Biochemical Pharmacology, Vol. 55, pp. 71–76, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(97)00402-4

Inhibitory Effect of Genistein on Bone Resorption in Tissue Culture

Masayoshi Yamaguchi and Ying Hua Gao*

LABORATORY OF ENDOCRINOLOGY AND MOLECULAR METABOLISM, GRADUATE SCHOOL OF NUTRITIONAL SCIENCES, UNIVERSITY OF SHIZUOKA, SHIZUOKA CITY 422, JAPAN

ABSTRACT. The effect of genistein on bone resorption *in vitro* was investigated. Femoral-metaphyseal tissues obtained from elderly female rats were cultured for 48 hr in Dulbecco's modified Eagle's medium (high glucose, 4.5%) supplemented with antibiotics and bovine serum albumin. The experimental cultures contained 10^{-7} to 10^{-3} M genistein. The bone-resorbing factors parathyroid hormone (1-34) (PTH; 10^{-7} M), prostaglandin E_2 (PGE_2; 10^{-5} M), and lipopolysaccharide (10 μg/mL) caused a significant decrease in bone calcium content. The decrease in bone calcium content induced by bone-resorbing factors was inhibited completely by genistein (10^{-7} to 10^{-5} M). In addition, this isoflavonoid (10^{-5} M) completely inhibited the PTH (10^{-7} M)- or PGE_2 (10^{-5} M)-induced increase in medium glucose consumption and lactic acid production by bone tissues. Moreover, genistein (10^{-5} M) blocked both PTH (10^{-7} M)-increased acid phosphatase and -decreased alkaline phosphatase activities of bone tissues. The inhibitory effect of genistein (10^{-5} M) on PTH (10^{-7} M)-stimulated bone resorption was clearly prevented by the presence of 10^{-6} M tamoxifen, an anti-estrogen reagent. Genistein (10^{-5} M) did not further enhance the inhibitory effect of estrogen (10^{-9} M) on PTH-stimulated bone resorption. These findings indicate that genistein has a direct inhibitory effect on bone resorption in tissue culture *in vitro*. BIOCHEM PHARMACOL **55**;1:71–76, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. genistein; parathyroid hormone; estrogen; bone resorption; bone tissue culture

Osteoporosis is widely recognized as a major public health problem. The most dramatic expression of this disease is represented by fractures of the proximal femur for which the number increases as the population ages [1, 2]. Malnutrition or undernutrition is often observed in the elderly, and it appears to be more severe in patients with hip fracture than in the general ageing population [3–5]. Deficiency in both micronutrients and macronutrients appears to be strongly implicated in the pathogenesis and the consequences of hip fracture in the osteoporotic elderly [6]. Nutritional and pharmacological factors are needed to prevent bone loss with increasing age. The chemical compounds that act on bone metabolism as nutrients in food, however, are poorly understood.

Genistein is a natural isoflavonoid phytoestrogen found in *Leguminosae*. This isoflavonoid has been shown to have a strong inhibitory effect on protein tyrosine kinases [7, 8], and it can produce cell cycle arrest and apoptosis in leukemic cells [8, 9]. The biological effect of genistein, however, has not been fully clarified. More recently, it has been shown that genistein may have an influence on bone metabolism [10, 11]. Blair *et al.* [10] have reported that genistein is not effective on avian osteoclastic activity and on reduction of bone loss in ovariectomized rats. Dietary soybean protein has been shown to prevent bone loss in ovariectomized rats [11]. Moreover, it has been found that genistein has an anabolic effect on bone formation and mineralization in the tissue culture system [12]. Whether genistein has a direct effect on bone resorption, however, has not been clarified thus far. Therefore, the present study was undertaken to determine the effect of genistein on bone-resorbing factor-induced bone resorption *in vitro*. It was found that genistein can directly inhibit bone resorption in femoral-metaphyseal tissues obtained from elderly rats *in vitro*.

MATERIALS AND METHODS

Chemicals

Dulbecco's modified Eagle's medium and penicillin-streptomycin solution (5000 units/mL penicillin; 5000 μg/mL streptomycin) were obtained from Gibco Laboratories (Grand Island, NY, U.S.A.). Bovine serum albumin (fraction V), tamoxifen, 17β-estradiol (estrogen), okadaic acid, genistein, and PGE_2† were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Other chemicals were of reagent grade and were obtained from Wako Pure Chemical Industries (Osaka, Japan). Synthetic human PTH was

* Corresponding author: Dr. Masayoshi Yamaguchi, Laboratory of Endocrinology and Molecular Metabolism, Graduate School of Nutritional Sciences, University of Shizuoka, 52-1 Yada, Shizuoka City 422, Japan. Tel. (Fax): 81-54-264-5580.

