Hesperidin, a Citrus Flavonoid, Inhibits Bone Loss and Decreases Serum and Hepatic Lipids in Ovariectomized Mice[1]

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ABSTRACT The purpose of this study was to examine whether hesperidin inhibits bone loss in ovariectomized mice (OVX), an animal model of postmenopausal osteoporosis. Forty 8-wk-old female ddY mice were assigned to five groups: a sham-operated group fed the control diet (AIN-93G), an OVX group fed the control diet, an OVX+HesA group fed the control diet containing 0.5 g/100 g hesperidin, and an OVX+HesB group fed the control diet containing 0.7 g/100 g α-glucosylhesperidin and an OVX+17β-estradiol (E_2) group fed the control diet and administered 0.03 μg E_2/d with a mini-osmotic pump. After 4 wk, the mice were killed and blood, femoral, uterine and liver were sampled immediately. Hesperidin administration did not affect the uterine weight. In OVX mice, the bone mineral density of the femur was lower than in the sham group ($P < 0.05$) and this bone loss was significantly prevented by dietary hesperidin or α-glucosylhesperidin. The Ca, P and Zn concentrations in the femur were significantly higher in the hesperidin-fed and E_2 groups than in the OVX group. Histomorphometric analyses showed that the trabecular bone volume and trabecular thickness in the femoral distal metaphysis were markedly decreased ($P < 0.05$) by OVX, and α-glucosylhesperidin significantly prevented this bone loss. Furthermore, hesperidin decreased the osteoclast number of the femoral metaphysis in OVX mice, as did E_2. Serum and hepatic lipids were lower in mice that consumed the hesperidin-containing diets ($P < 0.05$) than in the OVX group fed the control diet. These results suggest a possible role for citrus flavonoids in the prevention of lifestyle-related diseases because of their beneficial effects on bone and lipids. J. Nutr. 133: 1892–1897, 2003.

KEY WORDS: • *bone mineral density* • *estrogen* • *hesperidin* • *osteoporosis* • *uterus*

Osteoporosis is the most common bone disease, characterized by reduced bone mineral density (BMD)[4] and an increased risk of fracture. In particular, in postmenopausal women, osteoporosis is one of the critical disorders involving high bone turnover and bone loss attributed to estrogen deficiency (1). Although estrogen replacement therapy can prevent bone loss caused by menopause, some adverse effects such as uterine bleeding and carcinogenesis accompany it (2,3).

Several lines of evidence show that nonsteroidal, estrogen-like compounds such as phytoestrogens can prevent bone loss in osteoporotic animal models and postmenopausal women (4–8). Furthermore, certain vegetables, such as onion and Italian parsley, can prevent bone resorption in ovariectomized (OVX) rats (9,10). These vegetables are rich in flavonoids such as quercetin and rutin. In fact, rutin inhibits trabecular bone loss caused by estrogen deficiency in ovariectomized rats (9).

Among the naturally occurring citrus flavonoids, hesperidin, by pharmacological determination, is a potential anti-inflammatory agent (11). Furthermore, hesperidin may be associated with potential health benefits, such as the prevention of atherosclerosis progression, lowering cancer risks and positive effects on vaginal symptoms (12–14). Hesperidin also regulates hepatic cholesterol synthesis by inhibiting the activity of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase (15–17). Recently, Mundy et al. (18) reported that statins, cholesterol-lowering agents, induce bone formation and inhibit bone resorption both in vitro and in vivo. Furthermore, it has been confirmed that the BMD in patients treated with statins is higher than in untreated subjects (19–21). Thus, considerable attention has focused on the relationship between the inhibitory activity on HMG-CoA reductase and bone metabolism. In this study, we examined the effects of hesperidin and α-glucosylhesperidin, which is 10,000 times more water soluble than hesperidin, on bone metabolism in ovariectomized mice.

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[4] BMD, bone mineral density; BV/TV, bone volume/tissue volume; E_2, 17β-estradiol; ER, estrogen receptor; HesA, hesperidin; HesB, α-glucosylhesperidin; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA; OC.N/BS, osteoclasts number/bone surface; OVX, ovariectomized; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; TC, total cholesterol; TRAP, tartrate-resistant acid phosphatase.

