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Effects of soy protein and isoflavones on insulin resistance and adiponectin in male monkeys

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Abstract

Isoflavones may influence insulin action by means of their well-known receptor-mediated estrogenic activity. However, isoflavones also bind to peroxisome proliferator–activated receptors (PPARs) that are strongly associated with insulin action. Soy protein with its isoflavones has previously been shown to improve glycemic control in diabetic postmenopausal women and to improve insulin sensitivity in ovariectomized monkeys. The purpose of the current report was to extend our studies of dietary soy protein to male monkeys and determine effects of the soy isoflavones on insulin resistance. Two studies are reported here. Study one involved 91 male monkeys consuming 3 diets differing only by the source of protein (casein-lactalbumin, soy protein with a low isoflavone concentration, or soy protein with a high isoflavone concentration). Intravenous glucose tolerance tests were done, and plasma adiponectin and lipoprotein concentrations were determined after 25 months of study. Samples of visceral fat were obtained at 31 months for assessment of adiponectin and PPARγ expression. The second study involved 8 monkeys in a Latin-square design that compared the effects of diets with casein/lactalbumin, soy protein with a high isoflavone concentration, or soy protein that was alcohol-washed to deplete the isoflavones. After 8 weeks of treatment, insulin sensitivity and plasma lipoproteins were assessed. At 10 weeks, a biopsy of the skeletal muscle was performed for determination of insulin receptor, PPARα, and PPARγ content. The major findings were that consumption of isoflavone-containing soy protein dose-dependently increased insulin responses to the glucose challenge and decreased plasma adiponectin, whereas isoflavone-depleted soy protein decreased body weight and had no effect on plasma adiponectin concentrations. Muscle PPARα and γ expression was also increased with the isoflavone-depleted soy relative to either casein or soy protein containing the isoflavones. Further studies are needed to determine the mechanisms involved in these effects of a high–soy isoflavone diet and to optimize dietary isoflavone content for maximal health benefits in male subjects.

1. Introduction

Current estimates are that 50% of the adult population of the United States is obese [1]. About half of the obese population also has prediabetes or the metabolic syndrome [2]. Insulin resistance and obesity are key features of the metabolic syndrome and type 2 diabetes mellitus (T2DM) [2,3]. One potential mechanism involves the production of hormones or adipokines by adipose tissue. Plasma concentrations of many adipokines, such as leptin, tumor necrosis factor–α, and plasminogen activator inhibitor–1, have been associated positively with insulin resistance, whereas adiponectin is associated negatively [4,5]. Furthermore, lower plasma concentrations of adiponectin are associated with increased incidence of metabolic syndrome, diabetes, and vascular disease [5-7].

Pharmacologic agents, such as thiazolidinediones, are potent agonists of peroxisome proliferator–activated receptor (PPAR) γ and have become useful clinical tools to improve insulin resistance and raise adiponectin concentrations [5,7]. Interestingly, the isoflavones genistein and daidzein, plant estrogens found in soy beans and processed soy protein, have also been shown to bind to PPARγ as well as PPARα and δ [8-10], suggesting the potential value of

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isoflavones as a nutritional approach to modulating insulin action. Soy is the most commonly used botanical in the United States; and the Food and Drug Administration has approved a health claim for soy protein and soy-based food products based largely on the evidence that soy consumption improves plasma lipid and lipoprotein concentrations and might reduce risk of coronary heart disease, yet does not appear to increase cancer risk [11].

The earliest report of soy beans having beneficial effects on glycemic control was in 1910 [12], when the consumption of soy beans was found to decrease glycosuria in diabetics. Until relatively recently, however, there have been few studies of the effects of soy or its isoflavones on glycemic indices. Among the somewhat limited data is the finding that consumption of soy protein containing isoflavones was associated with improved lipoprotein and glycemic control in T2DM postmenopausal women [13]. In nondiabetic postmenopausal women, soy consumption was associated with decreased fasting insulin concentrations; and isoflavone intake was inversely associated with postchallenge insulin concentrations [14]. Similarly, we have found improved plasma lipoprotein profiles and insulin sensitivity in premenopausal monkeys fed soy-rich diets [15]. However, in smaller studies of predominantly male T2DM subjects (14 men and 6 women), the improvement in lipoprotein profiles has been seen, but not the improved glycemic control [16]. Similarly, in a small study of diabetic and nondiabetic male monkeys, soy protein improved plasma lipoproteins and atherosclerosis but did not affect glycemic control [17].

