 ± 2.93	1.25 ± 0.88
ANOVA	Phenotype (Ph)	$P < 0.035$	$P < 0.0001$	NS
	Diet (D)	NS	NS	NS (0.081)
	Ph × D	NS	NS	NS

Values are means ± SEM of 8 rats.
Abbreviations as in Table 1.

the effect of isoflavones appear to be tissue specific and may also depend on species and gender. Our previous studies have shown no significant differences in body weight or tissue weight between rats fed casein or soy protein concentrate [31,32]. Thus, it appears that isoflavones but not the type of protein affect the body weight or the tissue weights.

The striking finding in our study is the significant reduction in all four fat depots measured in the study (namely, subdiaphragmatic, perirenal, ileal, and epididymal fat pads) by isoflavones. Our results are in agreement with those observed by Naaz et al. [30]. Adipose tissue metabolism is under the control of estrogen and the present study show that soy isoflavones have similar effect even though phytoestrogen have several-fold less estrogenic and antiestrogenic effect than the endogenous estrogen estradiol. In addition, genistein has been shown to decrease lipoprotein lipase mRNA in parametrial fat pads indicating antilipogenic effect [30]. Whether isoflavones also affect lipolysis is not clear. Other isoflavones, daidzin, and glycetin also affect adipose tissue weight [33]. High consumption of isoflavones in postmenopausal women has been shown to have lower body mass index than those consuming little or no isoflavones [34]. In addition to isoflavones, saponins also inhibit fat deposition in adipose tissues [35]. Though we have not measured the effect of isoflavones on fat accumulation in liver in the present study, we have previously shown that soy protein concentrate containing isoflavones, reduced fat accumulation in liver as compared to casein [32].

There are numerous studies showing hypocholesterolemic effects of soy protein in humans and animals [10,11,36–40]. The data in the present study show that isoflavones also reduce total as well as LDL and HDL cholesterol in rats. This confirms the earlier findings by others that isoflavones lower total and LDL cholesterol in humans and animals [37,41–46]. However, the effect of isoflavones on HDL cholesterol is equivocal. In many studies a small increase in HDL cholesterol is observed after feeding soybean or isoflavones alone, whereas in others either no change or a small decrease in HDL cholesterol is observed as in the present study [32,37,44–46]. In ovariectomized cynomolgus monkeys soy protein but not isoflavones have a hypocholesterolemic effect, which may be due to decreased cholesterol absorption [47]. In healthy men and postmenopausal women, soy protein isolate lowered LDL/HDL cholesterol ratio and increased LPa compared to casein but had no significant effect on total, LDL, or HDL cholesterol compared to casein [48]. Besides protein and isoflavones, other constituents of soybean may also be responsible for lowering cholesterol.

There are reports that probiotics lower LDL cholesterol, but the data are conflicting [49–54]. Agerholm-Larsen et al. [55] reported that the consumption of yogurt fermented with different probiotics had variable effects on LDL cholesterol in obese subjects. For example, they observed that a yogurt fermented with one strain of *Enterococcus faecium* and two strains of *Streptococcus thermophilus* reduced LDL cholesterol by 8.4%, an effect that was different from that in yogurt fermented with different strains, suggesting that hypolipidemic effect is dependent on the type of probiotics. Other studies have also shown that some but not all strains of probiotic microorganisms reduce cholesterol levels in humans and animals [56,57]. In the present study we used probiotic mixture containing *Lactobacillus acidophilus, Lactobacillus casei,* and *Bifidobacterium bifidum* but found no significant effect on body weight, fat deposition, plasma cholesterol levels, or steroid and thyroid hormones. Whether other types of probiotic mixtures containing different bacterial strains (other than used in the present study) have effects on lipid metabolism deserves further investigation.

Several previous studies in humans and animals have suggested that dietary soy protein or isoflavones affect the endocrine system or circulating hormones that may affect obesity or lipid metabolism. For example, Weber et al. [58] have reported that phytoestrogens from soy decreased plasma testosterone and androstenedione in rats. In human studies in premenopausal women, isoflavones have been

shown to decrease estradiol and estrone sulfate levels but to elevate serum hormone binding globulin (SHBG) levels [59,60]. However, in hypercholesterolemic postmenopausal women, soy protein isolate containing isoflavones had no effect on estradiol, estrone sulfate, or SHBG [61]. Nagata et al. [62] reported that in Japanese men soy milk lowered estrone levels but had no effect on estradiol, testosterone, or SHBG. Thus isoflavone intake affects estrogen metabolism by altering some steroidal hormones and thereby affects menstrual cycle length [60]. However, the effects of isoflavones on plasma hormones do not appear to be of clinical significance [59].

In the present study isoflavones had no significant effect on any of the three thyroid hormones. Klein et al. [63] reported that soy protein isolate had no effect on T_3 and free T_3 but significantly decreased T_4 compared to casein in rats. Forsythe [64] reviewed the effect of soy protein on thyroid hormone regulation and plasma cholesterol levels and observed that soy protein increased T_4, and that this increase in T_4 may be responsible in part for cholesterol lowering effect of soy protein. Many other studies have also reported that soy protein affect T_3, T_4, and TSH in animals and humans, but the effects were inconsistent [64–69]. In hyperlipidemic postmenopausal women isoflavones have been shown to increase T_3, T_4, and TSH, but the increase may not of clinical significance [61]. However, in premenopausal women daily intake of isoflavones decreased free T_3 during early follicular phase [70]. Thus both soy protein and isoflavones affect thyroid hormones. However, there was no significant effect of probiotics, given alone or with isoflavones, either on lipid metabolism or on steroid and thyroid hormones.

In summary, the present study has shown that ingestion of isoflavones affect lipid metabolism. The strains of probiotics used in the present study do not appear to have significant effect on body weight, fat deposition, lipid metabolism, or the endocrine system. In addition, they do not seem to potentiate the effects of isoflavones on these parameters.

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