Soybean Isoflavones Dose-Dependently Reduce Bone Turnover but Do Not Reverse Established Osteopenia in Adult Ovariectomized Rats[1]

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ABSTRACT We assessed the dose-dependent effects of daily soybean isoflavone (IF) consumption in reversing bone loss in adult ovariectomized rats. On d 0, female Wistar rats (7 mo old; $n = 55$) were either sham-operated (SH; $n = 14$) or ovariectomized ($n = 41$). On d 80, intermediate rats (SH: $n = 5$; ovariectomized: $n = 5$) were killed to confirm the ovariectomy-induced bone loss. The remaining ovariectomized rats were randomly assigned to one of four groups of nine rats each and fed soybean IF (mixed with a soy protein–free semipurified diet) at 0 (OVX), 20 (IF20), 40 (IF40) or 80 (IF80) mg/(kg body · d) for 84 d. Simultaneously, SH rats were fed the semipurified diet without any additional compound and killed on d 164, as were the other rats. As expected, both bone mineral density in the total femur and in its diaphyseal and metaphyseal subregions and cancellous bone area/measured surface in the distal femur metaphysis were lower in OVX than in SH rats ($P < 0.05$). OVX rats had higher plasma osteocalcin concentration and urinary deoxypyridinoline excretion than SH rats ($P < 0.05$). On d 164, osteocalcin and deoxypyridinoline concentrations were lower in IF40 or IF80 rats than in OVX rats ($P < 0.05$). Nevertheless, neither bone mineral density nor cancellous bone area was greater in IF-fed rats than in OVX rats. Therefore, in adult ovariectomized rats, daily soybean IF consumption decreased bone turnover but did not reverse established osteopenia. J. Nutr. 131: 723–728, 2001.

KEY WORDS: • *soybean isoflavones* • *curative effects* • *bone* • *rats*

With the continuing demographic shift in populations toward an older society, osteoporosis has become a major public health problem. Hormone replacement therapy (1) remains the mainstay for the prevention of postmenopausal osteoporosis, because the biggest culprit in the process of bone loss is estrogen deficiency (2). However, hormone replacement therapy is not accepted universally due to the contraindications in some patients, low compliance and reluctance of many women because of the fear and dislike of possible side effects and long-term risks (1). On the other hand, the human diet contains a complex array of naturally occurring bioactive molecules, the phytochemicals, that may confer important long-term health benefits (3). Specifically, phytoestrogens, which are well represented in Leguminosae seeds (where they occur mainly as glycosides) and described as compounds with a weak estrogen-like activity associated with their ability to bind to the estrogen receptor (4), have attracted the most attention.

Soybeans (*Glycine max*), which were traditionally used for fermented and unfermented soy food preparations, are rich in flavonoids (the most common and widely distributed group of polyphenols) and, in particular, in the isoflavones (IF)[3] genistin and daidzin. Although extensive data on ipriflavone (a synthetic IF derivative) suggest that it is a useful and safe alternative to estrogen therapy in the treatment of existing low bone mass or osteoporosis in postmenopausal women, data on naturally occurring IF are very limited but suggest that including them in the diet results in a reduction in bone resorption caused by estrogen deficiency (5). Indeed, dietary supplementation with soybean IF might maintain bone mass in postmenopausal women (6,7).

Recently, the bone-sparing effects of soybean IF, either administered orally (8–13) or injected subcutaneously (14), have been widely examined in a preventive approach in the most commonly used animal model for postmenopausal osteoporosis, the ovariectomized rat (15–18). Little is known about their curative effects; only one study investigated the impact of the short-term consumption of soy proteins (with normal or reduced IF content) in reversing an established bone loss in the young ovariectomized rat, and that study demonstrated

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[3] Abbreviations used: BMD, bone mineral density; D-BMD bone mineral density in the femoral diaphysis; M-BMD, bone mineral density the distal femur metaphysis; T-BMD, bone mineral density in the total femur; DEXA, dual-energy x-ray absorptiometry; DPD, deoxypyridinoline; IC, initial control; IF, isoflavones; IF20, IF40 or IF80, ovariectomized rats fed isoflavones at 20, 40 or 80 mg/(kg body · d); OC, osteocalcin; OVX, ovariectomized; OVXi, intermediate ovariectomized; SH, sham-operated; SHi, intermediate sham-operated. .