Received 9 April 1997; accepted 24 June 1997.

† *Abbreviations:* PGE_2, prostaglandin E_2; PTH, parathyroid hormone (1-34); and LPS, lipopolysaccharide.

supplied by Asahi Chemical Industries (Shizuoka, Japan). Lipopolysaccharide B (*Escherichia coli*, 026:B6, containing 8.61% lipid A) was purchased from Difco Laboratories (Detroit, MI, U.S.A.). All water used was glass-distilled.

Animals

Elderly female Wistar rats, weighing 300–350 g (50 weeks old), were obtained from Japan SLC (Hamamatsu, Japan). The animals were fed commercial laboratory chow (solid) containing 1.1% calcium, 1.1% phosphorus, and 0.012% zinc, and distilled water. The rats were killed by decapitation (approved by the Animal and Use Committee).

Bone Culture

Femoral-metaphyseal tissues from 50 week-old female rats were removed aseptically. The metaphyseal tissues were cultured in a 35-mm dish in 2.0 mL of medium consisting of Dulbecco's modified Eagle's medium (high glucose; 4.5%) supplemented with 0.25% bovine serum albumin (fraction V) plus antibiotics, with either genistein, bone-resorbing factors, or vehicle (sterile distilled water). Cultures were maintained at 37° in a water-saturated atmosphere containing 5% CO_2 and 95% air for 48 hr. In separate experiments, the respective media contained 17β-estradiol, tamoxifen, or okadaic acid.

Bone Calcium

The bone tissues were dried for 16 hr at 120°, weighed, and then dissolved in nitric acid solution [13]. Calcium was determined by atomic absorption spectrophotometry. The bone calcium content was expressed as milligrams of calcium per gram dry bone.

Bone Alkaline and Acid Phosphatases

Alkaline and acid phosphatase activities in the bone tissues were determined by the method of Walter and Schutt [14]. The bone tissues were immersed in 3.0 mL of ice-cold 6.5 mM sodium barbital buffer (pH 7.4), cut into small pieces, homogenized in a Potter–Elvehjem homogenizer with a Teflon pestle, and disrupted for 60 sec with an ultrasonic device. The supernatant fraction, centrifuged at 600 × *g* for 5 min, was used for measurement of the enzyme activity. The efficiency of the enzyme extraction was greater than 90%, and the enzyme analysis was reproducible. The enzyme assay was carried out under optimal conditions in medium (2.05 mL) containing 97.5 mM diethanolamine (pH 9.8), 1.21 mM *p*-nitrophenylphosphate, and the supernatant solution of bone homogenate (5–10 μg/mL of protein). Enzyme reaction was stopped by the addition of ice-cold 0.05 N NaOH (10 mL). Enzyme activities were expressed as nanomoles of *p*-nitrophenol liberated per minute per milligram of protein. Protein was determined by the method of Lowry *et al.* [15].

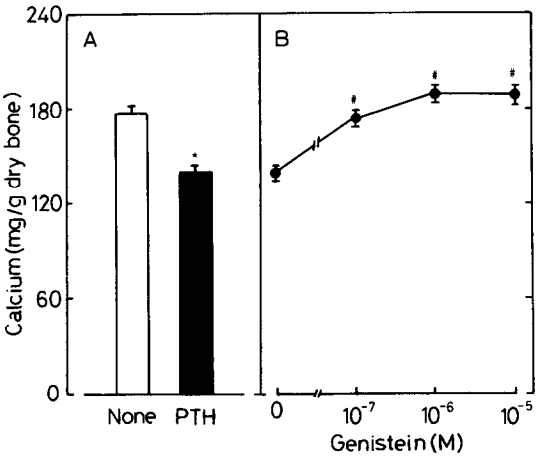

FIG. 1. Effect of genistein on PTH-decreased bone calcium content in femoral-metaphyseal tissues obtained from elderly rats *in vitro*. Bone tissues were cultured for 48 hr in a medium containing either vehicle or PTH (10^{-7} M) in the absence (A) or presence (B) of genistein (10^{-7} to 10^{-5} M). Each value is the mean ± SEM of five bone tissues from separate rats. Key: (*) $P < 0.01$, compared with the control (none) value; and (#) $P < 0.01$, compared with the value of PTH alone.