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MATERIALS AND METHODS

Animals and diets. Eight-wk-old female ddY mice were obtained from Shizuoka Laboratory Animal Center (Shizuoka, Japan). After a 3-d adaptation period, 40 mice were either sham-operated ($n = 8$) or OVX ($n = 32$) and fed an AIN-93G control diet with corn oil instead of soybean oil (22) (**Table 1**). The mice were housed in individual plastic cages in a temperature- and humidity-controlled room (23 ± 1°C and 60 ± 5% relative humidity) with a 12-h light/dark cycle, and were given free access to food and distilled water. Starting on d 0, the OVX mice ($n = 32$) were randomly divided into four groups of 8 each. The sham group and two OVX groups were fed the control diet (AIN-93G) (Table 1). One group of OVX mice received a hesperidin-containing diet [HesA: AIN-93G diet (22) containing 5.0 g/kg hesperidin] or an α-glucosylhesperidin diet (HesB: AIN-93G diet containing 7.0 g/kg α-glucosylhesperidin) (Table 1) for 4 wk. Some OVX mice received subcutaneously 0.03 μg/d 17β-estradiol (E_2) (Sigma Chemical Co., St. Louis, MO) with a mini-osmotic pump (Alza Corp., Palo Alto, CA), and fed the control diet. At the end of the experiment, the mice were killed with pentobarbital sodium (Nembutal; Dainippon Pharmaceutical Co., Osaka, Japan). In each experiment, body and uterine weights were measured, and the right and left femora were removed for the measurement of BMD and histomorphometric analyses. Both native hesperidin (hesperidin 7-rutinoside) and α-glucosylhesperidin were supplied by Toyo Sugar Refining (Tokyo, Japan).

Figure 1 shows the molecular structure of α-glucosylhesperidin. The dose of hesperidin in both the HesA and the HesB diets was the same, although the actual concentration of HesB was 0.2% higher than that of HesA because HesB is enzymatically modified hesperidin. To improve its low water solubility, native hesperidin (HesA) was hydrolyzed with β-glucosidase. This enzymatically modified hesperidin, α-glucosylhesperidin (HesB), has a high water solubility 10,000 times that of native hesperidin.

All procedures were in accordance with the National Institute of Health and Nutrition Guidelines for the Care and Use of Laboratory Animals in Japan.

Bone analysis. The femoral bones were carefully removed at necropsy. The right femur of each mouse was used for analysis of BMD and area by dual X-ray absorptiometry (DXA, Model DCS-600R; Aloka, Tokyo, Japan). BMD was calculated by the bone mineral content of the measured area. The scanned area of the femur was equally divided into three regions (proximal, midshaft, and distal femur) to assess the regional differences.

The femora were dried overnight at 100°C, weighed and then ashed at 550°C for 48 h. The ashed samples were extracted with 1 mol/L HCl. The amounts of Ca, Mg and Zn in the femur were determined by atomic absorption spectrophotometry (Spectra AA220FS; Varian, Melbourne, Australia) (23). Phosphorus in the femur was analyzed colorimetrically (24).

TABLE 1

Composition of experimental diets

Ingredient	Control	HesA	HesB
		g/kg diet	
Casein milk	200	200	200
Corn starch	529.5	529.5	529.5
Sucrose	100	95	93
Corn oil	70	70	70
Cellulose	50	50	50
Mineral mixture[1]	35	35	35
Vitamin mixture[1]	10	10	10
Choline	2.5	2.5	2.5
L-Cystine	3.0	3.0	3.0
Hesperidin[2]	—	5.0	7.0

[1] Prepared according to the AIN-93G formulation (22).

[2] Hesperidins (HesA; hesperidin, HesB; α-glucosylhesperidin); concentration of hesperidin was >95% of total mixture.

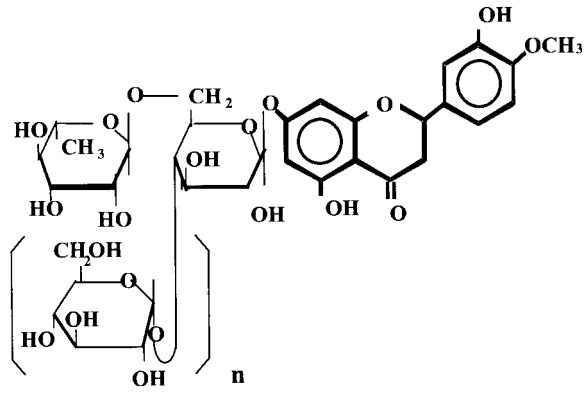

FIGURE 1 Molecular structure of α-glucosylhesperidin.

