The Soy Isoflavone Genistein Decreases Adipose Deposition in Mice

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Adipose tissue is responsive to estrogen and expresses both estrogen receptor α and β. To test the hypothesis that the estrogenic soy isoflavone genistein can have effects on adipose tissue, juvenile or adult C57BL/6 mice were ovariectomized and given daily injections of vehicle, 17β-estradiol (5 μg/kg/d) or genistein (8–200 mg/kg/d) sc for 21–28 d. To test effects of dietary genistein, 25- to 27-d-old mice were fed diets containing 0–1500 parts per million (ppm) genistein for 12 d. Mice were killed and fat pads weighed. Parametrial fat pads were used for morphometric and Northern analysis. Genistein injections decreased adipose weight and adipocyte circumference at higher doses; effects in adult and juvenile mice were similar. Genistein decreased lipoprotein lipase mRNA, which may be a critical aspect of its adipose effects. Juveniles fed 500-1500 ppm dietary genistein had dose-responsive decreases in fat pad weights of 37–57%, compared with controls; 300 ppm genistein did not cause decreases. Genistein doses of 300, 500, 1000, and 1500 ppm produced serum genistein concentrations of 1.02 ± 0.14 μM, 1.79 ± 0.32 μM, 2.55 ± 0.18 μM, and 3.81 ± 0.39 μM, respectively. These results indicate dietary genistein at 500-1500 ppm produces antilipogenic effects in mice at serum levels that humans are realistically exposed to. (Endocrinology 144: 3315–3320, 2003)

The factors that regulate white adipose tissue are of interest because of increasing obesity and obesity-related diseases (1). Adipose tissue is highly responsive to estrogen. Human and mouse adipose tissue expresses both estrogen receptor (ER)α and ERβ (2–5). Loss of circulating estrogen after ovariectomy leads to increased body and adipose weights, and this is prevented or reversed by estrogen replacement (6). Postmenopausal adipose increases in women can be similarly ameliorated or reversed by estrogen replacement (7). ERα knockout (αERKO) mice or mice lacking endogenous estrogen synthesis because of deletion of the aromatase or FSH receptor gene have more than a 100% increase in body fat (6–10), further confirming the role of estrogen in adipose tissue.

Estrogen can affect adipose tissue indirectly through modulating appetite (11) or energy expenditure (8, 9). Estrogen also directly decreases the activity of lipoprotein lipase (LPL), a lipogenic enzyme that regulates adipocyte lipid uptake. Ovariectomy increases LPL activity and lipid deposition in adipocytes, and 17β-estradiol (E2) reverses this (12). Similarly, LPL mRNA is increased in aromatase knockout mice lacking estrogen (13). Recently a negatively controlled estrogen response element that may mediate estrogen actions on lipogenesis has been reported in the LPL promoter element (14).

Phytoestrogens are plant-derived estrogens that can bind to ERα and ERβ and mimic the actions of E2 on target tissues. The isoflavone genistein is a phytoestrogen found in high concentrations in soy and soy products (15) and is a major source of phytoestrogen exposure for both humans and animals. Genistein is estrogenic in vivo and causes uterine growth, both in intact and ovariectomized animals, and stimulates growth of estrogen-responsive tumors (16, 17). Genistein binds both ERα and ERβ, although binding affinity is greater for ERβ than ERα (18). In addition to estrogenic effects, genistein has effects on protein tyrosine kinases, apoptosis, cell proliferation, and angiogenesis (19–21) and could potentially affect adipose tissue through these mechanisms.

Genistein and other soy isoflavones are consumed by Oriental populations at levels up to 1 mg/kg body weight (BW) per day, and human infants fed soy formula consume even higher quantities of isoflavones on a per-weight basis (22). Postmenopausal women consuming soy/isoalvone supplements as an alternative to hormone replacement therapy can also take in isoflavone levels that exceed those in people consuming a high-soy diet (23, 24). In addition, swine typically consume soy-rich diets, and certain pet foods used for companion animals such as cats contain high isoflavone levels (25). Thus, various human and animal populations are exposed to high amounts of genistein and other isoflavones, which might have estrogenic effects on adipose tissue.

The present study examined the effects of injected and dietary genistein on adipose tissue in mice. Our results indicate that genistein has clear antilipogenic effects on adipose tissue in mice, even when fed at levels that produce serum concentrations within the range reported for humans under various nutritional conditions.