These few studies suggest that the benefits of soy on carbohydrate metabolism are more apparent in female than male subjects. The studies did not assess potential mechanisms involved with changes in insulin action or whether the effect is due to the soy protein, its isoflavones, or both. In addition to the isoflavones binding to PPAR, they have estrogenic activity, binding to both estrogen receptor (ER) α and ERβ, but with greater affinity to ERβ [11]. Genistein is also a tyrosine kinase inhibitor [18], so high concentrations may inhibit insulin signaling pathways.

The purpose of the current studies was to extend our studies of dietary soy protein and isoflavones to address their effects on insulin resistance in male subjects. Furthermore, because adiponectin is strongly associated with insulin action and is also regulated by PPARγ, we explored whether changes in insulin resistance were related to changes in plasma concentrations of this adipokine.

2. Methods

2.1. Animal studies

2.1.1. Study 1

Ninety-one adult male cynomolgus monkeys (*Macaca fascicularis)* were imported from Indonesia (Institut Pertanian Bogor). Effects on plasma lipoprotein and isoflavone concentrations and the cardiovascular system have been reported previously [19]. All monkeys consumed a Western-type diet differing only by the source of dietary protein [19]. The major source of protein was casein-lactalbumin (casein, n = 30) for group 1, a mixture of unmodified soy protein isolate and alcohol-washed (isoflavone-depleted) soy protein isolate approximating human intake of 75 mg isoflavones per day (low ISO, n = 30) for group 2, and unmodified soy protein isolate containing an amount approximating human intake of 150 mg isoflavones per day (high ISO, n = 31) for group 3. Other than protein source, diets were equal in macronutrients, with 19% of calories from protein, 35% from lipid (0.28 mg cholesterol per kilocalorie), and 46% from carbohydrates. Detailed descriptions of the diet compositions have been published previously [19]. Glucose and insulin responses to an intravenous glucose tolerance test (IVGTT) and plasma adiponectin concentrations were determined after 25 months of study as described previously [20]. Visceral fat samples were collected after 31 months of treatment.

2.1.2. Study 2

Eight old, obese, hyperinsulinemic male monkeys were used in this study (note increased body weight and fasting insulin of monkeys in Table 2 vs Table 1). The study was a 3-phase Latin-square design, such that each monkey received each diet after a baseline period during which animals consumed the control diet. Animals were randomized to one of 3 diet groups containing (1) casein, (2) alcohol-washed (isoflavone-depleted) soy (SOY−, 8 mg isoflavones per day human equivalent), or (3) intact soy protein (SOY+, 132 mg isoflavones per day human equivalent). Diets were equal in macronutrients with the exception of the protein source and were composed of 19% of calories from protein, 20% from lipid (0.19 mg cholesterol per kilocalorie), and 61% from carbohydrates. Each phase lasted 10 weeks.

The first phase of diet interventions began the week after baseline measures were completed. After 4 and 8 weeks of treatment, measurement of body weight and blood collection for analysis of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) concentrations were done. At 8 weeks, minimal model analysis (frequently sampled IVGTT) was performed [21]. At 10 weeks of treatment, animals were sedated for measurement of body weight, plasma lipids, and lipoproteins, and for muscle biopsy of the vastus lateralis muscle.

All procedures involving animals were conducted in compliance with state and federal laws, standards of the US Department of Health and Human Services, and guidelines established by the Institutional Animal Care and Use Committee.

