Daidzein Is More Efficient than Genistein in Preventing Ovariectomy-Induced Bone Loss in Rats

Christel Picherit, Véronique Coxa, Catherine Benneau-Pelissero,* Séraphin Kati-Coulibaly,† Marie-Jeanne Davicco, Patrice Lebecque and Jean-Pierre Barlet†

ABSTRACT We investigated the ability of genistein and daidzein, two soybean isoflavones, compared with that of 17α-ethinylestradiol, to prevent bone loss in ovariectomized rats, a model for postmenopausal osteoporosis. Female Wistar rats (n = 65; 12 mo old) were either sham-operated (SH; n = 13) or ovariectomized (OVX; n = 52). On d 0, OVX rats were randomly assigned to groups as follows: 13 received genistein [G; 10 μg/(g body weight • d)], 13 were treated with daidzein [D; 10 μg/(g body weight • d)], 13 received 17α-ethinylestradiol [E2; 30 μg/kg body weight • d] and 13 were untreated (OVX). Compounds were mixed with a soy protein-free powdered semipurified diet and given orally for 3 mo. On d 90, the bone mineral density (BMD) in lumbar vertebrae, femur and its metaphyseal and diaphyseal zones (rich in cancellous and cortical bone, respectively) was lower in OVX than in SH (P < 0.01). In D or E2, the four BMD were not different from SH, whereas in G, only the diaphyseal BMD was not different from SH. Image analysis performed in the distal femur metaphysis revealed that the cancellous bone area was lower in OVX than in SH (P < 0.01). Only the area in D was not different from that in SH. Finally, the bone turnover, which was higher in OVX than in SH (P < 0.005 and P < 0.05 for plasma osteocalcin concentration and urinary deoxypyridinoline excretion, respectively), was not different in G, D or E2 compared with SH. Therefore, consumption of 17α-ethinylestradiol or daidzein was more efficient than genistein in preventing ovariectomy-induced bone loss in rats.

KEY WORDS: • genistein • daidzein • prevention • bone • rats

Osteoporosis is a reduction in the amount of bone tissue per unit volume (bone resorption outstripping bone formation and thus disrupting bone remodeling) (Hallworth 1998), and also constitutes a bone microarchitectural impairment (Riggs and Melton 1986). Hypoestrogenemia after menopause is an important cause of osteoporosis. For this reason, hormone replacement therapy (HRT)2 is often recommended for osteoporosis prevention and treatment. However, HRT may be associated with side effects. Thus, increased research into alternatives to estrogen for postmenopausal women is of clinical, scientific and health policy importance (Kessel 1998).

Broadly defined, phytoestrogens include isoflavones, coumestans and lignans, found mainly in soybeans, clover or alfalfa sprouts, and oilseeds such as flaxseed, respectively (Kurzer and Xu 1997). Although few foods containing coumestrol (the major coumestan) are consumed by humans, both lignans and isoflavones were identified in many human physiological fluids after the consumption of ordinary diets. Because soybean consumption in postmenopausal women could be associated with potential health benefits, such as prevention of atherosclerosis progression, lowering of cancer risks, positive effects on hot flushes, vaginal symptoms, cognitive function or dementia, and bone preservation, isoflavones are under intensive investigation. Furthermore, although phytoestrogens are 100- to 10,000-fold less estrogenic than 17β-estradiol, their plasma concentrations were found to be high in Japanese men eating a traditional Asian diet (Adlercreutz et al. 1993b), and even up to 1000-fold higher than the highest levels of circulating 17β-estradiol in premenopausal women (Anderson and Garner 1997). Moreover, phytoestrogens were shown to interact with sex hormone production, metabolism or action at the cellular level (Murkies et al. 1998). In effect, they inhibit enzymes involved in steroid metabolism, such as 5α-reductase (Evans et al. 1995) or aromatase (Adlercreutz et al. 1993a), whereas they stimulate sex hormone binding globulin (SHBG) production, reducing the proportion of free estrogens circulating in plasma. On the other hand, because of a hydroxyl group