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that soy diets were somewhat effective (19). Nevertheless, the dose-dependent bone-curative effects of IF were never assessed for a long period in adult rats. Therefore, we investigated the ability of long-term daily intake of soybean IF to reverse established osteopenia in the adult ovariectomized rat, according to the IF ingestion level.

MATERIALS AND METHODS

Animals and diets. The study was conducted in accordance with current legislation on animal experiments in France. Female Wistar rats ($n = 60$; 195 d old) were purchased from I.N.R.A. (Clermont-Ferrand/Theix, France) and housed individually at 21°C with a 12-h light/dark cycle in metallic cages. Throughout the experimental period, rats had free access to water and were fed a daily humidified (1 mL water/g food) soy protein–free powdered semipurified diet (I.N.R.A., Jouy en Josas, France; **Table 1**). At 210 d old (~ 299 g), five rats designated as initial controls (IC) were killed by cervical dislocation; simultaneously (on d 0), the 55 remaining rats were anesthetized intraperitoneally with chloral hydrate (Fluka Chemie AG, Buchs, Switzerland; 80 g/L in saline solution; 0.4 mL/100 g body) and either sham-operated (SH; $n = 14$) or ovariectomized (OVX; $n = 41$). On d 80, rats designated as intermediate controls (SHi: $n = 5$; OVXi: $n = 5$) were killed to confirm the ovariectomy-induced bone loss. Then, the remaining OVX rats ($n = 36$; ~ 366 g) were randomly assigned into one of four groups of nine rats each, fed IF at 0 (OVX), 20 (IF20), 40 (IF40) or 80 (IF80) mg/(kg body · d) for 84 d and killed on d 164. IF were fed as a powdered soy IF concentrate (Novasoy Isoflavone compound 152-400; Archer Daniels Midland Company, Decatur, IL) containing 348 mg/g as total IF (genistin, 159; daidzin, 156; glycitin, 33) and mixed with the semipurified diet. Simultaneously, SH and OVX rats were fed the semipurified diet without any additional compounds. Throughout the experiment, the quantity of food distributed to each rat each day was adjusted to the mean level consumed by SH rats the previous day, to prevent ovariectomy-induced hyperphagia. Rats were weighed weekly to allow adjustment of IF doses to body weight during the second part of the experiment. At 48 h before the rats were killed, body composition was estimated with dual-energy x-ray absorptiometry (DEXA) (20). On d 0, 40, 80, 122 and 164, a 24-h urine sample was collected from rats that were housed individually for 24 h in metabolic cages, to measure the urinary excretion of calcium and/or deoxypyridinoline (DPD), a marker of bone resorption (21). Simultaneously, blood samples were harvested at 0900 h into ice-cooled heparinized plastic tubes containing 200 peptidase inhibitory units of aprotinin (Iniprol; Choay, Paris, France) per mL blood and centrifuged immediately (3500 *g* for 5 min at 4°C). Then, plasma was frozen at −20°C until measurements were made of phytoestrogens, calcium and/or osteocalcin (OC), a marker of osteoblast activity (22). On d 80 or 164, uterine horns were removed from each rat and weighed. Right or left femurs were cleaned from adjacent tissues and used for bone mineral density (BMD) measurements or mechanical testing, followed by image analysis, respectively.

TABLE 1

Composition of the soy protein–free powdered semipurified diet

Ingredient[1]	
	g/kg
Casein	180
Sucrose	210
Cornstarch	430
Cellulose fiber	100
Peanut oil	25
Rapeseed oil	25
Vitamin mixture[2]	10
Mineral mixture[3]	18.5
DL-Methionine	1.5

1 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) and DL-methionine (Jerafrance, Jeufosse, France).

2 Expressed in mg/kg of mixture: retinyl palmitate (250 IU/mg), 2000; cholecalciferol (400 IU/mg), 312; DL-α-tocopherol acetate (0.25 IU/mg), 20,000; menadione, 100; thiamine HCl, 1000; riboflavin, 1000; nicotinic acid, 4500; D-calcium pantothenate, 3000; pyridoxine HCl, 1000; inositol, 5000; D-biotin, 20; folic acid, 200; cyanocobalamin, 1.35; ascorbic acid, 10,000; *p*-aminobenzoic acid, 5000; choline chlorhydrate, 75,000; and sucrose, finely powdered, 871.9 g.