2.2. Clinical chemistry measures

Animals were fasted overnight and sedated with ketamine hydrochloride (15 mg/kg intramuscularly) (Ketaset; Fort

Dodge Animal Health, Fort Dodge, IA) before blood collection. Total cholesterol, HDL-C, and TG were determined by enzymatic techniques [19]. Lipoprotein fractions were separated by ultracentrifugation and high-performance liquid chromatography, and the cholesterol content of each fraction was determined enzymatically [19]. Plasma glucose, fructosamine, insulin, C-peptide, leptin, and adiponectin were determined as described [20-22].

Measurements for the IVGTT included the glucose area under the curve (AUC), calculated as the total area including all time points; the disappearance rates (*K* value) for glucose and insulin were calculated from the linear portion of the curve [20]. Measurements for the frequently sampled IVGTT included the insulin sensitivity index as described previously [15,21].

2.3. Western blot analyses

Biopsies of visceral fat (study 1) and skeletal muscle (study 2) were snap frozen in liquid nitrogen and stored at −70°C until processed as described previously [23]. Fat samples were assessed for adiponectin (mouse monoclonal antiadiponectin; BioVision Research Products, Mountain View, CA) and PPARγ (rabbit polyclonal anti-PPARγ; Santa Cruz Biotechnology, Santa Cruz, CA). Skeletal muscle homogenates were assessed for insulin receptor (IR) expression (mouse monoclonal anti-IR; Research Diagnostics, Flanders, NJ). Basal IR activity was determined using a phosphorylation state–specific antibody generated against the phosphorylated tyrosine residue 1158 of the human IR (anti-IRpY1158; Biosource International, Camarillo, CA). The blots were also probed with anti-PPARα (rabbit polyclonal anti-PPARα, Santa Cruz Biotechnology) or anti-PPARγ antibody (rabbit polyclonal anti-PPARγ; Biomol International, Plymouth Meeting, PA). To account for equal protein loading, blots were stripped (Re-Blot Plus; Chemicon International, Temecula, CA) and reprobed for actin in muscle (monoclonal actin Ab-1; Oncogene Research Products, Boston, MA) or guanidine disassociation inhibitor in fat (rabbit anti-Rho guanidine disassociation inhibitor polyclonal antibody, Santa Cruz Biotechnology) [24]. As described previously [23], signals were detected using a Storm Phosphorimager 860 (Molecular Dynamics, Sunnyvale, CA); and densitometry was quantified using ImageQuant Software (Version 5.2; Amersham Biosciences; Sunnyvale, CA). Densitometry results are presented as arbitrary scanning units after correcting for loading; however, results were similar regardless of this correction.

3. Statistics

All data are reported as mean ± SEM.

3.1. Study 1

The glucose tolerance test outcomes (glucose response curve and insulin response curve) were analyzed using linear mixed effects models. Other outcomes were analyzed using 1-way analysis of variance, adjusting for baseline measure when available.

3.2. Study 2

As the study design was a Latin-square design, treatment means were assessed for time trends, treatment by phase interactions, and homogeneity of variance (Levene). Data were analyzed for the difference between treatments by paired *t* test because there were no treatment by period interactions. Analyses were performed by SAS 9.1 (Cary, NC).

4. Results

4.1. Study 1

There were no treatment effects on fasting glucose, insulin, or overall glycemic control as assessed by fructosamine concentrations (Table 1). As reported previously [19], plasma low-density lipoprotein cholesterol (LDL-C) was decreased by 21% and 17% and HDL-C was increased by 36% and 18% in the groups fed low ISO and high ISO, respectively (all *P*s < .05). Plasma TG was unaffected [19]. Fig. 1 depicts the glucose and insulin responses to the IVGTT. There was no treatment effect on glucose responses (Fig. 1 and Table 1). However, insulin responses were significantly increased ($P < .05$) with treatment (Fig. 1), with a dose-dependent increase in the maximal insulin response ($P = .01$). The ratio of insulin-glucose AUC, an index of insulin resistance, was 17% and 41% greater with increasing dietary isoflavone content (); but this did not reach statistical significance ($P > .05$). Male monkeys fed high ISO also tended to gain more weight (Table 1). Consistent with an insulin-resistant condition, plasma adiponectin concentra-