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2 Abbreviations used: BMD, bone mineral density in the femoral diaphysis (D-BMD) or the distal femur metaphysis (M-BMD) or the total femur (T-BMD); D, ovariectomized rats given daidzein; DEXA, dual-energy X-ray absorptiometry; DPD, deoxypyridinoline; E2, ovariectomized rats given 17α-ethinylestradiol; ER, estrogen receptor; G, ovariectomized rats given genistein; HRT, hormone replacement therapy; IC, initial control rats; OC, osteocalcin; OXV, ovariectomized rats; SERM, selective estrogen receptor modulators; SH, sham-operated rats; SHBG, sex hormone binding globulin.
The composition of the diet is given in powdered semipurified diet (I.N.R.A., Jouy en Josas, France) for 1 mo. 21°C, with a 12-h light:dark cycle. Rats were fed a soy protein–free diet in metallic cages that allowed separation and collection of urine, at I.N.R.A. (Clermont-Ferrand/Theix, France) and housed individually or surgically ovariectomized (OVX; n = 52), under anesthesia using chloral hydrate (80 g/L in saline solution; 0.4 mL/100 g body weight, intraperitoneally). In the sham procedure, the ovaries were exteriorized and replaced to create a stress similar to that obtained with bilateral ovariectomy. On d 1 after surgery (designated as d 0), the OVX rats were randomly assigned to groups as follows: 1) treated with genistein at 10 μg/g body weight · d (n = 13; G); 2) received daidzein at 10 μg/g body weight · d (n = 13; D); 3) treated with 17α-ethinylestradiol at 30 μg/kg body weight) (n = 13; E2); or 4) untreated (n = 13; OVX: ovariectomized controls). During the 3-mo experimental period, all compounds were given orally. Diets were prepared by mixing the powdered genistein (Sigma, L’ille d’Abeau, France), daidzein (Sigma) or 17α-ethinylestradiol (Sigma) with the soy protein–free powdered semipurified diet. SH and OVX rats were fed the soy protein–free powdered semipurified diet without any additional compound. To prevent ovariectomy-induced hyperphagia, the daily diet quantity distributed to each rat was adjusted to their mean level consumed by SH the previous day. Food was humidified (1 mL/g), and each rat had free access to water. Every week, rats were weighed to adjust the genistein, daidzein or 17α-ethinylestradiol doses to body weight. On d 89, a 24-h urine sample was collected to measure urinary excretion of deoxypyridinoline, a marker of bone resorption (Robins 1994). On d 90, at 0900 h, rats were killed by cervical dislocation. Blood samples were collected into ice-cold heparinized plastic tubes containing 200 peptide inhibitor units of aprotinin (Iniprol, Choay, Paris, France) and milliliters blood, and centrifuged immediately (3500 g for 5 min at 4°C). Then, plasma was frozen at –20°C until measurements of phytoestrogens and osteocalcin, a marker of osteoblastic activity, were made. Uterine horns were removed from each rat and immediately weighed. Femurs and lumbar vertebrae were cleaned from adjacent tissues and used for physical and chemical measurements. Successes of ovariectomy and phytoestrogen treatment were confirmed by uterine weight and plasma genistein or daidzein concentrations, respectively.