Medium Glucose and Lactic Acid

The concentration of glucose in the medium cultured with bone for up to 48 hr was determined by the colorimetric method using *o*-toluidine [16]. The dry weight of the bone tissue was measured after extraction with 5.0% trichloroacetic acid, acetone, and ether. The medium glucose consumed by bone culture in 48 hr was expressed as milligrams of glucose per gram of dry bone tissue. Likewise, the medium lactic acid was measured by the enzymatic method [17]. Data were expressed as milligrams of lactic acid per gram of dry bone tissue.

Statistical Methods

Data are expressed as means ± SEM. Statistical differences were analyzed using Student's paired *t*-test. *P* values of less than 0.05 were considered to indicate statistically significant differences.

RESULTS

The effect of genistein on the bone-resorbing factor-induced decrease of calcium content in the femoral-metaphyseal tissues obtained from elderly female rats was examined *in vitro*. Bone tissues were cultured for 48 hr in medium containing either vehicle, PTH (10^{-7} M), PGE_2 (10^{-5} M), or LPS (10 μg/mL of medium) in the absence or presence of genistein (10^{-7} to 10^{-5} M). Bone calcium content was decreased significantly by the presence of PTH (Fig. 1A), PGE_2 (Fig. 2A), or LPS (Fig. 3A). These decreases were prevented completely by genistein (10^{-7} to 10^{-5} M), as shown in Figs. 1B, 2B, and 3B, respectively. An appreciable effect of genistein was seen at 10^{-7} M. At 10^{-6}

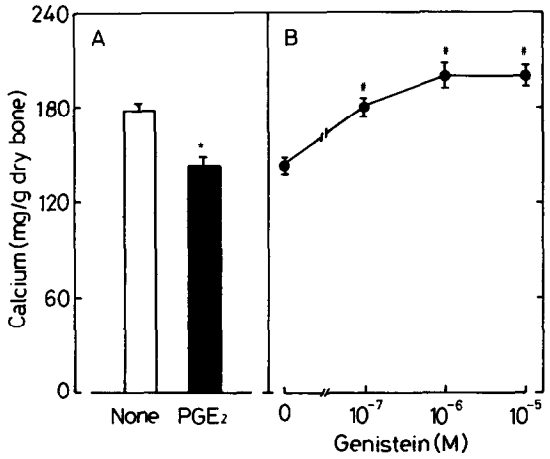

FIG. 2. Effect of genistein on PGE_2-decreased bone calcium content in femoral-metaphyseal tissues obtained from elderly rats *in vitro*. Bone tissues were cultured for 48 hr in a medium containing either vehicle or PGE_2 (10^{-5} M) in the absence (A) or presence (B) of genistein (10^{-7} to 10^{-5} M). Each value is the mean ± SEM of five bone tissues from separate rats. Key: (*) $P < 0.01$, compared with the control (none) value; and (#) $P < 0.01$, compared with the value for PGE_2 alone.

and 10^{-5} M, the genistein effect was saturated. Thus, genistein had an inhibitory effect on bone resorption induced by various bone-resorbing factors. Moreover, genistein (10^{-5} M) alone caused a significant increase of bone calcium content in the absence of bone-resorbing factors (Table 1).

The effect of genistein on acid phosphatase and alkaline phosphatase activities in the femoral-metaphyseal tissues *in vitro* is shown in Fig. 4. Bone tissues were cultured for 48 hr in medium containing either vehicle, PTH (10^{-7} M), or PGE_2 (10^{-5} M) in the absence or presence of genistein