3 Expressed in g/kg of mixture: $CaHPO_4 \cdot 2H_2O$, 308; K_2HPO_4, 194; $CaCO_3$, 146; $MgSO_4 \cdot 7H_2O$, 109; NaCl, 168; MgO, 24.3; $FeSO_4 \cdot 7H_2O$, 20.9; $ZnSO_4 \cdot H_2O$, 12.1; $MnSO_4 \cdot H_2O$, 12.1; $CuSO_4 \cdot 5H_2O$, 2.4; NaF, 1.9; $CrK(SO_4)_2 \cdot 12H_2O$, 1.2; KI, 0.097; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.0005; $CoCO_3$, 0.0005; Na_2SeO_3, 0.0005.

Plasma phytoestrogen concentrations. Genistein, daidzein and equol were measured with enzyme-linked immunosorbent assays (23,24). The sensitivity was 35, 40, and 10 nmol/L for genistein, daidzein and equol, respectively. The intra-assay variation was 4.8, 5 and 5% for genistein, daidzein and equol, respectively, whereas the interassay variation was 13.1, 12.8 and 13.6% for genistein, daidzein and equol, respectively.

Plasma OC concentrations. The OC concentrations were assessed by radioimmunoassays with rat ^{125}I-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 variations were 7 and 9%, respectively.

Urinary DPD excretion. DPD excretion was determined by competitive radioimmunoassay with rat monoclonal anti-DPD antibody coated to the inner surface of a polystyrene tube and ^{125}I-labeled DPD (Pyrilinks-D RIA kit; Metra Biosystems, Mountain View, CA). The sensitivity was 2 nmol/L. The intra- and interassay variations were 4 and 6%, respectively. Results were expressed as nmol DPD/mmol creatinine (21). The urinary creatinine assay, based on a modified Jaffés method in which picric acid forms a colored solution in the presence of creatinine, was used to adjust DPD values for variation in urine volume.

Plasma and urinary calcium concentrations. These concentrations were measured by atomic absorption spectrophotometry with a Perkin-Elmer 400 spectrophotometer (Perkin-Elmer Cetus, Norwalk, CT). Samples were previously diluted with lanthanum oxide solution (1 g/L; Carlo Erba Reagenti, Val de Reuil, France).

BMD. BMD was assessed with DEXA with a Hologic QDR-4500A x-ray bone densitometer (Hologic, Massy, France). The total femur BMD (T-BMD), as well as the BMD of two subregions, one corresponding to the diaphysis (D-BMD) (rich in cortical bone) and the other corresponding to the distal femur metaphyseal zone (M-BMD) (mainly cancellous bone), were determined (25).

Mechanical testing. Immediately after collection, the femoral length and the mean diaphyseal diameter were measured with precision calipers (Mitutoyo, Shropshire, U.K.). Then, the femoral failure load was determined with a Universal Testing Machine (Instron 4501; Instron, Canton, MA), according to a three-point bending test (26).

Image analysis. Distal femurs were first dehydrated in a graded series of ethanol solutions and embedded in methyl metacrylate (Sigma, L'Isle d'Abeau, France) (27). Then, 100- to 200-μm frontal sections were cut with a low speed saw (Isomet 2000; Buehler, Krautkramer, Champagne-Mont d'Or, France) (with a diamond-tipped cutting blade), ground to 80-μm sections with a polishing machine (Metaserv 2000; Buehler) and stained according to the Von Kossa silver method ($AgNO_3$; Sigma). To characterize static cancellous bone, image analysis was performed in the secondary spongiosa of the distal femur metaphysis with an image-analysis system with OsteoLab software (Biocom, Paris, France) that allows an evaluation of cancellous bone area/measured surface and the number, separation or thickness of trabeculae.

Statistical methods. Results were expressed as means ± SEM. All data were analyzed with GraphPad InStat software (Microsoft, San Diego, CA). An ANOVA was first performed to test for any significant differences among groups. When significant, the Student-Newman-Keuls multiple comparison test was used to determine the specific differences between means. Parametric ANOVA was performed when data were sampled from populations with equal variance. Otherwise, nonparametric methods were selected. Thus, a Kruskal-Wallis test was first performed. If it indicated a significant difference among groups, the Mann-Whitney *U* test was used to determine specific differences. To test for any significant differences among days within a group, repeated measures ANOVA was performed and, when significant, the Student-Newman-Keuls multiple comparison test was used to determine the specific differences between means. The level of significance was set at $P < 0.05$ for all statistical tests.