### TABLE 1
Composition of the soy protein–free powdered semipurified diet consumed by female Wistar rats

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>225</td>
</tr>
<tr>
<td>Casein</td>
<td>225</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>450</td>
</tr>
<tr>
<td>Sucrose</td>
<td>20</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>20</td>
</tr>
<tr>
<td>Fiber</td>
<td>20</td>
</tr>
<tr>
<td>Cellulose</td>
<td>20</td>
</tr>
<tr>
<td>Fat</td>
<td>25</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>25</td>
</tr>
<tr>
<td>Rapeseed oil</td>
<td>25</td>
</tr>
<tr>
<td>Vitamin mixture2</td>
<td>10</td>
</tr>
<tr>
<td>Mineral mixture(Ca-P deficient)3</td>
<td>18.5</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>1.5</td>
</tr>
</tbody>
</table>

1 From I.N.R.A., (Jouy en Josas, France); casein (Union des caséineries, Surgères, France), sucrose (Eurosucre, Paris, France), cornstarch (Cerestar, Saint-Maur, France), cellulose (Durieux, Marne la Vallée, France), oil (Bailly, Aulnay sous Bois, France), vitamin mixture (Roche, Neuilly sur Seine, France), mineral mixture (Prolabo, Fontenay sous Bois, France), DL-methionine (Jerofrance, Jeufosse, France).

2 With cholecalciferol, 1250 IU/kg.

3 With calcium, 2.3; phosphorus, 1.6; magnesium, 0.42.

Thus, isoflavones appear to have potential promise for maintaining or modestly improving bone mass of human subjects when consumed at optimal dosages (Anderson and Garner 1997). Moreover, in ovariectomized rats used classically as an animal model for postmenopausal osteoporosis (Kalu 1991), dietary soybean proteins were found to prevent bone loss (Arjmandi et al. 1996), and this bone-sparing effect was mediated by the isoflavone content of soybean (Arjmandi et al. 1998). Therefore, this study investigated the effects of the two major soybean isoflavones (genistein and daidzein), given orally and separately at equal dose, on ovariectomy-induced bone loss in adult rats.

### MATERIALS AND METHODS

#### Animals and treatments.
The study was conducted in accordance with current legislation on animal experiments in France. Female Wistar rats (n = 75; 11 mo old; ~ 375 g) were purchased from I.N.R.A. (Clermont-Ferrand/Theix, France) and housed individually in metallic cages that allowed separation and collection of urine, at 21°C, with a 12-h light-dark cycle. Rats were fed a soy protein–free powdered semipurified diet (I.N.R.A., Jouy en Josas, France) for 1 mo. The composition of the diet is given in Table 1. After the adaptation period, 10 rats designated as initial controls (IC) were killed; the remaining 65 rats were either sham-operated (SH: controls; n = 13) or surgically ovariectomized (OVX; n = 52), under anesthesia using chloral hydrate (80 g/L in saline solution; 0.4 mL/100 g body weight, intraperitoneally). In the sham procedure, the ovaries were exteriorized and replaced to create a stress similar to that obtained with bilateral ovariectomy. On d 1 after surgery (designated as d 0), the OVX rats were randomly assigned to groups as follows: 1) treated with genistein at 10 μg/g body weight · d (n = 13; G); 2) received daidzein at 10 μg/g body weight · d (n = 13; D); 3) treated with 17α-ethinylestradiol at 30 μg/kg body weight (n = 13; E2); or 4) untreated (n = 13; OVX: ovariectomized controls). During the 3-mo experimental period, all compounds were given orally. Diets were prepared by mixing the powdered genistein (Sigma, L’ille d’Abeau, France), daidzein (Sigma) or 17α-ethinylestradiol (Sigma) with the soy protein–free powdered semipurified diet. SH and OVX rats were fed the soy protein–free powdered semipurified diet without any additional compound. To prevent ovariectomy-induced hyperphagia, the daily diet quantity distributed to each rat was adjusted to the mean level consumed by SH the previous day. Food was humidified (1 mL/g), and each rat had free access to water. Every week, rats were weighed to adjust the genistein, daidzein or 17α-ethinylestradiol doses to body weight. On d 89, a 24-h urine sample was collected to measure urinary excretion of deoxypyridinoline, a marker of bone resorption (Robins 1994). On d 90, at 0900 h, rats were killed by cervical dislocation. Blood samples were collected into ice-cold heparinized plastic tubes containing 200 peptide inhibitor units of aprotinin (Iniprol, Choay, Paris, France) and milliliters blood, and centrifuged immediately (3500 g for 5 min at 4°C). Then, plasma was frozen at –20°C until measurements of phytoestrogens and osteocalcin, a marker of osteoblastic activity, were made. Uterine horns were removed from each rat and immediately weighed. Femurs and lumbar vertebrae were cleaned from adjacent tissues and used for physical and chemical measurements. Successes of ovariectomy and phytoestrogen treatment were confirmed by uterine weight and plasma genistein or daidzein concentrations, respectively.