Table 1
Study 1: measures (mean ± SEM) for male monkeys consuming protein from casein-lactalbumin (casein), soy with low isoflavones (Low Iso Soy), or soy with high isoflavones (High Iso Soy)

Outcome	Casein (n = 30)	Low Iso Soy (n = 30)	High Iso Soy (n = 31)
Glucose (mg/dL), fasting	74.5 ± 1.68	73.6 ± 1.94	74.2 ± 1.91
Glucose, IVGTT, max	398.4 ± 6.59	399.5 ± 7.82	416.1 ± 6.80
Glucose, IVGTT, K value	3.63 ± 0.19	3.55 ± 0.21	3.87 ± 0.23
Insulin (IU/mL), fasting	28.00 ± 3.73	27.30 ± 3.55	28.92 ± 9.00
Insulin, IVGTT, max	115.6 ± 10.36	134.7 ± 12.49	172.4 ± 14.96 *
Fructosamine, baseline	263.9 ± 6.12	272.9 ± 6.28	271.2 ± 6.05
Fructosamine, treatment	260.1 ± 6.53	252.7 ± 4.61	253.1 ± 6.59
Body weight (kg), baseline	5.51 ± 0.12	5.70 ± 0.13	5.68 ± 0.13
Body weight (kg), treatment	5.95 ± 0.14	6.05 ± 0.16	6.22 ± 0.17
Fat adiponectin (ASU)	0.58 ± 0.07	0.58 ± 0.08	0.58 ± 0.08
Fat PPARγ (ASU)	10.80 ± 0.52	9.70 ± 0.78	11.20 ± 1.05

ASU indicates arbitrary standardized unit.
* $P < .05$ vs casein.