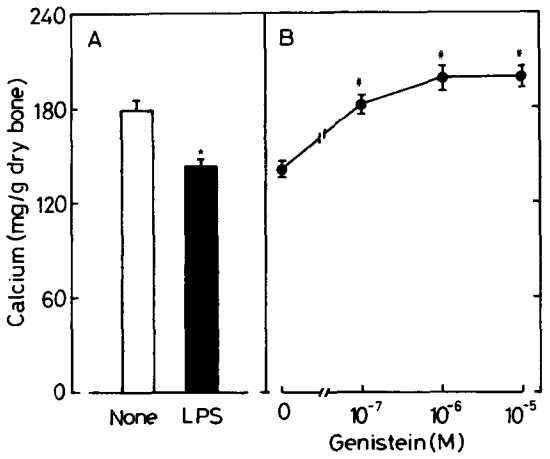

FIG. 3. Effect of genistein on LPS-decreased bone calcium content in femoral-metaphyseal tissues obtained from elderly rats *in vitro*. Bone tissues were cultured for 48 hr in a medium containing either vehicle or LPS (10 μg/mL of medium) in the absence (A) or presence (B) of genistein (10^{-7} to 10^{-5} M). Each value is the mean ± SEM of five bone tissues from separate rats. Key: (*) $P < 0.01$, compared with the control (none) value; and (#) $P < 0.01$, compared with the value of LPS alone.

TABLE 1. Effect of genistein on PTH-induced alterations of bone calcium content and bone glucose consumption in the presence of tamoxifen *in vitro*

Treatment	Calcium (mg/g dry bone)	Glucose consumed (mg/g dry bone)
None		
Control	175.6 ± 2.7	42.1 ± 2.1
PTH (10^{-7} M)	143.8 ± 5.0*	79.8 ± 5.7*
Genistein (10^{-5} M)		
Control	211.0 ± 4.3*	40.3 ± 3.1
PTH (10^{-7} M)	190.3 ± 7.1*	37.3 ± 3.5
Genistein (10^{-5} M) + tamoxifen (10^{-6} M)		
Control	172.9 ± 4.7	36.9 ± 3.2
PTH (10^{-7} M)	151.0 ± 3.5*	67.3 ± 6.3*
Tamoxifen (10^{-6} M)		
Control	177.2 ± 5.8	38.2 ± 3.3
PTH (10^{-7} M)	147.3 ± 6.1*	74.5 ± 4.1*

Femoral-metaphyseal tissues were cultured for 48 hr in a medium containing either vehicle, PTH (10^{-7} M), genistein (10^{-5} M), or PTH plus genistein in the absence or presence of tamoxifen (10^{-6} M). Each value is the mean ± SEM of five bone tissues from separate animals.

* $P < 0.01$, compared with the control (none) value.

(10^{-5} M). The presence of PTH or PGE_2 caused a significant increase in bone acid phosphatase activity and an appreciable decrease in bone alkaline phosphatase activity. These alterations were prevented significantly by genistein. The presence of genistein alone caused a significant elevation of bone alkaline phosphatase activity, although it had no effect on bone acid phosphatase activity.

The effect of PTH or PGE_2 on medium glucose consumption and lactic acid production by bone tissues is shown in Fig. 5. The femoral-metaphyseal tissues were cultured for 48 hr in medium containing either vehicle, PTH (10^{-7} M), or PGE_2 (10^{-5} M) in the absence or presence of genistein (10^{-5} M). Medium glucose consumption by the bone tissues was elevated significantly by the presence of PTH or PGE_2 (Fig. 5A). This elevation was blocked completely by genistein (10^{-5} M). Lactic acid production by the bone tissues also was increased significantly by the presence of PTH (10^{-7} M) or PGE_2 (10^{-5} M) (Fig. 5B), and this increase, too, was prevented completely by genistein (10^{-5} M).

The effect of estrogen (17β-estradiol) on calcium content and glucose consumption in the femoral-metaphyseal tissues *in vitro* is shown in Fig. 6. The bone tissues were cultured for 48 hr in medium containing either vehicle or PTH (10^{-7} M) in the absence or presence of estrogen (10^{-9} M). PTH-decreased bone calcium content was clearly prevented by estrogen (Fig. 6A). In addition, PTH-increased bone glucose consumption was blocked completely by estrogen (Fig. 6B). These preventive effects by estrogen were not further enhanced by genistein. Estrogen alone caused a significant increase of bone calcium content