RESULTS

Body composition. Although body weight increased between d 0 and 80 in both SH and OVX rats (d 80; $P < 0.0001$), the former were lighter than the latter ($P < 0.05$) at the end of the first experimental period (**Fig. 1**). As body weight increased between d 80 and 164 in both SH and OVX rats ($P < 0.001$), SH rats were still lighter than OVX rats ($P < 0.05$) at the end of the experiment. Furthermore, except in the IF20 group, body weight did not significantly vary between d 80 and 164 in ovariectomized rats fed IF. As a result, on d 164, rats in the IF80 group had a body weight not different from that of SH rats and lower than that in OVX rats ($P < 0.05$), whereas values in IF20 or IF40 rats were not different from those in SH or OVX rats. On the other hand, no significant differences were observed among groups for fat and lean relative masses (28 ± 1 and 69 ± 1 g/100 g body, respectively), except in rats in the IC group, which had a lower relative fat mass and a higher relative lean mass than the others (14 ± 2 and 83 ± 2 g/100 g body, respectively; $P < 0.001$).

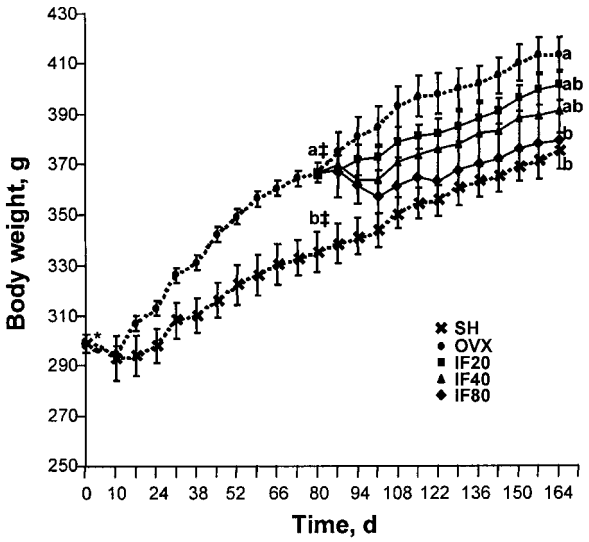

FIGURE 1 Body weight in sham-operated (SH), ovariectomized (OVX), ovariectomized + isoflavones at 20 mg/(kg body · d) (IF20), ovariectomized + isoflavones at 40 mg/(kg body · d) (IF40) and ovariectomized + isoflavones at 80 mg/(kg body · d) (IF80) rats. Values are means ± SEM, $n = 9$ (or 36 in OVX from d 0 to d 80). *Within SH or OVX groups, means on d 0 were significantly different from d 80, $P < 0.0001$. ‡Within SH, OVX or IF20 groups, means on d 80 were significantly different from d 164, $P < 0.001$; means on d 80 or d 164 not sharing a letter differ significantly, $P < 0.05$.

TABLE 2

Effects of ovariectomy and dietary soy isoflavones (IF) on uterine relative weight in rats[1]

Group[2]	Uterine weight
	g/100 g body
SHi	0.27 ± 0.02[a]
OVXi	0.05 ± 0.01[b]
SH	0.38 ± 0.04[a]
OVX	0.08 ± 0.01[b]
IF20	0.10 ± 0.01[b]
IF40	0.06 ± 0.01[b]
IF80	0.11 ± 0.01[b]

[1] Values are means ± SEM, $n = 5$–9. Means not sharing a letter differ significantly, $P < 0.001$.

[2] Group designations: SHi, intermediate sham-operated control rats; OVXi, intermediate ovariectomized control rats; SH, sham-operated rats; OVX, ovariectomized rats; IF20 rats ovariectomized plus IF fed at 20 mg/(kg body · d); IF40 rats, ovariectomized plus IF fed at 40 mg/(kg body · d); IF80 rats, ovariectomized plus IF fed at 80 mg/(kg body · d).

Uterine relative weight. It was lower in OVX than in SH rats ($P < 0.001$), on both d 80 and d 164 (**Table 2**). Moreover, on d 164, uterine weights in ovariectomized rats fed IF were not different from that of OVX rats.