The femoral cancellous bone of the distal metaphysis was analyzed two-dimensionally by use of a μCT system (μCT; Scanco Medical, Zurich, Switzerland) (25). The mean tissue volume of the scanned areas was 0.44 mm^3 in the trabecular bone of the femoral distal metaphysis.

For histomorphometry of the femoral distal metaphysis, undecalcified 5-μm sections were prepared from the femora and stained with tartrate-resistant acid phosphatase (TRAP). TRAP was used as a marker for the osteoclasts. The fixed sections were incubated in an acetate buffer (0.1 mol/L sodium acetate, pH 5.0) containing naphthol AS-MX phosphate (Sigma) as a substrate and fast red violet LB salt (Sigma) as stain for the reaction product in the presence of 50 mmol/L sodium tartrate. The mean number of osteoclasts in each millimeter of trabecular bone surface was determined in the area (1.08 mm^2) of the secondary spongiosa of the distal metaphysis (26). Histomorphometry was performed with a semiautomatic image system (Osteoplan II; Carl Zeiss, Thornwood, NY) linked to a light microscope (27). Histomorphometric parameters were quantified in cancellous bone tissue at the secondary spongiosa. The region in the trabecular bone within one cortical width from the endosteal surface was excluded from the measurements. Trabecular bone volume/tissue volume (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp) and the number of TRAP-positive multinucleated osteoclasts/bone surface, mm^{-1} (Oc.N/BS) were measured.

Serum and liver lipids. Lipids in the liver were extracted quantitatively with an ice-cold mixture of chloroform and methanol (2:1, v/v) by the method of Folch et al. (28). Total cholesterol (TC) and triglyceride (TG) concentrations in the liver and serum were measured by enzymatic colorimetric methods [cholesterol C test (Wako Pure Chemicals, Osaka, Japan); triglyceride E test (Wako Pure Chemicals)] (29,30). The HDL cholesterol level was measured by an enzymatic method (HDL cholesterol test; Wako Pure Chemicals) (31).

Serum hesperetin. Serum hesperetin was measured using the method of Adlercreutz et al. (32) combined with the modified HPLC method described by Gamache et al. (33). For the recovery calculation, 20 μL of ^{3}H-estradiol glucuronide and 10 μL of 10 mmol/L flavone (as the internal standard) were added to tubes containing 200 μL of serum and 200 μL enzyme solution. After mixing, the sample was hydrolyzed overnight at 37°C, and then unconjugated hesperetin was extracted with diethyl ether. The ether fraction was evaporated completely and dissolved in 200 μL methanol. A 10-μL sample of the solution was injected into the HPLC with electrochemical detection (Coulochem 2; ESA, Chelmsford, MA) and UV detection (SPD-10A; Shimadzu, Tokyo, Japan). Another 20 μL of the solution was used for liquid scintillation counting to determine recovery. Peaks were detected at 280 nm. The HPLC column was an MCM C_{18} (150 × 4.6 mm I.D.; MC-Medical, Tokyo, Japan, column oven temperature, 30°C). HPLC was carried out in the mobile phase with 50 mmol/L acetate buffer (pH 4.8)/methanol/acetonitrile (50/35/15). The flow rate was 1.0 mL/min. Quantification was done by measuring the peak areas based on calibration plots of the peak area of standards at various concentrations (from 8.5 to 70 μmol/L), and corrected for losses during hydrolysis and extraction based on the recovery data.