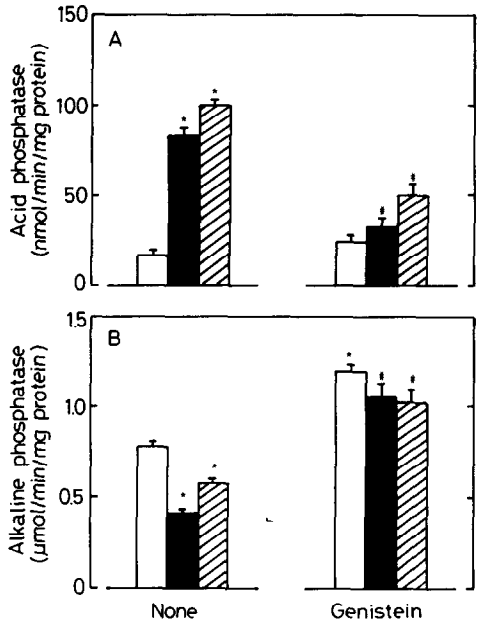

FIG. 4. Effect of genistein on PTH- and PGE_2-altered acid (A) and alkaline (B) phosphatase activities in femoral-metaphyseal tissues obtained from elderly rats *in vitro*. Bone tissues were cultured for 48 hr in a medium containing either vehicle, PTH (10^{-7} M), or PGE_2 (10^{-5} M) in the absence or presence of genistein (10^{-5} M). Each value is the mean ± SEM of five bone tissues obtained from separate rats. Key: (*) $P < 0.01$, compared with the control (none) value; and (#) $P < 0.01$, compared with the value of PTH or PGE_2 alone. Columns: (□) control, (■) PTH, and (▨) PGE_2.

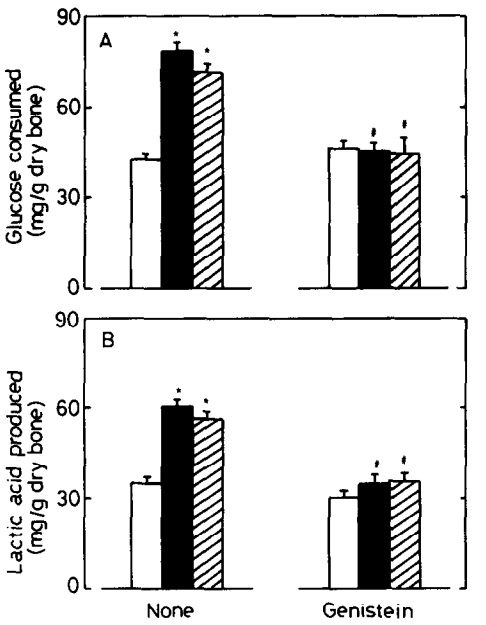

FIG. 5. Effect of genistein on PTH- and PGE_2-stimulated glucose consumption (A) and lactic acid production (B) by femoral-metaphyseal tissues obtained from elderly rats *in vitro*. Bone tissues were cultured for 48 hr in a medium containing either vehicle, PTH (10^{-7} M), or PGE_2 (10^{-5} M) in the absence or presence of genistein (10^{-5} M). Each value is the mean ± SEM of five bone tissues obtained from separate rats. Key: (*) $P < 0.01$, compared with the control (none) value; and (#) $P < 0.01$, compared with the value of PTH or PGE_2 alone. Columns: (□) control, (■) PTH, and (▨) PGE_2.

in the absence of PTH, although the steroid did not have an appreciable effect on bone glucose consumption *in vitro*.

The effect of tamoxifen on the inhibitory action of genistein on bone resorption *in vitro* is shown in Table 1. The femoral-metaphyseal tissues were cultured for 48 hr in medium containing either vehicle, PTH (10^{-7} M), genistein (10^{-5} M), or PTH (10^{-7} M) plus genistein (10^{-5} M) in the absence or presence of tamoxifen (10^{-6} M). The inhibitory effect of genistein on the PTH-induced decrease in bone calcium content was clearly abolished by the presence of tamoxifen. Also, genistein did not have an appreciable effect on the PTH-increased bone glucose consumption in the presence of tamoxifen. Tamoxifen alone had no effect on bone calcium and bone glucose consumption in the absence or presence of PTH.

The effect of okadaic acid on the inhibitory action of genistein on bone resorption *in vitro* is shown in Table 2. The femoral-metaphyseal tissues were cultured for 48 hr in medium containing either vehicle, PTH (10^{-7} M), genistein (10^{-5} M), or PTH (10^{-7} M) plus genistein (10^{-5} M) in the absence or presence of okadaic acid (10^{-5} M). The effect of genistein to inhibit both PTH-decreased bone calcium and -increased bone glucose consumption was not altered appreciably by okadaic acid. Okadaic acid alone did not have a significant effect on bone calcium content and bone glucose consumption in the absence of PTH. However, the PTH-induced alterations of bone calcium content and bone glucose consumption was blocked by the presence of okadaic acid.