Plasma phytoestrogen concentrations. Values on d 164 were lower in IF20 rats than in IF40 or IF80 rats ($P < 0.0001$) but did not differ among IF40 and IF80 rats (**Table 3**). Simultaneously, very low levels of genistein, daidzein and equol were detected in the plasma of untreated rats (0.08 ± 0.01, 0.09 ± 0.01 and 0.05 ± 0.01 μmol/L, respectively, $P < 0.0001$).

Plasma OC concentrations. On each day of measurement, plasma OC values were higher in OVX than in SH rats (**Fig. 2**). Moreover, although plasma OC concentrations in IF20 or IF80 rats were not different from that in SH rats on d 122, values in the three groups of rats fed IF also did not differ from that in OVX rats. By contrast, as circulating OC levels in treated rats decreased between d 122 and 164, although they were not modified in OVX or SH rats, concentrations at d 164 in IF-fed rats were not different from that in SH rats and lower than that in OVX rats (except in rats from the IF20 group).

Urinary DPD excretion. Values were significantly higher in OVX than in SH rats at all time points (**Fig. 3**). On the other hand, although urinary DPD excretion at d 122 was

TABLE 3

Effects of dietary soy isoflavones (IF) on plasma genistein, daidzein and equol concentrations[1]

Group[2]	Genistein	Daidzein	Equol
		μmol/L	
IF20	0.73 ± 0.06[b]	0.72 ± 0.09[b]	0.95 ± 0.12[b]
IF40	1.32 ± 0.16[a]	1.38 ± 0.11[a]	1.50 ± 0.19[a]
IF80	1.39 ± 0.29[a]	1.62 ± 0.22[a]	1.93 ± 0.30[a]

[1] Values are means ± SEM, $n = 9$. Means not sharing a letter differ significantly, $P < 0.0001$.

[2] Group designations: IF20 rats, ovariectomized plus IF fed at 20 mg/(kg body · d); IF40 rats, ovariectomized plus IF fed at 40 mg/(kg body · d); IF80 rats, ovariectomized plus IF fed at 80 mg/(kg body · d).

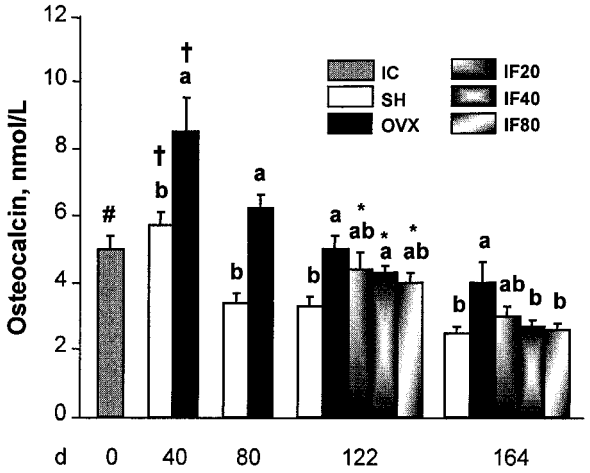

FIGURE 2 Plasma osteocalcin concentrations in initial control (IC), sham-operated (SH), ovariectomized (OVX), ovariectomized + isoflavones at 20 mg/(kg body · d) (IF20), ovariectomized + isoflavones at 40 mg/(kg body · d) (IF40) and ovariectomized + isoflavones at 80 mg/(kg body · d) (IF80) rats. Values are means ± SEM, $n = 9$ (or 5 in IC, or 36 in OVX on d 40 and d 80). #Different from SH rats on d 80, d 122 or d 164, $P < 0.05$. †Within a group, means on d 40 were significantly different from d 80, d 122 or d 164 (SH) or from d 80 (OVX), $P < 0.05$. *Within a group, means on d 122 were significantly different from d 164, $P < 0.05$; means on each day not sharing a letter differ significantly, $P < 0.05$.

higher in IF-treated rats than in SH rats, concentrations in IF80 rats, but not in IF20 or IF40 rats, were significantly lower than that in OVX rats ($P < 0.05$). Moreover, DPD excretion in IF40 rats decreased between d 122 and 164 ($P < 0.05$). As a result, although on d 164 DPD excretion was greater in all ovariectomized rats than in SH rats, urinary DPD excretion in IF40 or IF80 rats (but not in IF20 rats) was significantly lower than that in OVX rats ($P < 0.05$).