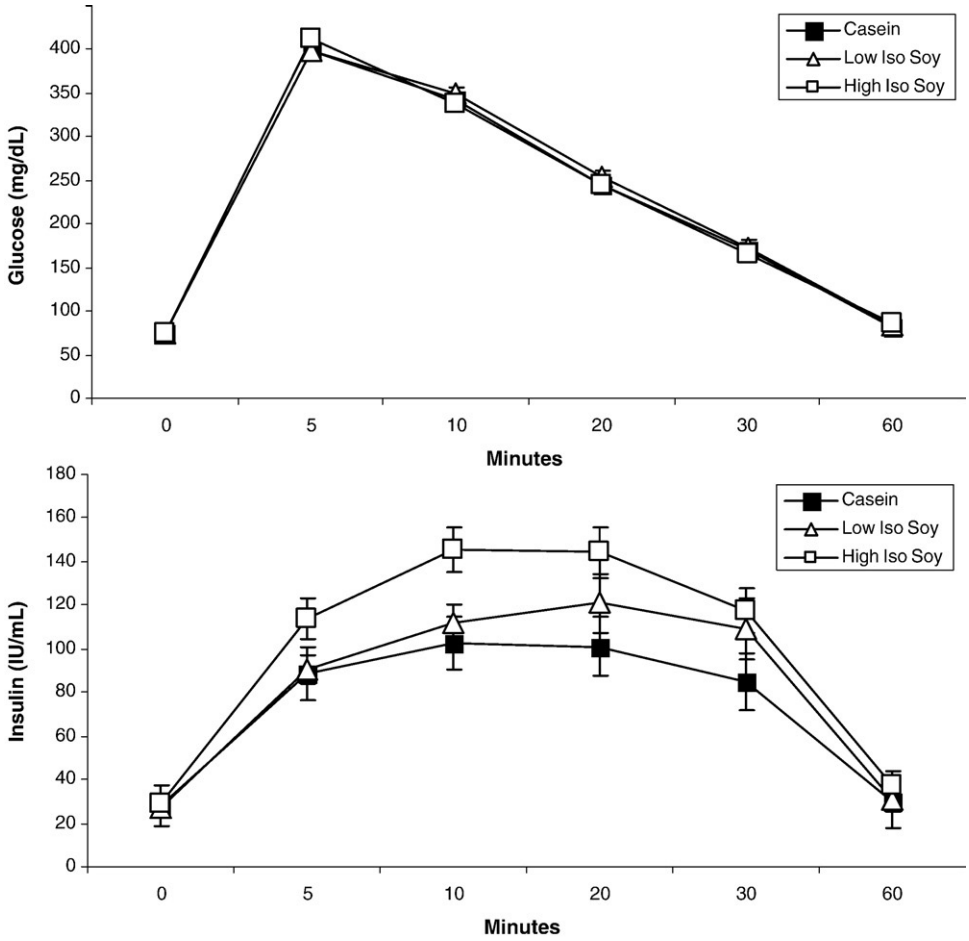

Fig. 1. Glucose (top) and insulin (bottom) responses to an IVGTT for monkeys consuming casein, soy protein with low isoflavone dose (Low Iso Soy), and soy protein with high isoflavone dose (High Iso Soy). There were no changes in glucose response, but a dose-dependent increase in insulin responses was found with isoflavone intake (analysis of variance, $P < .05$). The treatment differences were due to High Iso Soy compared with casein ($P = .03$) and an intermediate response with Low Iso Soy compared with casein ($P = .11$).

tions (Fig. 2) were significantly decreased with isoflavone consumption ($P = .02$).

Despite the lower plasma adiponectin concentrations with soy isoflavones, there was no difference in the abundance of adiponectin in fat, the primary source of circulating adiponectin. Adiponectin levels are controlled in part by PPARγ, the expression of which was also not affected by soy isoflavones (Table 1).

4.2. Study 2

Treatment with SOY− decreased body weight compared with SOY+ ($P = .02$) but not casein ($P = .17$) (Table 2, Fig. 3), despite no effect on leptin concentrations (Table 2). Compared with casein, SOY− treatment resulted in lower LDL-C ($P = .02$) but had no effect on TG or HDL-C. There was no significant effect of SOY+ treatment on LDL-C, HDL-C, or TG compared with casein. There was also no significant effect of SOY− or SOY+ treatment on insulin sensitivity, glucose effectiveness, fasting blood glucose, insulin, or C-peptide concentrations compared with casein (all Ps > .05).

SOY− treatment increased PPARα and PPARγ expression in skeletal muscle compared with casein ($P = .009$, $P = .03$); but SOY+ treatment had no effect ($P = .61$, $P = .86$),

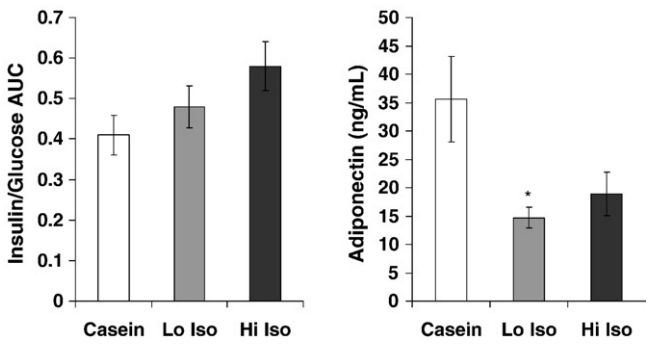

Fig. 2. Changes in insulin resistance (as determined by insulin-glucose AUCs) after a glucose challenge (as depicted in Fig. 1) and plasma adiponectin concentrations with monkeys consuming casein, Low Iso Soy, and High Iso Soy. Adiponectin was significantly less in Low Iso compared with casein ($P = .02$), with a similar trend for High Iso compared with casein ($P = .08$).