DISCUSSION

It has been reported that PTH, PGE_2, and LPS have a stimulatory effect on bone resorption in a culture system *in vitro* [18–20]. The presence of PTH (10^{-7} M), PGE_2 (10^{-5} M), and LPS (10 μg/mL) clearly stimulated bone resorption in femoral-metaphyseal tissues cultured for 48 hr, when bone resorption was estimated by a decrease in bone calcium content. It has been shown that the concentration of bone-resorbing factors used can reveal a maximum effect on bone resorption in tissue culture *in vitro* [21]. The present data coincided with studies reported previously [21]. The effect of bone-resorbing factors (PTH, PGE_2, and LPS) to stimulate bone resorption was inhibited completely by the presence of genistein (10^{-7} to 10^{-5} M). Thus, genistein may have an inhibitory effect on bone resorption in the tissue culture system *in vitro*.

The presence of PTH or PGE_2 caused a significant decrease in bone alkaline phosphatase activity and a corresponding increase in bone acid phosphatase activity. This result is in agreement with other reports [22, 23] in which PTH was found to cause alterations in alkaline and acid phosphatase activities in bone tissues. The PTH- or PGE_2-altered alkaline and acid phosphatase activities were

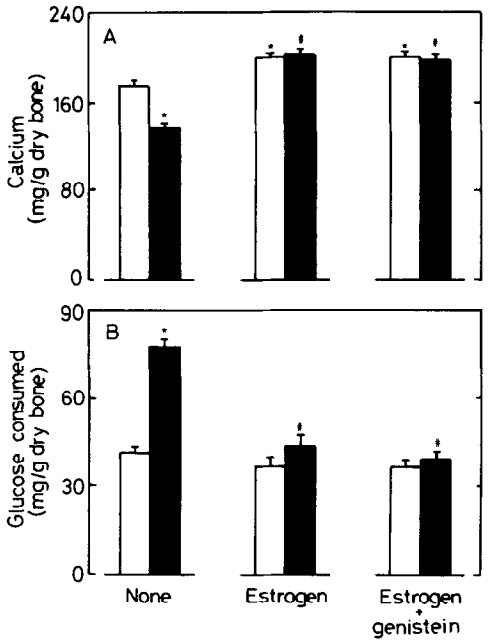

FIG. 6. Effect of genistein and estrogen on the PTH-altered bone calcium content (A) and bone glucose consumption (B) in femoral-metaphyseal tissues obtained from elderly rats *in vitro*. Bone tissues were cultured for 48 hr in a medium containing either vehicle or PTH (10^{-7} M) in the absence or presence of genistein (10^{-5} M) with estrogen (10^{-9} M). Each value is the mean ± SEM of five bone tissues from separate rats. Key: (*) $P < 0.01$, compared with the control (none) value; and (#) $P < 0.01$, compared with the value of PTH alone. Columns: (□) control, and (■) PTH.

TABLE 2. Effect of genistein on PTH-induced alterations of bone calcium content and bone glucose consumption in the presence of okadaic acid *in vitro*

Treatment	Calcium (mg/g dry bone)	Glucose consumed (mg/g dry bone)
None		
Control	178.1 ± 3.3	43.4 ± 3.5
PTH (10^{-7} M)	140.9 ± 4.6*	73.9 ± 4.3*
Genistein (10^{-5} M)		
Control	209.3 ± 5.9*	39.2 ± 3.0
PTH (10^{-7} M)	196.2 ± 8.3*	37.8 ± 4.3
Genistein (10^{-5} M) + okadaic acid (10^{-5} M)		
Control	221.5 ± 6.3	38.1 ± 3.7
PTH (10^{-7} M)	217.2 ± 4.4*	31.6 ± 4.9
Okadaic acid (10^{-5} M)		
Control	175.3 ± 4.5	40.2 ± 4.0
PTH (10^{-7} M)	217.6 ± 8.7*	36.7 ± 2.4*

Femoral-metaphyseal tissues were cultured for 48 hr in a medium containing either vehicle, PTH (10^{-7} M), genistein (10^{-5} M), or PTH plus genistein in the absence or presence of okadaic acid (10^{-5} M). Each value is the mean ± SEM of five bone tissues from separate animals.

* $P < 0.01$, compared with the control (none) value.

blocked completely by the presence of genistein. Moreover, genistein produced a significant elevation of alkaline phosphatase activity in bone tissues cultured in the presence of PTH. Such an effect was also seen in the presence of PGE_2. From these results, it is assumed that the inhibitory effect of genistein on bone resorption is partly involved in the restoration of bone phosphatase activity by PTH or PGE_2.