Abbreviations: BW, Body weight; DMSO, dimethyl sulfoxide; E2, 17β-estradiol; ER, estrogen receptor; αERKO, ERα knockout; LC-ES/MS, liquid chromatography electrospray mass spectrometry; LPL, lipoprotein lipase; QPCR, quantitative PCR.
**Materials and Methods**

**Study 1: effects of injected genistein on adult females**

**Animals and treatment.** All experiments were approved by the Institutional Animal Care and Use Committee of the University of Illinois. Age-matched (12–13 wk old) nulliparous C57/BL6 female mice were purchased from Harlan (Indianapolis, IN), individually caged, and maintained under standard conditions in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Mice were ovarioectomized, and 5 d later placed on a semipurified phytoestrogen-free, casein-based diet (AIN-93G). Beginning a week after ovarioectomy, mice were given daily sc injections of DMSO vehicle (0.02 ml), E2 (5 μg/kg BW/d) or genistein (200 mg/kg BW) for 21 d. Feed consumption was measured in the control; E2, and 20, 80, and 200 mg/kg genistein groups from a week after ovarioectomy until end of treatment. At the end of treatment, animals were weighed and killed and three fat pads (inguinal, parametrial, and perirenal) were collected and weighed. The parametrial fat pad was snap frozen in liquid nitrogen for subsequent quantitative PCR (QPCR) or Northern analysis. The glutinous musculus muscle from one limb of each mouse used in this experiment was dissected out and weighed to the nearest 0.1 g to test the hypothesis that the changes in adipose tissue following genistein or E2 injections were accompanied by similar decreases in other tissues such as muscle.

To determine whether the effect of genistein on adipose tissue was mediated through ERα, oERKO mice (12–13 wk old) were ovarioectomized and beginning 1 wk later were given 21 daily injections of genistein (80 mg/kg BW/d) or DMSO (control). Mice were then killed, and inguinal fat pad weights were measured in control and genistein-treated mice.

QPCR. RNA from parametrial fat pads was extracted using Trizol reagent (Invitrogen, Carlsbad, CA), quantified, and 2 μg RNA for each sample were reverse transcribed using Retroscript kit (Ambion, Inc., Austin, TX). QPCR was done using the SYBR green assay with SYBR green PCR master mix (Applied Biosystems, Foster City, CA) using the ABI PRISM 7000 sequencing system (Applied Biosystems). Cyclophilin was used as the reference housekeeping gene, and calculations were done as described for the Comparative Method in the User Bulletin 2 of ABI PRISM sequence detection system.

**Northern analysis.** Primers for the LPL cDNA probe were designed using primer designer software from a published nucleotide sequence of the LPL gene (26). The LPL cDNA probe was prepared by RT-PCR using the Retroscript kit (Ambion, Inc.) with mouse adipose tissue total RNA as template. The PCR products were run on a 1% agarose gel and extracted using the Qiaquick gel extraction kit (QIAGEN, Valencia, CA). The cDNA concentration was quantified using a spectrophotometer. The cDNA was labeled with 32P-DCTP using the prime-a-gene labeling system (Promega Corp., Madison, WI), and excess probe was removed using the Qiaquick nucleotide removal kit (QIAGEN). Total RNA was extracted from parametrial fat pads using guanidium isothiocyanate (27). Hybridizations were carried out in Quikhyb (Stratagene, La Jolla, CA) according to the manufacturer’s recommendations in a hybridization oven (Robbins Scientific Inc., Sunnyvale, CA). The hybridized membrane was washed and exposed to X-ray film (Kodak, Rochester, NY) with intensifying screens for 8 h. Probe was removed from the membrane using formamide-sodium chloride/sodium phosphate/EDTA, and the membrane was reprobe with labeled 28S rRNA cDNA probe. Autoradiographic bands were scanned and quantitated using a computer-linked laser densitometer and RFLPrint software (Pdi, Huntington Station, NY). The 28S rRNA bands were used to normalize for differences in gel loading.