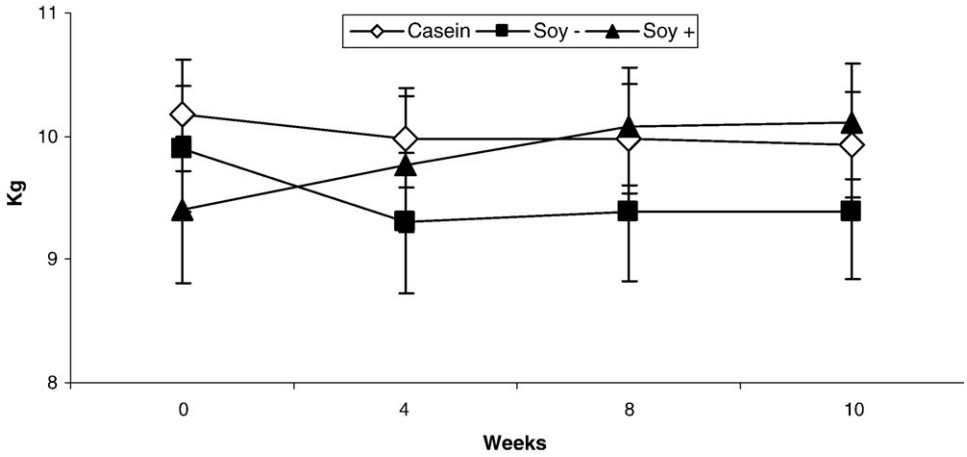

Fig. 3. Change in body weight in monkeys with consumption of casein, SOY−, and SOY+ over a 10-week period using a Latin-square design. Body weights decreased ($P < .05$) with consumption of SOY− compared with SOY+.

suggesting that isoflavones attenuated the effect of soy protein. There were no significant correlations between PPARα and PPARγ expression and insulin sensitivity, or fasting glucose and insulin concentrations, or body weight.

SOY+ treatment resulted in less skeletal muscle IR expression compared with casein treatment ($P = .01$), with a similar tendency for SOY− treatment ($P > .05$). However, there was no difference in basal IR activity (tyrosine phosphorylation) with either of the soy treatments (Table 2).

5. Discussion

The major findings from these studies are that, in male monkeys, consumption of soy protein with its isoflavones increases insulin secretion after a glucose challenge (Fig. 1). Despite the increased insulin secretion, there were no changes in glucose disposal and a dose-dependent increase in the ratio of insulin-glucose AUCs determined from the IVGTTs (Fig. 2), indicating an increase in peripheral insulin resistance due to the isoflavones. Furthermore, consistent with an insulin resistance state, there is a significant decrease in plasma adiponectin concentrations with soy isoflavones (Fig. 2). In contrast, consumption of isoflavone-depleted soy protein resulted in loss of body weight and no effect on plasma adiponectin concentrations. Muscle PPARα and γ expression was also increased in this group compared with either casein or soy protein containing the isoflavones.

The increase in insulin secretion after the glucose challenge was not unexpected. As earlier studies have shown with estradiol [25], studies of genistein [26,27] and, to a lesser extent, daidzein [27] found increased insulin secretion from islet preparations. A more recent study by Liu et al [28] found that genistein increases glucose-stimulated insulin secretion in cell lines and mouse pancreatic islets at micromolar concentrations via a cyclic adenosine monophosphate–dependent protein kinase mechanism. This action could be beneficial and may be the basis for the early report suggesting clinically relevant decreases in glucosuria in diabetics [12], consistent with a secretagogue-like effect. However, in the current studies, the lack of increased glucose removal despite the increased insulin secretion indicates peripheral insulin resistance.

Potential mechanisms for increased insulin resistance could relate to the fact that genistein is a potent tyrosine kinase inhibitor for both platelet-derived growth factor and epidermal growth factor [18,29]. Our data (Table 2) and those of others [30] suggest that basal IR activity is not affected by soy isoflavones. However, high concentrations of genistein could be inhibitory, whereas daidzein, which is not a tyrosine kinase inhibitor, likely would not be inhibitory.