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TABLE 2

Final body weight and wet weight of the uterus in sham-operated mice (Sham) and ovariectomized (OVX) mice fed the control diet or diets containing hesperidin (HesA) or α-glucosylhesperidin (HesB) or subcutaneously administered 0.03 μg/d 17β-estradiol (E_2)[1]

Group	Final body weight	Uterus
	g	*mg*
Sham	35.7 ± 1.5[b]	128.0 ± 8.3[b]
OVX	39.1 ± 1.3[a]	23.3 ± 1.4[c]
OVX+HesA	37.4 ± 0.4[a,b]	23.7 ± 2.2[c]
OVX+HesB	37.3 ± 0.8[a,b]	23.7 ± 2.0[c]
OVX+E_2	36.4 ± 0.9[a,b]	184.3 ± 0.7[a]

[1] Values are means ± SEM, $n = 8$. Means in a column without a common letter differ, $P < 0.05$.

TABLE 3

BMD in the femur of sham-operated mice (Sham) and ovariectomized (OVX) mice fed the control diet or diets containing hesperidin (HesA) or α-glucosylhesperidin (HesB) or subcutaneously administered 0.03 μg/d 17β-estradiol (E_2)[1]

Group	Proximal	Middle	Distal	Total
	mg/cm²			
Sham	43.2 ± 0.8[a]	35.0 ± 0.6[a,b]	44.6 ± 1.3[a,b]	40.9 ± 0.8[a]
OVX	36.0 ± 0.2[b]	30.5 ± 0.2[c]	37.2 ± 0.5[d]	34.9 ± 0.3[b]
OVX+HesA	42.2 ± 0.2[a]	33.5 ± 1.1[b,c]	40.2 ± 0.6[b,c]	38.7 ± 0.6[a]
OVX+HesB	43.8 ± 1.4[a]	34.7 ± 1.2[a,b]	39.1 ± 1.0[c,d]	39.1 ± 1.2[a]
OVX+E_2	43.2 ± 1.7[a]	37.4 ± 0.3[a]	46.1 ± 3.1[a]	43.3 ± 2.5[a]

[1] Values are means ± SEM, $n = 8$. Means in a column without a common letter differ, $P < 0.05$.

Statistical analysis. Data are expressed as means ± SEM. The effects of treatments were determined by use of one-way ANOVA. Differences among treatment groups were assessed by the Sheffé test (SPSS version 11.0; SPSS, Chicago, IL). Differences were considered significant at $P < 0.05$.

RESULTS

Body and organ weights. Initial body weights of the five groups of mice did not differ. The final body weight of the OVX group was higher than that of the sham-operated group ($P < 0.05$) (**Table 2**). Intake of hesperidin for 4 wk did not affect the body weight in OVX mice. Food intakes did not differ among the groups throughout the experiment. Uterine weight was lower ($P < 0.05$) in OVX mice than in sham-operated mice, indicating that they were estrogen deficient. Uterine weights of the OVX + hesperidin groups (HesA and HesB) did not differ from that of the OVX group. Uterine weight of the E_2 group was significantly higher than those of the other groups (Table 2). Liver weights among the groups did not differ.

Bone mass and minerals. Marked bone loss occurred in the femoral cancellous bone in OVX mice, and the loss was prevented by treatment with hesperidin or α-glucosylhesperidin. Administration of E_2 also prevented the bone loss in OVX mice (**Fig. 2**).

Total femoral BMD in the OVX group was markedly lower than in the sham-operated group ($P < 0.05$) (**Table 3**). The total BMD in all other groups was greater than in the OVX group and did not differ from that of the sham group. The reduction in BMD attributed to OVX was in the proximal, middle and distal regions of the femur, and hesperidin prevented bone loss at all three regions.

The femur Ca concentrations in the OVX groups was significantly lower than in the sham group, and those in the HesA and HesB-fed groups were greater than in the OVX group fed the control diet (**Table 4**, $P < 0.05$). There were no differences in Mg concentration between OVX and OVX fed hesperidin groups. The femur P concentration in the HesB group was higher than in the OVX group fed the control diet ($P < 0.05$). The femoral Zn concentration was greater in the HesB group than in the OVX or HesA groups ($P < 0.05$).

Bone histology. Intake of HesA, HesB or E_2 restored the loss of trabecular bone in the distal femur in OVX mice fed the control diet (**Fig. 3***A*). Histomorphometric analysis of the femoral metaphysis showed that bone volume/tissue volume (BV/TV) (Fig. 3*B*) and trabecular thickness (Tb.Th) in OVX mice (Fig. 3C) was much less than in sham-operated mice, whereas trabecular separation (Tb.Sp) in OVX mice (Fig. 3*D*) was much greater than in sham-operated mice. Intake of HesB but not HesA significantly decreased Tb.Sp in OVX mice, although HesB did not restore it to the level in the sham-operated mice ($P < 0.05$). Administration of E_2 prevented all of these OVX-induced changes.