**Study 2: effects of injected genistein on juvenile females**

**Animals and treatment.** Age-matched juvenile C57/BL6 female mice (25–27 d old) purchased (Harlan) or raised in our colony were ovarioectomized and placed on a phytoestrogen-free diet (AIN-93G) 5 d later. Beginning a week after ovarioectomy, mice were given daily sc injections of DMSO or genistein (8–80 mg/kg BW) for 28 d. At the end of treatment, body weight and parametrial fat pad weight were determined, and this fat pad was fixed in 10% neutral buffered formalin for morphometric analysis.

**Adipocyte size.** To measure adipocyte circumference, hematoxylin and eosin-stained histological sections of adipose tissue were photographed using a Spot digital camera interfaced with a microscope (Olympus Corp., Melville, NY) and a Power Macintosh G4 computer. The circumference of approximately 100 cells from each section was measured using the public domain NIH IMAGE program.

**Study 3: effects of dietary genistein on juvenile females**

**Animals and treatment.** Age-matched juvenile C57/BL6 females (25–27 d old) were ovarioectomized and placed on a phytoestrogen-free diet (AIN-93G) 5 d later. One week after ovarioectomy, mice were randomly divided into five groups and fed AIN-93G diet supplemented with 0, 300, 500, 1000, or 1500 parts per million (ppm) genistein ad libitum for 12 d. Mice were killed at lights on (0800 h) on d 12 of feeding; serum genistein levels at this time reflect levels seen during the night when the mice are eating (28). Blood was collected by cardiac puncture and serum separated for genistein measurement. Body, uterine, and parametrial and inguinal fat pad weights were determined. Parametrial fat pads from all groups were fixed and then subsequently analyzed to determine adipocyte circumference as in Study 2 above.

**Serum genistein measurement.** Serum concentrations of total genistein were measured following enzymatic deconjugation using a validated isotope dilution liquid chromatography electrospray mass spectrometry (LC-ESI/MS) method (29).

**Statistics.** Data were expressed as mean ± SEM. Fat pad, uterine and body weights, adipocyte size, QPCR results, feed consumption, and muscle weight were analyzed using the general linear model followed by a one-way Dunnett’s test using the SYSTAT (version 10) statistical package. Northern analysis results were analyzed using t test. A P value less than 0.05 was considered significant.

**Results**

**Study 1**

Weights of parametral fat pads in adult females treated with E2 were approximately 45% less than controls (Fig. 1A). The 200 mg/kg BW-d genistein-treated groups showed significant 23% and 37% decreases, respectively, compared with controls. The 20 mg/kg BW-d genistein-treated group showed the same trend, although the decrease did not reach significance (P = 0.10). The decrease induced by 200 mg/kg BW genistin was not statistically different from that in the E2 group (Fig. 1A). Effects of E2 and 200 mg/kg BW-d genistein were similar in all three fat pads, and muscle (gluteus maximus) showed no significant differences (Fig. 1B). Body weights of genistein-treated animals were slightly reduced at the higher genistein doses, similar to the E2 effect, but these decreases did not reach statistical significance vs. control (final body weight = 23.2 ± 0.4 g, 22.3 ± 0.7 g, and 22.8 ± 0.9 g in DMSO, E2, and 200 mg/kg genistein-treated animals, respectively). There was no significant difference in feed consumption in the estrogen and 20–200 mg/kg genistein groups, compared with the controls (total feed consumption = 96.1 ± 0.8 g, 90.3 ± 1.4 g, 98.8 ± 2.0 g, 94.8 ± 1.1 g, and 87.6 ± 0.8 g for DMSO, E2, and 20, 80, and 200 mg/kg genistein-treated mice, respectively).

QPCR analysis indicated that E2 and genistein treatment decreased LPL mRNA by 60–70%, compared with DMSO controls (Fig. 2A). The inhibitory effect of genistein on LPL mRNA was confirmed by Northern analysis, which showed...
a 45% decrease in steady-state LPL mRNA in adipose tissue genistein-treated mice, compared with DMSO controls (Fig. 2B). Inguinal fat pad weights in ovariectomized αERKO mice treated with 80 mg/kg BW•d of genistein (181 ± 11 mg, n = 8) were not reduced, compared with similar ovariectomized mice given DMSO (185 ± 6 mg, n = 15). Thus, genistein did not decrease adipose weight in mice lacking ERα.