PTH and PGE_2 caused a remarkable increase in glucose consumption and lactic production by bone tissues. The production of lactic acid from bone tissues may be related to the augmentation of glucose consumption. Presumably, PTH- and PGE_2-stimulated lactic acid production by bone tissues can induce a decrease in bone calcium content, since the stimulatory mechanism of PTH on bone resorption is related to the extracellular release of acid by bone cells (osteoclasts) [24]. Genistein completely blocked the PTH- or PGE_2-induced increase in both glucose consumption and lactic acid production by bone tissues. These findings suggest that the inhibitory effect of genistein on bone resorption is partly related to the prevention of lactic acid production by bone tissues.

The presence of estrogen (17β-estradiol) caused a complete inhibition of the PTH-decreased bone calcium content and PTH-increased bone glucose consumption. The effect of estrogen was not further enhanced by genistein. Meanwhile, the inhibitory effect of genistein on the PTH-stimulated bone resorption was clearly blocked by the presence of tamoxifen, an anti-estrogen [25, 26]. These results suggest that the effect of genistein to inhibit bone resorption is mediated through an estrogen-like action. At present, it is unknown whether genistein can bind to the estrogen receptors in bone cells (osteoblasts). This remains to be elucidated.

Okadaic acid is an inhibitor of protein phosphatase [27]. The presence of okadaic acid significantly inhibited the PTH-stimulated bone resorption, although this chemical alone had no effect on bone calcium content and bone glucose consumption in the absence of PTH. This finding suggests that the stimulatory effect of PTH on bone resorption is partly involved in protein phosphatase. Meanwhile, the inhibitory effect of genistein on PTH-stimulated bone resorption was not further enhanced by okadaic acid. Genistein has been shown to have a strong inhibitory effect on protein tyrosine kinase [7, 8]. There may be a possibility that the inhibitory effect of genistein on bone resorption is mediated in part through the inhibition of protein tyrosine kinase.

Osteoclasts, bone-resorbing cells, are formed from bone marrow cells [28]. It is unknown whether genistein inhibits osteoclastic cell formation or whether the isoflavonoid has a direct inhibitory effect on mature osteoclasts. The cellular mechanisms by which genistein inhibits bone resorption remain to be elucidated.

Genistein had a stimulatory effect on bone formation and mineralization in tissue culture *in vitro* [10]. Also, the isoflavonoid could inhibit bone resorption in tissue culture. These effects were seen in the femoral-metaphyseal tissues obtained from elderly female rats *in vitro*. Presumably,

genistein is a useful tool in the prevention of and therapy for osteoporosis.