Other postulated mechanisms that could increase peripheral insulin resistance include changes in insulin receptor number, affinity, intracellular phosphorylation, and altera-

Table 2
Study 2: measures (mean ± SEM) for male monkeys consuming protein from casein-lactalbumin (casein), soy washed to remove isoflavones (SOY−), or soy with high isoflavones (SOY+)

Outcome	Casein (n = 8)	SOY− (n = 8)	SOY+ (n = 8)
Glucose (mg/dL), fasting	67.8 ± 4.8	81.9 ± 6.0	69.9 ± 5.2
Insulin (IU/mL), fasting	54.3 ± 8.6	53.0 ± 16.5	42.5 ± 8.8
C-peptide (ng/mL)	9.87 ± 2.7	7.31 ± 3.1	6.83 ± 1.7
SI (10^{-4} min^{-1} mU^{-1} mL)	3.10 ± 1.15	3.19 ± 0.85	2.85 ± 1.11
Glucose effectiveness (min^{-1})	0.037 ± 0.010	0.036 ± 0.014	0.020 ± 0.006
HDL-C (mg/dL)	71.8 ± 11.6	71.3 ± 12.1	71.5 ± 16.5
LDL-C (mg/dL)	106.1 ± 18.5	78.9 ± 15.4 *	96.3 ± 16.1
Adiponectin (ng/mL)	4.26 ± 1.39	5.42 ± 2.08	3.96 ± 1.43
Fasting leptin (ng/mL)	53.5 ± 11.9	40.6 ± 5.2	40.3 ± 6.4
Body weight (kg)	9.99 ± 0.43	9.53 ± 0.55 **	10.22 ± 0.47
Muscle IR activity (ASU)	16794 ± 645	31387 ± 3350	29454 ± 5317
Muscle IR expression (ASU)	4726 ± 820	2879 ± 268	2721 ± 596 *

SI indicates insulin sensitivity index.
* $P < .05$ vs casein.
** $P < .05$ vs SOY+.

tions in the glucose transport apparatus [31-36]. Insulin receptor number was found to be decreased in rat livers perfused with genistein [31]. This result is consistent with the in vivo finding reported here for skeletal muscle (Table 2). Other effects that could be detrimental to insulin action include genistein-induced inhibition of Glut4 translocation in rat adipocytes [32] and effects on glucose oxidation [30]. These inhibitory effects on hormone signal transduction could be due to inhibition of other protein kinases, such as those with adenosine triphosphate binding at the catalytic sites [30]. In support of this, genistein has been shown to inhibit Akt kinase activity [33]; and effects of soy diet on Akt activity has also been shown to result in worsening of heart disease in male but not female mice [34].

In vitro studies have shown that soy isoflavones increase expression of PPARs [8-10]. In murine macrophage-like RAW 264.7 cells expressing a peroxisome proliferator response element–containing reporter and either PPARα or PPARγ plasmids, unconjugated genistein and daidzein increased both PPARα- and γ-directed gene expression [10]. Consistent with a PPARγ effect, when obese Zucker rats were fed diets containing soy protein with isoflavones, the animals had improved lipid metabolism and glucose tolerance; but they gained weight consistent with PPARγ agonist treatment [10].

Genistein (>1 μm) was also shown to act as a ligand for PPARγ in mesenchymal progenitor cells (precursor cells for osteoblasts and adipocytes), resulting in up-regulation of adipogenesis and down-regulation of osteogenesis. Transfection experiments showed that activation of PPARγ by genistein at micromolar concentrations down-regulates its estrogenic transcriptional activity, whereas activation of ERα and ERβ by genistein down-regulates PPARγ transcriptional activity [8]. These same investigators reported similar effects with daidzein [9]. In addition, there were concentration-dependent biphasic effects of daidzein on osteogenesis and adipogenesis that were not apparent when ERs were blocked. As well as transactivating PPARγ, daidzein also transactivated PPARα and δ. These studies suggest cross talk between ER and PPAR, with outcomes dependent on the balance between activated ERs and PPARγ.