Plasma and urinary calcium concentrations. Plasma calcium levels did not differ among groups at any time point (2.58 ± 0.07, 2.53 ± 0.03, 2.61 ± 0.01 and 2.47 ± 0.01 mmol/L on d 0, 80, 122 and 164, respectively). Urinary calcium excretion

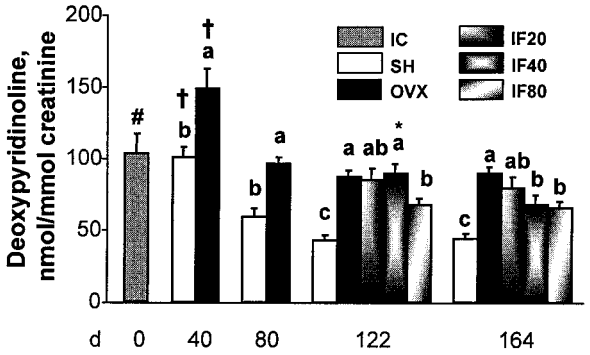

FIGURE 3 Urinary deoxypyridinoline excretion in initial control (IC), sham-operated (SH), ovariectomized (OVX), ovariectomized + isoflavones at 20 mg/(kg body · d) (IF20), ovariectomized + isoflavones at 40 mg/(kg body · d) (IF40) and ovariectomized + isoflavones at 80 mg/(kg body · d) (IF80) rats. Values are means ± SEM, $n = 9$ (or 5 in IC, or 36 in OVX on d 40 and d 80). #Different from SH on d 80, d 122 or d 164, $P < 0.005$. †Within a group, means on d 40 were significantly different from d 80, d 122 or d 164 (SH) or from d 80 (OVX), $P < 0.01$. *Within a group, means on d 122 were significantly different from d 164, $P < 0.05$; means on each day not sharing a letter differ significantly, $P < 0.05$.

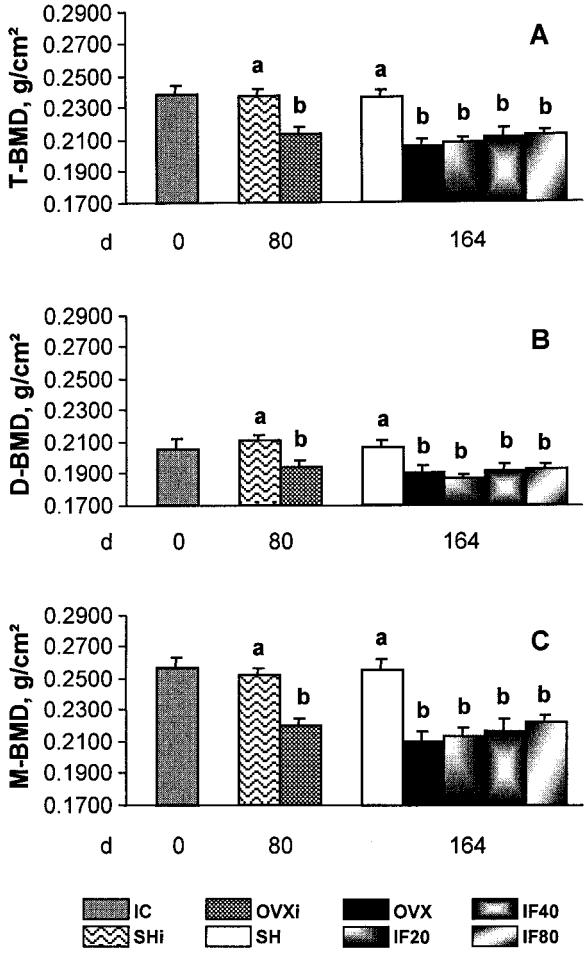

FIGURE 4 Bone mineral density (BMD) in initial control (IC), intermediate sham-operated control (SHi), intermediate ovariectomized control (OVXi), sham-operated (SH), ovariectomized (OVX), ovariectomized + isoflavones at 20 mg/(kg body · d) (IF20), ovariectomized + isoflavones at 40 mg/(kg body · d) (IF40) and ovariectomized + isoflavones at 80 mg/(kg body · d) (IF80) rats. *(A)* Total femur (T-BMD); *(B)* femoral diaphysis (D-BMD) and *(C)* distal femur metaphysis (M-BMD). Values are means ± SEM, $n = 5$–9; means on each day not sharing a letter differ significantly, $P < 0.005$ *(A)*, $P < 0.05$ *(B)* and $P < 0.001$ *(C)*.

also did not differ among groups (2.6 ± 0.2, 3.1 ± 0.2, 2.8 ± 0.2 and 3.9 ± 0.2 mg/d on d 0, 80, 122 and 164, respectively).