The estrogen deficiency caused by OVX stimulated marked osteoclast differentiation, resulting in an increase in the number of TRAP-positive multinucleated osteoclasts (Fig. 3*E*).

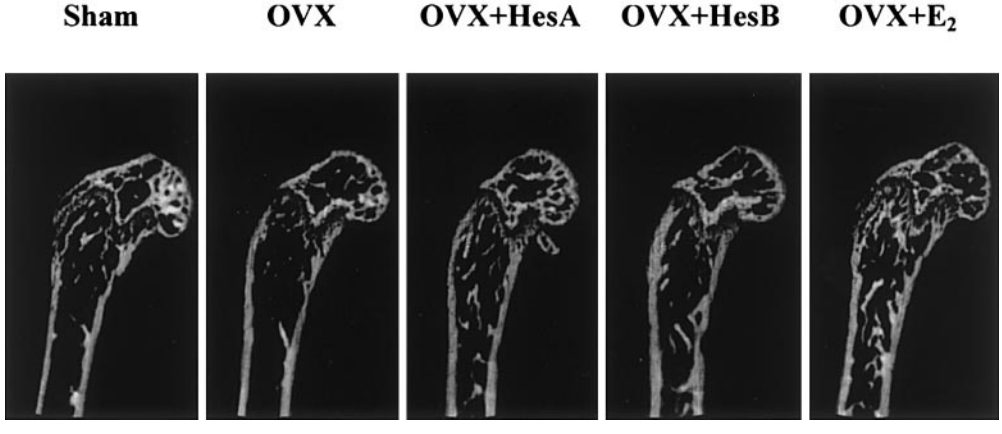

FIGURE 2 μCT scanning of the distal femoral metaphysis collected from sham-operated (Sham) mice; ovariectomized (OVX) mice and OVX mice fed diets containing hesperidin (HesA) or α-glucosylhesperidin (HesB); and OVX mice treated with 17β-estradiol (E_2) for 4 wk. Left to right: sham-operated, OVX, OVX+HesA, OVX+HesB, OVX+E_2.

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TABLE 4

Ca, Mg, P and Zn concentrations in the femur of sham-operated mice (Sham) and ovariectomized (OVX) mice fed the control diet or diets containing hesperidin (HesA) or α-glucosylhesperidin (HesB) or subcutaneously administered 0.03 μg/d 17β-estradiol (E_2)[1]

Group	Ca	P	Mg	Zn
	mmol/g dry bone		*μmol/g dry bone*	
Sham	7.81 ± 0.10[a]	3.58 ± 0.06[a,b]	185 ± 4.2[b]	3.49 ± 0.45[a,b]
OVX	7.24 ± 0.12[b]	3.40 ± 0.06[b]	182 ± 2.0[b]	2.93 ± 0.13[b]
OVX+HesA	7.98 ± 0.10[a]	3.51 ± 0.06[a,b]	189 ± 4.8[a,b]	2.81 ± 0.04[b]
OVX+HesB	8.08 ± 0.05[a]	3.65 ± 0.08[a]	190 ± 9.8[a,b]	3.74 ± 0.40[a]
OVX+E_2	7.96 ± 0.63[a]	3.45 ± 0.10[b]	217 ± 15[a]	3.99 ± 0.09[a]

[1] Values are means ± SEM, $n = 8$. Means in a column without a common letter differ, $P < 0.05$.

Administration of hesperidin, or E_2, restored it to the level in sham-operated mice.