#### Bone mineral density (BMD).
BMD was assessed by dual-energy X-ray absorptiometry (DEXA), with the Hologic QDR-4500 A X-ray densitometer (Hologic, Massy, France). The total right femur BMD (T-BMD), as well as the BMD of two subregions, one corresponding to the distal femur metaphyseal zone (M-BMD), rich in cancellous bone, and the other to the diaphyseal zone (D-BMD), rich in cortical bone, were determined (Pastoureau et al. 1995). These second, third, fourth and fifth lumbar vertebrae (mainly cancellous bone) were also scanned and the mean BMD measured.

#### Femoral calcium content.
Femoral Ca was determined with an atomic absorption spectrophotometer (Perkin Elmer 400, Norwalk, CT), in ashed femurs (dissolved in HCl and diluted with 1 g/L lanthanum oxide).

#### Femoral mechanical testing.
Immediately after collection, the length of the left femur and the mean diameter of the femoral diaphysis were measured with a precision caliper (Mitutoyo, Shropshire, UK). Bones were kept in NaCl (9 g/L) at 4°C, and femoral failure load was determined 24 h later, using a 3-point bending test (Turner and Burr 1993), with a Universal Testing Machine (Instron 4501, Intron, Canton, MA).

#### Image analysis.
To measure cancellous bone area in the distal femur metaphyseal zone, frontal sections were cut with a saw (Isomet 2000, Buehler, Krautkramer, Champagne-Mont d’Or, France), ground to 80-μm sections (Metaserv 2000 polisher, Buehler), and stained with Von Kossa’s reagent (AgNO₃, Sigma). The underlying zone to growth plate was then analyzed with an automated microscope image-analysis system, as previously described (Rose et al. 1996).

#### Marker of osteoblastic activity.
Osteocalcin (OC) in plasma was measured by RIA, using rat 121I-labeled OC, goat anti-rat OC antibody and donkey anti-goat second antibody (Biochemical Technologies, Stoughton, MA). The sensitivity was 0.01 nmol/L. The intra- and interassay precisions were 6.8 and 8.9%, respectively.

#### Marker of bone resorption.
Deoxypyridinoline (DPD) in urine was determined by competitive RIA, using rat monoclonal anti-DPD antibody coated to the inner surface of a polystyrene tube and
RESULTS

Body and uterine weights. During the experimental period, body weight increased compared with d 0 (P < 0.005), except in E2 (Fig. 1). As a result, although no significant difference was observed among SH, OVX, G and D, rats in the E2 group were lighter than the others (P < 0.01). Uterine weight, higher in IC than in SH (P < 0.05), was lower in OVX than in SH on d 90 (Table 2; P < 0.01). It was higher in E2 than in OVX, but lower than in SH (P < 0.01). Uterine weights in G and D were not different from OVX.

Plasma phytoestrogen concentrations. No significant difference in plasma phytoestrogen concentration was observed among IC and SH, or SH, OVX and E2 (Table 3). On the contrary, plasma concentrations of genistein and daidzein on d 90 were higher in G (P < 0.005) and D (P < 0.01), respectively, compared with all other groups.
Image analysis. The cancellous bone area in the distal femur metaphysis was not different between IC and SH (Fig. 3). This variable, lower in OVX than in SH on d 90 (P < 0.01), was not different among OVX, G, D and E2. However, the D group also was not different from SH. Photomicrographs of histological slides used for image analysis are shown in Figure 4.