Study 2

Genistein treatment of juvenile mice at 20 and 80 mg/kg BW•d for 28 d produced 36% and 47% decreases, respectively, in parametrial fat pad weight, compared with the controls (Fig. 3A), but the 8 mg/kg BW•d dose caused no decrease. Adipocyte circumference showed significant decreases of 18% and 25% in mice treated with 20 and 80 mg/kg BW•d genistein, respectively, compared with DMSO controls (Fig. 3B).

Study 3

Parametrial fat pad weights were compared in mice fed diet supplemented with 0, 300, 500, 1000, or 1500 ppm genistein (Fig. 4A). Parametrial fat pad weights in mice fed 500, 1000, or 1500 ppm genistein were 37%, 40%, and 57% less, respectively, than in control mice. Inguinal fat pad weights showed similar decreases in the genistein groups (data not shown). Uterine weights in mice given 300-1500 ppm genistein were increased, compared with controls (Fig. 4B), reflecting estrogenic effects on this organ. Adipocyte circumference was reduced by both 1000 and 1500 ppm genistein, compared with controls (80.7 ± 0.4 μm, 68.4 ± 0.3 μm, and 62.9 ± 0.3 μm in control, 1000, and 1500 ppm genistein groups, respectively).

Serum genistein levels in mice fed 0, 300, 500, 1000, and 1500 ppm genistein were 0.08 ± 0.02 μM, 1.02 ± 0.14 μM, 1.79 ± 0.32 μM, 2.55 ± 0.18 μM, and 3.81 ± 0.39 μM, respectively (Fig. 4C), which are comparable to concentrations reported in humans under certain nutritional conditions (22).

Discussion

Our results show that genistein injections cause dose-responsive decreases in adipose tissue in juvenile and adult ovariectomized mice. The estrogenicity of genistein is approximately 1/100 to 1/10,000 that of E2, depending on the...
end point used (22). However, the highest genistein dose produced decreases in adipose tissue equal to 80% of those seen with E2, indicating that genistein has antilipogenic effects on adipose tissue.

Previous work has indicated that genistein decreases insulin-induced lipogenesis in both primary adipocyte cultures (30) and 3T3-L1 preadipocyte cell lines (31). In addition, genistein enhanced epinephrine-induced lipolysis (30). However, these in vitro experiments involved high genistein concentrations, and it was unclear whether genistein effects on adipocytes would occur in vivo. Our present results indicate that the inhibitory genistein effects on adipose tissue are seen in vivo and are consistent with genistein effects on preadipocytes and adipocytes in vitro.

Genistein produced dose-responsive decreases in adipocyte circumference. Changes in adipose depots in adults usually occur by alteration in adipocyte size (32), and genistein’s effects here primarily or entirely reflect decreases in adipocyte size. However, estrogens influence proliferation and differentiation of adipocyte precursors during development (33), suggesting that genistein could influence adipocyte numbers at certain developmental stages.

The magnitude of the antilipogenic effect of genistein was similar in all three fat pads (inguinal, parametrial, and renal).

Thus, genistein produces qualitatively and quantitatively similar changes in sc (inguinal) as well as visceral (parametrial and renal) fat pads.

Estrogen has been reported to inhibit postovariectomy body weight increases (11), similar to our observations. Genistein injections also produced a small decrease in body weight, although this did not reach significance. The inhibitory genistein effect on adipose tissue contrasted with the lack of effect on skeletal muscle, indicating that the adipose effects of genistein are not nonspecific.

A recent study by Misso et al. (13) indicated that E2 inhibited LPL mRNA and antilipogenic effects mediated through changes in LPL are the critical mechanism for the adipose effects, rather than increases in lipolysis. Genistein produces decreases in LPL mRNA in adipose tissue. Thus, a critical aspect of genistein’s effects on adipose tissue may involve inhibition of LPL, similar to estrogen.

Genistein binds ERα and ERβ and has other effects (34). This raises the question of how genistein affects LPL and adipose tissue. αERKO mice have increased adipose tissue (8), indicating that antilipogenic actions of E2 are mediated through ERα. Genistein did not decrease adipose tissue in
ovariectomized aERKO mice, showing that genistein’s adipose effects require ERα. Injected genistein is antilipogenic. Humans and other species consume genistein, so it is important to determine whether dietary genistein in quantities producing physiological serum genistein concentrations can have adipose effects. Our results show that dietary genistein produces dose-dependent decreases in adipose tissue of 37–57% after a 12-d treatment. A description of body weights in mice given dietary genistein has been reported (35); body weights in the 300–1000 ppm genistein group were not different from control, and the 1500 ppm genistein group had a body weight slightly (5%) but significantly lower than controls. These results are consistent with previous reports that dietary genistein at 1000 ppm did not affect body weight (17) and genistein at 100 or 1500 ppm did not affect food consumption (17, 36). Thus, effects of dietary genistein on adipose tissue are not simply a reflection of decreased body weight because adipose decreases exceed body weight changes even in the 1500 ppm genistein group.