The PPAR action is modified by cofactors such as PPARγ coactivator–1 (PGC-1). The PGC-1 is also estrogen responsive and may mediate some of the ER transcriptional effects [37]. As isoflavones also have estrogenic activity, some effect on glycemic control may be mediated through PGC-1 [38]. Taken together, these studies suggest an intriguing mechanism pathway whereby soy isoflavones and endogenous hormones may interact to affect PPAR action, resulting in different action in male and female subjects.

Because genistein and daidzein have both been shown to bind to and activate PPARγ [8-10], it is likely that changes in insulin sensitivity could then be modified by adiponectin, which is increased in response to PPARγ agonists [5-7]. Studies in mice have shown that soy protein isolate containing isoflavones increased both plasma concentrations and adipose tissue messenger RNA abundance of adiponectin [39,40]. This is opposite of our finding in monkeys of lower plasma adiponectin concentrations with no difference in adipose tissue expression (Fig. 2, Table 1). The only data we are aware of in humans suggest that soy isoflavones do not affect plasma adiponectin concentrations [41]. Interestingly, low adiponectin levels have been shown to be associated with impaired vasodilation in people [6]. In this study, soy isoflavones did not improve arterial vasodilation in male monkeys [19]; but plasma lipids and atherosclerosis were improved with soy isoflavones in this study [19] and those of others [11].

There are also sex-specific differences in metabolism of soy isoflavones. Stroud et al [42] found that male monkeys had higher plasma genistein, daidzein, and total isoflavones concentrations compared with premenopausal female monkeys fed the same soy isoflavone–containing diet. It is not known how these varying plasma isoflavone concentrations relate to different tissue levels, but it is likely that tissue differences occur.

Although soy isoflavones have often been thought to be the active, beneficial ingredient of soy beans, others have proposed components of soy protein to be the health-beneficial component. For example, Moriyama et al [43] suggest that soy protein, in particular the 7S component, has therapeutic benefits for treatment of obesity and metabolic syndrome. Likewise, Lovati et al [44] have shown beneficial effects with the 7S component on lipoprotein metabolism. Thus, it is likely that, although soy protein both is heart-healthy and improves a number of aspects of the metabolic syndrome, these effects are attenuated by the soy isoflavones in male but not necessarily in female subjects. We show here that the isoflavones resulted in a dose-dependent increase in insulin resistance in male monkeys (Fig. 1) and lower plasma adiponectin concentrations (Fig. 2). However, the smaller study with isoflavone-depleted soy (study 2) resulted in lower body weight (Fig. 3) and greater muscle PPARα and γ expression (Fig. 4). Future studies with a comparison of soy

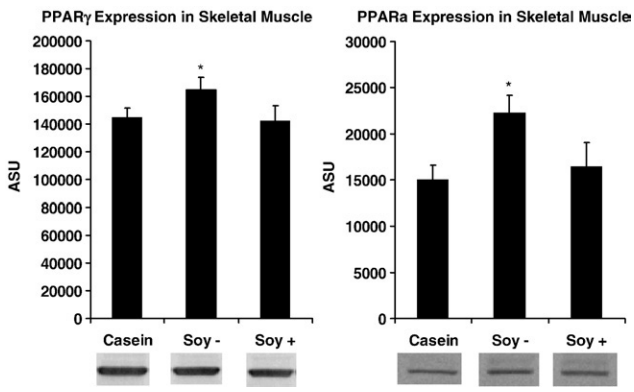

Fig. 4. The PPARγ and PPARα expression in skeletal muscle of monkeys with consumption of casein, SOY−, and SOY+ after a 10-week period using a Latin-square design. Expression is significantly greater ($P < .05$) for SOY− compared with casein.

protein, in particular the 7S component, and soy protein containing lower isoflavone concentrations would be warranted, especially in male subjects, where the plasma isoflavone concentrations are higher than in female subjects. The adverse effects of a high–soy isoflavone diet on insulin and glucose metabolism in male monkeys are of potential public health relevance, and studies are needed to determine the mechanisms involved and to optimize dietary isoflavone content for maximal health benefits.

Acknowledgment/Conflict of Interest

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