Bone turnover. Plasma OC concentration was higher in IC than in SH (P < 0.01) (Fig. 5A). On d 90, it was also higher in OVX than in SH (P < 0.005) and the effect of OVX was prevented by daidzein because no difference was observed between SH and D. Concentrations in G and E2 were not different from OVX or SH. The urinary DPD excretion did not differ between IC and SH (Fig. 5B). On d 90, it was significantly higher in OVX than in SH (P < 0.05) and this effect was prevented by genistein and daidzein because no difference was observed between SH and G or D. Excretion in E2 was not different from OVX or SH.

DISCUSSION

Ovariectomized rats are classically used as an animal model for postmenopausal bone loss (Kalu 1991, Miller et al. 1995).
Mosekilde, Wronska, and Yen 1991). Furthermore, they may provide a useful model for investigating the biological effects of soy isoflavones because of the similarity in plasma genistein concentrations attainable in rats (King et al. 1996) and humans (Xu et al. 1994). Isoflavones are degraded by gut microflora, which profoundly influences their bioavailability (Xu et al. 1995). In rats, genistein is highly bioavailable; not only is it well absorbed from the intestines, but it is also extracted efficiently from the portal blood into the liver and excreted into bile (Sfákianos et al. 1997). This enterohepatic cycle leads to a new circulation of genistein in the general circulation. However, differences in bioavailability occur when we consider the conjugate or glycoside forms of genistein and daidzein (genistin and daidzin, respectively). These forms occur naturally in vegetables and are then metabolized into genistein and daidzein, respectively, by gut bacteria glycodieses). Daidzein was reported to be more bioavailable than genistein in rats (King 1998) and humans (Xu et al. 1994). Because dietary soybean proteins (Arjmandi et al. 1996), their isoflavones (Arjmandi et al. 1998), and genistin and daidzin (Ishida et al. 1998) prevent bone loss in young ovariectomized rats, this study investigated the potential preventive effects of genistein and daidzein, given orally and separately at equal dose, and compared these effects with those of orally administered 17α-ethinylestradiol on ovariectomy-induced bone loss in adult rats. The T-, M- and D-BMD were lower in 15- than in 12-month-old female rats. This could be attributed in part to a decrease in osteoblastic activity, as shown by plasma OC concentrations. In addition, an increase in fecal and urinary calcium excretions, as well as a decrease in calcium absorption efficiency with age, might contribute to the reduction of BMD (Aivioli et al. 1965, Gaumet et al. 1997). For cancellous bone, the BMD reduction in the distal femur metaphysis was not sufficient, however, to reduce the corresponding cancellous bone area. Again, the BMD decrease in the cortical bone was not sufficient to affect its mechanical properties.

At both the cancellous and cortical sites, ovariectomy greatly reduced BMD resulting from increased bone turnover as indicated by the higher plasma OC concentration and urinary DPD excretion in the O VX group compared with the SH group. These results are in agreement with those of Wronska et al. (1985), which demonstrated that bone remodeling in rats is accelerated after the cessation of ovarian function. Moreover, related to the M-BMD reduction, ovariectomy also decreased cancellous bone area, probably by lowering trabeculae number rather than by thinning them. In cortical bone, however, the BMD decrease was less pronounced than that in cancellous bone and was not sufficient to impair its mechanical properties. In contrast, ingestion of 17α-ethinylestradiol prevented the BMD reduction at both the cancellous and cortical sites in rats, by suppressing the increase in bone turnover. In effect, in ovariectomized rats, estradiol prevents bone loss by depressing bone turnover (Wronski et al. 1988). However, under our experimental conditions, the decrease in cancellous bone area of the distal femur metaphysis was not prevented by ingestion of 17α-ethinylestradiol.