BMD. BMD values did not differ among IC, SHi or SH rats but were significantly lower in ovariectomized rats than in SH rats, on both d 80 and 164 (**Fig. 4**). Furthermore, BMD values on d 164 were not greater in IF-treated rats than in OVX rats.

Mechanical testing. No significant difference among groups was demonstrated for femoral length, diaphyseal diameter or femoral failure load throughout the experiment (37.1 ± 0.1 mm, 3.62 ± 0.04 mm or 115 ± 2 N, respectively).

Image analysis. Cancellous bone area/measured surface in the distal femur metaphysis was not different among IC, SHi and SH groups. By contrast, it was significantly lower in ovariectomized rats than in SH rats on both d 80 and d 164 (**Fig. 5**). Furthermore, values on d 164 were not greater in IF-treated rats than in OVX rats. Similarly, trabecular number was lower in all ovariectomized rats than in all nonovariectomized rats (1.6 ± 0.1 versus 3.2 ± 0.2 trabeculae/mm, $P < 0.0001$). Trabecular separation was higher in all ovariecto-

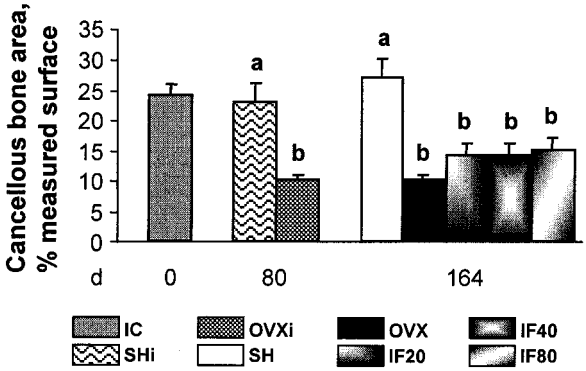

FIGURE 5 Cancellous bone area/measured surface in the distal femur metaphysis in initial control (IC), intermediate sham-operated control (SHi), intermediate ovariectomized control (OVXi), sham-operated (SH), ovariectomized (OVX), ovariectomized + isoflavones at 20 mg/(kg body · d) (IF20), ovariectomized + isoflavones at 40 mg/(kg body · d) (IF40) and ovariectomized + isoflavones at 80 mg/(kg body · d) (IF80) rats. Values are means ± SEM, $n = 5–9$; means on each day not sharing a letter differ significantly, $P < 0.05$.

mized rats than in all nonovariectomized rats (613 ± 36 versus 242 ± 19 μm, $P < 0.0001$). By contrast, although trabecular thickness (which did not differ among IC, SHi and SH rats) was lower in OVXi than in SHi rats (65 ± 3 versus 77 ± 3 μm, $P < 0.05$), no significant difference was observed among groups on d 164 (84 ± 2 μm).

DISCUSSION

Various studies have demonstrated preventive effects of dietary soybean IF on ovariectomy-induced osteopenia in rats (8–13). However, only one experiment showed that once bone loss has occurred, its reversal through daily consumption of soy proteins (with normal or reduced IF content) from d 35 to d 100 after surgery was difficult to obtain (19). Nevertheless, this short-term study in young rats was performed with only one dose. Indeed, several consumption levels associated with a long-term ingestion should be tested. Furthermore, an experiment in adult rather than in young rats should be required to assess bone loss. Therefore, the present study investigated the dose-dependent bone-curative effects of daily soybean IF intake in rats that underwent ovariectomy at the age of 7 mo and were fed IF for 84 d from d 80 after surgery.