Serum and liver lipids. Serum and hepatic total cholesterol (**Fig. 4***A, B*) and triglyceride concentrations (Fig. 4*C, D*) were low in the hesperidin-treated groups compared with the OVX group. The serum HDL cholesterol concentrations did not differ among the groups (Fig. 4*E*). The ratios of serum HDL to total cholesterol (Fig. 4*F*) in the groups treated with hesperidin were higher than in the OVX group ($P < 0.05$), and not different from that in the sham-operated group. Ad-

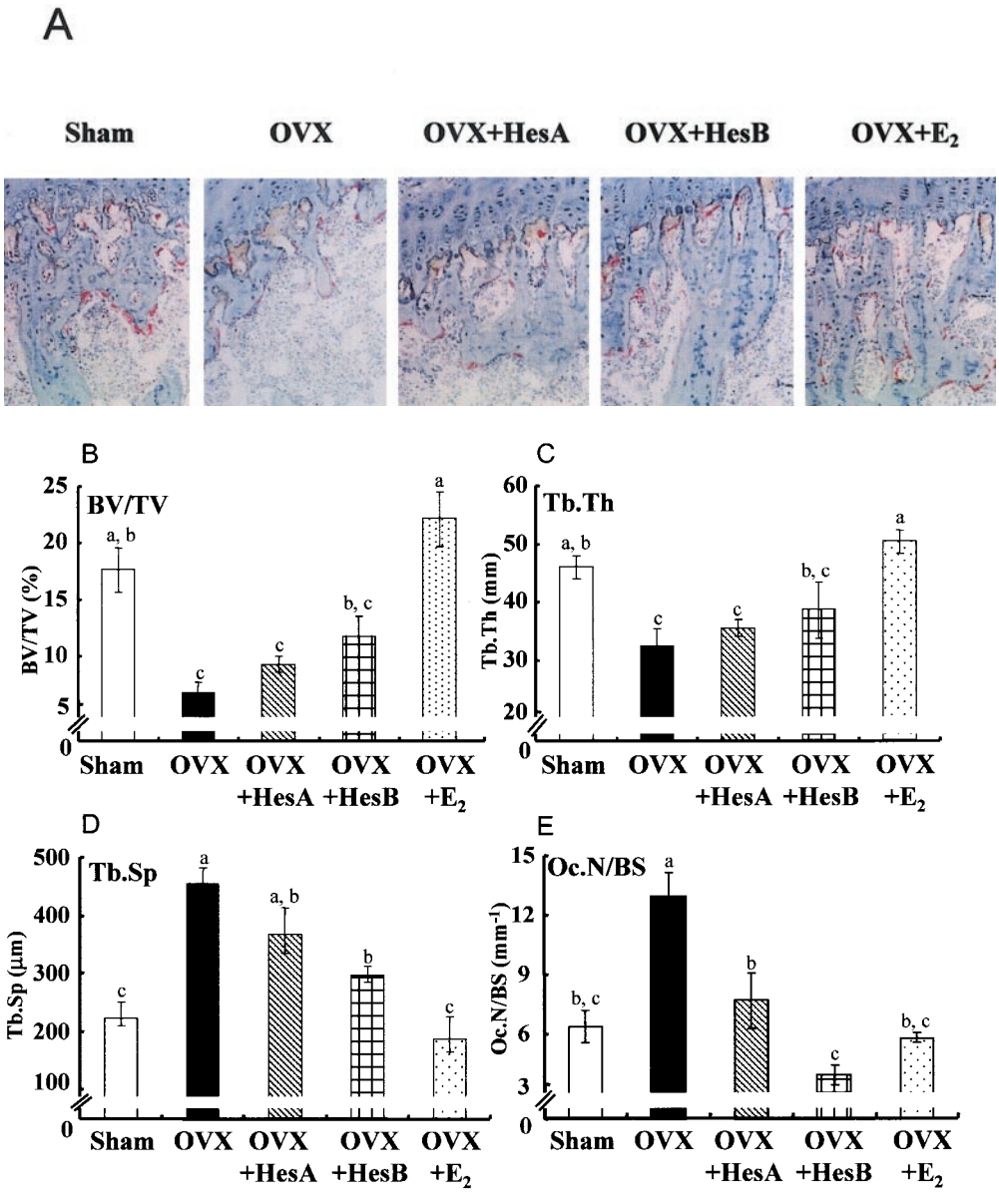

FIGURE 3 Histological analysis of the trabecular bone collected from sham-operated (Sham) mice; ovariectomized (OVX) mice and OVX mice fed diets containing hesperidin (HesA) or α-glucosylhesperidin (HesB); and OVX mice treated with 17β-estradiol (E_2) for 4 wk. (*A*) Sections of trabecular bone stained for tartrate-resistant acid phosphatase (TRAP) (×85). Left to right: sham-operated, OVX, OVX+HesA, OVX+HesB, OVX+E_2. (*B–E*) Two-dimensional histomorphometric parameters of trabecular bone shown in *A*. (*B*) Bone volume/tissue volume (BV/TV). (*C*) Trabecular thickness (Tb.Th). (*D*) Trabecular separation (Tb.Sp). (*E*) Osteoclasts number/bone surface (Oc.N/BS). Values are means ± SEM, $n = 8$. Means without a common letter differ, $P < 0.05$.