Uterine weight increases were seen at 300 ppm genistein and higher, but decreases in adipose tissue were seen only when mice were fed 500 ppm genistein or more. Thus, increases in uterine weight are a more sensitive indicator of genistein exposure than the adipose changes. Adipose changes only occur at doses above those that produce uterotropic effects, a complicating factor that must be considered in determining whether genistein and soy could potentially be used for weight reduction in humans.

An important question is whether these results may have relevance for humans. Our present results show that the serum levels measured using LC-ES/MS in mice fed 500–1500 ppm genistein were 1.79–3.51 μM; these values are higher than serum genistein values we reported previously using HPLC-UV (35). This likely is due to methodological differences; the LC-ES/MS method used here should be more accurate because of higher specificity and the use of isotonic internal standards. However, the values reported by this method, although higher than reported earlier, are still well within the range encountered in humans under certain nutritional conditions. Serum genistein levels in mice fed 500–1500 ppm dietary genistein exceed those reported in Japanese men (0.16–0.89 μM) (37). However, humans consuming three meals per day containing soy milk have serum genistein concentrations up to 4.6 μM (38). Likewise, consumption of one soy-based meal resulted in peak serum genistein concentrations of 4.1 and 2.4 μM, respectively (39, 40). Consumption of 100 mg/d of the isoflavone supplement Novosoy by men also resulted in peak serum genistein concentrations in the range shown here to produce adipocyte effects in mice (41). Finally, human infants fed soy-based infant formula have plasma genistein levels ranging from 1.5–4.4 μM (22). Thus, there are numerous situations in which human soy/isoالفانون consumption produces serum genistein levels equaling or exceeding those causing antilipogenic effect in mice.

Some previous literature is consistent with the possibility that isoflavones could have beneficial effects on body weight and lipid metabolism in humans. Postmenopausal women with relatively high isoflavone consumption in their normal diet had a body mass index over 9% lower (P < 0.05) than similar women not consuming appreciable quantities of isoflavones (42). Other studies have shown beneficial effects of soy on serum lipid levels in obese women, although it is still unclear whether these effects are accompanied by decreases in body weight (43–45).

Laboratory rodents are also exposed to large amounts of isoflavones because soy is an inexpensive source of high-quality protein (46). Commercial rodent chows contain up to 830 ppm total isoflavones and total plasma isoflavone levels up to 8.5 μM were reported in mice fed commercial diets (46). These concentrations of both dietary and plasma isoflavones exceed those of genistein shown to have adipose effects, although both the dietary and plasma isoflavones measured in these studies reflect contributions of other isoflavones in addition to genistein. However, daidzin, the glycoside form of daidzein found in high levels in soy, has been shown to inhibit the postovariectomy adipose increase in rats (47). In addition, other compounds, such as glycetin (47) and sapo[nis (48), are reported to inhibit adipose deposition. Rats or mice fed soy-based diets weighed less than those fed similar diets without soy (49, 50). Our present results indicate that the most abundant dietary isoflavone, genistein, can have pronounced antilipogenic effects, but other soy constituents may also contribute to this effect. Thus, isoflavones and other soy constituents present in high quantities in commercial rodent diets appear likely to have an effect on adipose tissue, and this should be considered in the design and interpretation of rodent studies involving analysis of adipose tissue.

In summary, our results show that genistein is antilipogenic, and this effect requires ERα. Genistein decreases adipocyte size, caused at least in part by inhibitory effects on the lipogenic enzyme LPL. Although our results indicate that genistein can modulate adipose deposition in mice, it is not clear whether genistein could have antilipogenic effects in human populations. However, our results indicate that the serum genistein concentrations that produce adipose changes in mice are within the range of those reported in humans under various nutritional conditions.

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