As reported previously in cortical bone (Ishida et al. 1998), daidzein, like estrogen, prevented the ovariectomy-induced BMD reduction at both the cancellous and cortical sites by suppressing the bone turnover increase. Moreover, daidzein consumption also resulted in the preservation of cancellous bone area and in bone distal femur metastasis. By contrast, genistein did not prevent cancellous bone loss; however, as previously reported (Ishida et al. 1998), it did prevent ovariectomy-induced cortical bone loss. Our results obtained in cancellous bone are consistent with those of Anderson et al. (1998) demonstrating that, in ovariectomized lactating rats, orally administered genistein induced a cancellous bone tissue retention at a low dose [1.5 μg/(g body weight · d)], whereas there were no effects at a higher dose [from 5 to 15 μg/(g body weight · d)]. In this study, because the exchange surface with plasma was higher in cancellous than in cortical bone, it is possible that cancellous bone was overexposed to genistein, thus inducing potential mechanisms of ER saturation or impairments of cellular activity (such as protein phosphorylation). Genistein was found to suppress osteoclastic activity through tyrosine-kinase inhibition (Blair et al. 1996, Williams et al. 1998). However, the hypothesis based on the inefficiency of a high genistein dose is unlikely because injections of 5g (Fanti et al. 1998) or 20 μg/(g body weight · d) (Ishimi et al. 1999), which likely provided higher plasma genistein concentrations than oral administration of 5 or 15 μg/(g body weight · d) (Anderson et al. 1998), respectively, induced cancellous bone-sparing effects. Moreover, as reported by Ishimi et al. (1999), but contrary to results from Fanti et al. (1998) and Ishida et al. (1998), the bone loss preventive effects of genistein in this study resulted from a suppression in bone turnover increase; thus, these effects could be due to a mechanism similar to that of estrogen. Nevertheless, our results do not explain why genistein and daidzein exhibited different effects on cancellous bone and similar effects on cortical bone, whereas both molecules reduced the increase in bone turnover. It is possible that the difference in cancellous and cortical bone responsiveness to phytoestrogens depends on the ER subtype. Indeed, some differences in both the binding affinity of phytoestrogens to ERα or ERβ and the ERα or ERβ content in cancellous and cortical bones could be involved. Thus, genistein possesses a higher affinity for ERβ than for ERα (Ishimi et al. 1998), and although both ERα and ERβ mRNAs are expressed in osteoblasts, the expression of ERα mRNA is higher in cancellous bone of the rat distal femoral metaphysis and lumbar vertebrae than in cortical bone of the femoral diaphysis (Onoe et al. 1997). Again, recent studies also showed that ERα mRNA was expressed predominantly in rat osteoblasts covering the metaphyseal bone trabecular surface (Windahl et al. 2000), and neither ERα or ERβ mRNA was detected in rat cortical bone (Lim et al. 1999). Moreover, an ERβ-like immunoreactivity was demonstrated not only in the nuclei of human and murine osteoblasts, but also in the osteoclast cytoplasm (Vidal et al. 1999). Thus, the response of target tissues to phytoestrogens could be modulated by the ERα/ERβ ratio in each tissue. Further studies are required to determine whether phytoestrogens act via ER-dependant mechanisms.