As expected, Figure 4 indicates that in intermediate or experimental rats, ovariectomy (confirmed with uterine atrophy; Table 2) induced femoral osteopenia, in both in the distal femur metaphysis (rich in cancellous bone that is mainly involved in metabolic functions) and the femoral diaphysis (rich in cortical bone that fulfills essentially mechanical and protective functions), as shown by M- and D-BMD, respectively. However, the D-BMD reduction was not associated with an impairment of mechanical properties, as indicated by the femoral failure load, which remained constant. On the other hand, the M-BMD drop was associated with a decrease in cancellous bone area (Fig. 5). Simultaneously, trabeculae number decreased, whereas trabecular separation increased. By contrast, although trabecular thickness was lower in OVXi than in SHi rats, no significant difference was observed between SH and OVX rats on d 164. It might indicate that bone loss has occurred by lowering the trabeculae number rather than by reducing them. Moreover, osteopenia probably resulted from an increase in bone turnover, as shown by the higher plasma OC concentration and urinary DPD excretion in OVX than in SH rats (Figs. 2, 3).

Unlike Arjmandi et al. (19), who reported that soy diets that provide per os genistin plus daidzin at ~25 or ~2.5 μg/(g body · d) during a 65-d period were somewhat effective in reversing the femoral bone density loss (assessed with the Archimedes principle), IF consumption in the present experiment did not elicit any curative effect on femoral BMD (assessed by DEXA), at both the cortical and cancellous sites (Fig. 4). Nevertheless, our results are in accordance with those of Arjmandi et al. (19), who also reported that ash weight (g/100 g dry bone) in the right femur was lower in ovariectomized rats whether fed or not fed soy diets than in SH rats. Moreover, associated with M-BMD data, no curative effect of IF on changes in cancellous bone area (Fig. 5) or number and separation of trabeculae was observed in the present study. By contrast, bone turnover was lower in IF-fed rats than in OVX rats (Figs. 2, 3). Although osteocalcinemia in IF20 rats was not different from that in OVX rats, values at d 164 in the three groups of rats fed IF were similar to that in SH rats. Furthermore, plasma OC concentrations were decreased between d 122 and d 164 in IF-fed rats, whereas they remained constant in OVX rats (Fig. 2). Although urinary DPD excretion was higher in IF-fed ovariectomized rats than in SH rats, values were lower in IF80 than in OVX rats on both d 122 and 164 (Fig. 3). Moreover, they decreased between d 122 and 164 in the IF40 group, whereas they remained stable in OVX rats, resulting in a d-164 DPD excretion lower in IF40 than in OVX rats. These data suggest that the IF-induced antiosteoclastic activity occurred in a dose-dependent manner, because only the two highest levels of consumption reduced bone resorption. However, Arjmandi et al. (19) reported that the daily intake of genistin plus daidzin at ~25 or ~2.5 μg/(g body · d) did not slow down the ovariectomy-induced higher rates of bone turnover. Nevertheless, considering the first soy group in this later study and the IF20 group in the present experiment, results do not differ completely in that in both groups, bone resorption was similar to that measured in OVX rats and higher than that in SH rats.

In parallel with bone turnover parameters, plasma phytoestrogen concentrations at d 164 were increased in a dose-dependent manner between IF20 and IF40 groups but not between IF40 and IF80 groups (Table 3). It could be in part explained by a maximal IF level beyond which IF absorption might not be further increased and by the possibility of an elimination system of plasma phytoestrogens more efficient with high levels than with low doses, in 10- to 13-mo-old ovariectomized rats. Moreover, in the early part of soybean feeding and, therefore probably in association with a small adaptation period to the diet, a weak (and nonsignificant) reduction in body weight was observed in the IF80 group (Fig. 1). As a result and because OVX rats exhibited a greater body weight than SH rats regardless of whether the pair-feeding to SH rats had minimized the ovariectomy-induced hyperphagia, body weight at d 164 in IF80 rats was lower than that in OVX rats and did not differ from that in SH rats. Finally, these present results also indicate that IF feeding was unable to reverse the ovariectomy-induced uterine atrophy (Table 2). In the same way, 65 d of soy feeding providing per os genistin plus daidzin at ~25 μg/(g body · d) did not result in any uterotrophic activity in young ovariectomized rats (19).

In conclusion, the present study demonstrated that a daily soybean IF intake in adult ovariectomized rats reduced bone turnover but did not reverse a previously established bone loss. Furthermore, it appeared that the two highest consumption levels were more effective in depressing the ovariectomy-

induced increase in bone turnover (and in bone resorption specifically) than the lowest dose. Therefore, ingestion levels of soybean IF should be considered to improve bone health in a preventive rather than a curative approach of human postmenopausal osteoporosis.

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