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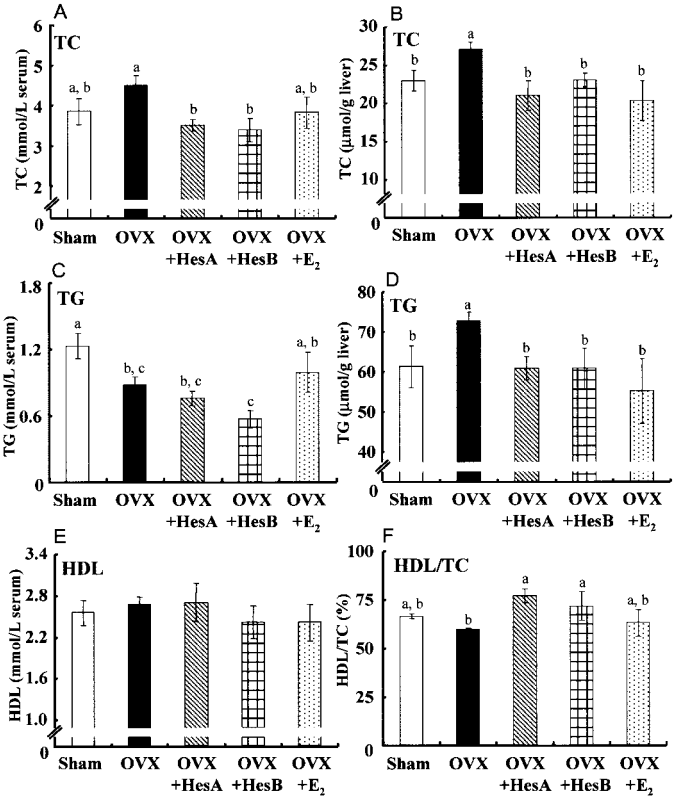

FIGURE 4 Serum and hepatic total cholesterol and triglyceride concentrations in sham-operated (Sham) mice; ovariectomized (OVX) mice and OVX mice fed diets containing hesperidin (HesA) or α-glucosylhesperidin (HesB); and OVX mice treated with 17β-estradiol (E_2) for 4 wk. (*A*) Total cholesterol (TC) level in serum. (*B*) TC level in liver. (*C*) Triglycerides (TG) in serum. (*D*) TG in liver. (*E*) HDL cholesterol in serum. (*F*) HDL cholesterol/total cholesterol ratio in serum. Values are means ± SEM, $n = 8$. Means without a common letter differ, $P < 0.05$.

ministration of E_2 decreased hepatic total cholesterol and triglyceride concentrations in OVX mice (Fig. 4*B, D*).

Serum hesperetin. The serum concentrations of hesperidin in mice fed HesA and HesB were 5.59 ± 1.60 and 9.82 ± 2.53 μmol/L, respectively. The concentration tended to be higher in the HesB group than in the HesA group ($P = 0.10$). Serum hesperetin was not detected in the other three groups.

DISCUSSION

In the present study we clearly demonstrated that hesperidin not only has cholesterol-lowering effects, but also prevents bone loss in OVX mice without substantial effects on the uterus, indicating that intake of the citrus flavonoids might be useful in preventing symptoms arising from estrogen deficiency. However, because the dose of hesperidin used in this study was much higher than the usual daily intake, it is unlikely to be consumed in a normal diet.