References

1. Cooper C and Melton J III, Epidemiology of osteoporosis. *Trends Endocrinol Metab* **3:** 224–229, 1992.
2. Cummings SR, Kelsey JL, Nevitt MC and O'Dowd KJ, Epidemiology of osteoporosis and osteoporotic fracture. *Epidemiol Rev* **7:** 178–199, 1985.
3. Young GA, Chem C and Hill GL, Assessment of protein-caloric malnutrition in surgical patients from plasma proteins and anthropometric measurements. *Am J Clin Nutr* **31:** 429–435, 1978.
4. Older MWJ, Delyth E and Dickerson JWT, A nutrient survey in elderly women with femoral neck fracture. *Br J Surg* **67:** 884–886, 1980.
5. Jensen JE, Jensen TG, Smith TK, Johnston DA and Dudrick SJ, Nutrition in orthopedic surgery. *J Bone Joint Surg Am* **64:** 1263–1272, 1982.
6. Bonjour J-P, Schurch M-A and Rizzoli R, Nutritional aspects of hip fractures. *Bone* **18:** 139S–144S, 1996.
7. Liu Y, Bhalla K, Hill C and Priest DG, Evidence for involvement of tyrosine phosphorylation in taxol-induced apoptosis in a human ovarian tumor cell line. *Biochem Pharmacol* **48:** 1265–1272, 1994.
8. Spinozzi F, Pagliacci MC, Migliorati G, Moraca R, Grignami F, Riccardi C and Nicoletti I, The natural tyrosine kinase inhibitor genistein produces cell cycle arrest and apoptosis in Jurkat T-leukemia cells. *Leuk Res* **18:** 431–439, 1994.
9. Bergamaschi G, Rosti V, Danova M, Ponchio L, Lucotti C and Cazzola M, Inhibitors of tyrosine phosphorylation induce apoptosis in human leukemic cell lines. *Leukemia* **7:** 2012–2018, 1993.
10. Blair HC, Jordan SE, Peterson TG and Barnes S, Variable effects of tyrosine kinase inhibitors on avian osteoclastic activity and reduction of bone loss in ovariectomized rats. *J Cell Biochem* **61:** 629–637, 1996.
11. Arjmandi BH, Alekei L, Hollis B, Amin D, Stacewicz-Sapuntzakis M, Guo P and Kukreja SC, Dietary soybean protein prevents bone loss in an ovariectomized rat model of osteoporosis. *J Nutr* **126:** 161–167, 1996.
12. Yamaguchi M and Gao YH, Anabolic effect of genistein on bone metabolism in the femoral-metaphyseal tissues of elderly rats is inhibited by anti-estrogen tamoxifen. *Res Exp Med*, in press.
13. Yamaguchi M, Oishi H and Suketa Y, Stimulatory effect of zinc on bone formation in tissue culture. *Biochem Pharmacol* **36:** 4007–4012, 1987.
14. Walter K and Schutt C, Acid and alkaline phosphatase in serum. In: *Methods of Enzymatic Analysis* (Ed. Bergmeyer HU), Vol. 1–2, pp. 856–860. Academic Press, New York, 1965.
15. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
16. Hyvarimen A and Nikkila EA, Specific determination of blood glucose with *o*-toludine. *Clin Chim Acta* **7:** 140–143, 1962.
17. Noll F, Determination with LDH, GPT and NAD. In: *Methods of Enzymatic Analysis* (Ed. Bergmeyer HU), Vol. 3, pp. 1475–1479. Academic Press, New York, 1965.
18. Klein-Nulend J, Fall PM and Raisz LG, Comparison of the effects of synthetic human parathyroid hormone (PTH)-(1-34)-related peptide of malignancy and bovine PTH-(1-34) on bone formation and resorption in organ culture. *Endocrinology* **126:** 223–227, 1990.
19. Klein DC and Raisz LG, Prostaglandins: Stimulation of bone resorption in tissue culture. *Endocrinology* **86:** 1436–1440, 1970.
20. Meryon SD and Perris AD, Lipopolysaccharide-induced bone resorption is mediated by prostaglandins. *Life Sci* **28:** 1061–1065, 1981.
21. Klaushofer K, Hoffmann O, Stewart PJ, Czerwenka E, Koller K, Peterlik M and Stern PH, Cyclosporin A inhibits bone resorption in cultured neonatal calvaria. *J Pharmacol Exp Ther* **243:** 584–590, 1987.
22. Thomas HL and Ramp WK, Increased ATPase and decreased alkaline phosphatase activities by parathyroid hormone in cultured chick embryo tibiae. *Proc Soc Exp Biol Med* **157:** 358–362, 1978.
23. Graves L III and Jilka RL, Comparison of bone and parathyroid hormone as stimulators of osteoclast development and activity in calvarial cell cultures from normal and osteopetrotic (mi/mi) mice. *J Cell Physiol* **145:** 102–109, 1990.
24. Vaes G, On the mechanisms of bone resorption. The action of parathyroid hormone on the excretion and synthesis of lysosomal enzymes and on the extracellular release of acid by bone cells. *J Cell Biol* **39:** 676–697, 1968.
25. Reddel RR, Murphy LC, Hall RE and Sutherland RL, Differential sensitivity of human breast cancer cell lines to the growth-inhibitory effects of tamoxifen. *Cancer Res* **45:** 1525–1531, 1985.
26. Yamaguchi M and Kitajima T, Effect of estrogen on bone metabolism in tissue culture: Enhancement of the steroid effect by zinc. *Res Exp Med* **191:** 145–154, 1991.
27. Cohen P, Klumpp S and Schelling DL, An improved procedure for identifying and quantitating protein phosphatases in mammalian tissues. *FEBS Lett* **250:** 596–600, 1989.
28. Takahashi N, Yamana H, Yoshiki S, Roodman GD, Mundy GR, Jones SJ, Boyde A and Suda T, Osteoclast-like cell formation and its regulation by osteotropic hormones in mouse bone marrow cultures. *Endocrinology* **122:** 1373–1382, 1988.