Because a nutritional approach was used to meet the goals of this experiment, all treatments were given orally. Because 17β-estradiol is a weak oral estrogen (Barnes 1998), we used 17α-ethinylestradiol, which is at least 200-fold more active than 17β-estradiol when equivalent doses are given orally (Messina et al. 1994). We chose the dose of 30 μg/(kg body weight · d) because it prevents bone loss in ovariectomized rats (Ke et al. 1997). The dose choice for genistein and daidzein was based on data computed from studies by Anderson et al. (1998), Fanti et al. (1998), and Ishimi et al. (1999). In this experiment, oral administration of 10 μg/(g body weight · d) for 3 mo induced high plasma genistein and daidzein concentrations in the G and D groups, respectively. These concentrations are equivalent to 1000- to 10,000-fold the concentrations by ingestion of 3 mg of pure daidzein per kg of diet. Therefore, plasma daidzein levels usually observed in rats (basal and peak estradiol concentrations during the estrous cycle are 7-17 and 50-88 pg/L, respectively) (Butcher et al. 1974). On the other hand, the
presence of both daidzein and genistein in the G and D groups could be explained by an unspecific cross-reaction between genistein and anti-daidzein antibody, and vice versa (Benne- tetau-Pelissero et al. 2000). Moreover, because equol is obtained naturally by a gut microbial transformation of daidzein (Axelson et al. 1984, Braden 1967) in ruminants, monogastrics and therefore humans, it is absorbed in the gut, conjugated in the liver and excreted in urine (Axelson et al. 1984). Because orally administered daidzein in rats can be metabolized to equol (Yasuda and Oshawa 1998), we also measured plasma equol concentrations in the D group. The mean level obtained with a very specific antibody (Bennetau-Pelissero et al. 2000) was 281 ± 55 nmol/L, indicating that equol was produced in D. Moreover, in various estrogenic tests in many species, the estrogenicity of isoflavone and isoflavane compounds can be ordered as follows: daidzein < genistein < equol. Indeed, the last-mentioned is at least 10- to 100-fold more estrogenic than daidzein and at least 10-fold more estrogenic than genistein in fish (Pelissero et al. 1991). It is possible, then, that the bone estrogenic effects observed in D could be due to equol rather than daidzein, or to both equol and daidzein. However, it would be also reasonable to consider that the degree of estrogenic activity of genistein, daidzein and equol is not universal, and there could be tissue differences in the effects of these compounds.

Ovariectomized rats may exhibit some protection against bone loss by obesity (Kalu 1991). However, under our experimental conditions, ovariectomy did not influence the body weight by ovariectomy (Kalu 1991). However, under our experimental conditions, ovariectomy did not influence the body weight, indicating that pair-feeding to SH avoided the ovariectomy-induced hyperphagia. On the contrary, rats in the E2 group were significantly lighter than the others because of a reduced food consumption (daily mean food intake was 75% of the daily SH diet consumption), which was likely due to the palatability of 17α-ethinylestradiol. Uterine weight was significantly decreased by ovariectomy, confirming the effects of the surgical intervention. In these rats, the 17α-ethinylestradiol intake [22–23 rather than 30 μg/kg body weight (d)] induced an uterotrophic activity. Furthermore, when results are expressed as g/100 g body weight rather than as grams, values in E2 were not different from SH (data not shown). On the contrary, genistein and daidzein did not exhibit any uterotrophic activity, confirming results obtained in previous studies (Anderson et al. 1998, Arjmandi et al. 1996 and 1998, Ishimi et al. 1999, Tansey et al. 1998). Effectively, these phytoestrogens are weakly estrogenic in the rat uterus. In the study of Fanti et al. (1998), a high genistein dose [25 μg/g (body weight × d)] subcutaneously was required to cause an increase in uterine mass. In the same way, in the study of Ishida et al. (1998), only the highest orally administered daidzein dose [50 μg/g (body weight × d)] increased uterine weight in ovariectomized rats. However, when genistein was administered at the same dose, no uterotrophic activity was demonstrated (Ishida et al. 1998). Again, it is possible that ER subtype–dependant mechanisms could be involved because both ERα and ERβ are present in the rat uterus (Hiroi et al. 1999).

In conclusion, we demonstrated that both cancellous and cortical bone loss or only cortical bone loss was prevented by orally administered daidzein or genistein, respectively, in the ovariectomized rat model of postmenopausal osteoporosis. Like estradiol, these phytoestrogens suppressed the ovariectomy-induced increase in bone turnover. Moreover, neither genistein nor daidzein exhibited estrogenic activity on the uterus, demonstrating the usefulness of a soybean isoflavone to prevent postmenopausal bone loss without any adverse effects on the uterus.

LITERATURE CITED
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