Recently, Horcajada-Molteni et al. (10) found that rutin, which is the main flavonol in onion, inhibited OVX-induced trabecular bone loss in rats, both by slowing bone resorption and increasing osteoblastic activity. The onion extract has also been shown to inhibit bone resorption in vitro and in vivo (34,35). In this study, we demonstrated that a citrus flavonoid could prevent bone loss in OVX mice without affecting the reproductive organs. Hesperidin prevented bone loss at all three regions of the femur, indicating that this citrus flavonoid was effective on both cortical and trabecular bones (Table 3). It prevented trabecular bone resorption by a decrease in the osteoclast number at the metaphysis of the femur of OVX mice (Fig. 3*B–E*). Furthermore, the calcium concentration in the femur of mice fed the diet containing hesperidin was significantly higher than in OVX mice fed the control diet, and restored to a level similar to that in the sham-operated mice (Table 4). This indicates that hesperidin not only inhibits bone resorption, but also increases the mineral concentrations in the femur of OVX mice.

We assumed that the protective effect of α-glucosylhesperidin on bone resorption in OVX mice would be slightly higher than that of native hesperidin but in general, the HesA and HesB groups did not differ (Fig. 3*B-3E*). Because the solubility of α-glucosylhesperidin is 10,000 times higher than that of hesperidin, we assume that the bioavailability of the transglycosylated compound is higher than that of the corresponding aglycone. In fact, the serum concentration of hesperetin, the aglycone of hesperidin, in the mice fed the diet containing α-glucosylhesperidin tended to be higher than that in the mice fed native hesperidin. These results suggest that enzymatic transglycosylation of the flavonoids with α-glucosidase can increase the efficacy of the compounds by improving their water solubility.

The molecular mechanism of the inhibitory effects of hesperidin on bone resorption is not clear. Among the naturally occurring flavonoids, hesperidin has been pharmacologically evaluated as a potential anti-cancer and anti-inflammatory agent because of its antioxidant activity (11,36). Because the inhibition of osteoclastic superoxide availability reduces bone resorption (37–39), it is possible that hesperidin inhibits bone resorption by its antioxidant activity. However, the antioxidant capacity of both hesperidin and hesperetin is not as high as that of quercetin, myricetin or genistein (40). Another possibility is that hesperidin acts on bone cells through estrogen receptors (ER). ER have been found in osteoblasts and bone marrow stromal cells (41,42). The binding affinity of some flavonoids for ERβ is high, although there are no data concerning the binding affinity of hesperetin for ER. Kuiper et al. (43) reported that the binding affinity of naringenin (flavanone) for ERβ was 0.11, whereas those of genistein (isoflavone) and kaemferol (flavonol) were 87 and 3, respectively, when the binding affinity for E_2 was arbitrarily set at 100. The affinity of hesperetin may be low because hesperetin belongs to the same group (flavanone) as naringenin. Concerning the estrogenic activity of flavonoids evaluated by growth stimulation of estrogen-dependent human MCF-7 breast cancer cells, Breinholt and Larsen (44) hypothesized that the main feature required to confer estrogenicity was the presence of a single hydroxyl group at the 4′-position of the B-ring of the flavan nucleus. Given that hesperetin has the methoxy group at the 4′-position of the B-ring, it is not likely to show estrogenic potency through ER.

It has also been reported that a high consumption of citrus flavonoids decreases the risk of coronary heart disease, given that hesperidin lowers serum cholesterol and triglycerides in rats (15–17). Bok et al. (15) demonstrated that a mixture of naringin and hesperidin significantly lowered the levels of plasma and hepatic cholesterol and triglycerides as well as the HMG-CoA reductase activity in rats. Recently, attention has centered on the effects of cholesterol-lowering agents, statins, which are inhibitors of hepatic HMG-CoA reductase and stimulate bone formation and suppress bone resorption in animals as well as in patients with hyperlipidemia (18). It was demonstrated that statins stimulate bone formation by the production of bone morphogenic protein (18). Because hes-

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peridin lowered serum and hepatic cholesterol and triglycerides in the present study, we hypothesize that hesperidin acts on bone by the same mechanism as that of statins. Further studies are needed to define hesperidin's mechanism of action on bone.

In conclusion, hesperidin added to the diet not only lowered serum and hepatic cholesterol, but also inhibited bone loss by decreasing the osteoclast number in OVX mice. Examination of the effects of flavonoids on bone metabolism in osteoporotic women is required in future investigations to establish the effects of hesperidin in humans.

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