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Bone and Mineral 24 (1994) 43–58

BONE AND MINERAL

Gender dependent effects of testosterone and 17β-estradiol on bone growth and modelling in young mice

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(Received 1 June 1993; accepted 4 October 1993)

Abstract

This study examined the effects of estrogen (17β-estradiol) and testosterone on the growth of long bones in male and female mice, with and without gonadectomy. Weight and nose-to-tail length were determined at 3 weeks of age at time of gonadectomy, 7 days later at the onset of hormone therapy, and throughout the treatment period. Gonadectomized mice exhibited an initial weight gain during the pretreatment period but length was unaffected. Hormone treatment altered weight gain in surgical and intact animals in a gender- and hormone-dependent manner. Estradiol enhanced weight gain in intact mice, but inhibited weight gain in ovariectomized mice. Lower doses of estradiol increased weight gain in orchiectomized mice at early time points. Testosterone increased weight in intact females and males, but not in gonadectomized mice. Estradiol increased nose-to-tail length in intact females at early time points, but inhibited length in ovariectomized females at later times, and it decreased length in intact males. Testosterone increased length in normal females and normal males. Serum Ca was unaffected by ovariectomy, but orchiectomy resulted in decreased levels. Estradiol reduced serum Ca in gonadectomized animals; serum Ca was increased by estradiol treatment in intact females. Changes in tibial bone weight, ash weight and mineral composition, and relative sizes of epiphyseal and metaphyseal bone were gender-, gonadectomy- and hormone-specific. Bone weight was greater in ovariectomized mice. Ash weight per bone was com-

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0169-6009/94/$07.00
SSDI 0169-6009(93)00644-I

parable, but there was an increase in Ca and P content with ovariectomy. Estradiol increased bone weight, ash content, and bone Ca and P in ovariectomized and intact females. Orchiectomy alone did not alter bone weight, ash content, or Ca and P, but orchiectomized mice were sensitive to estradiol; all parameters were increased in the orchiectomized animals treated with estradiol. Analysis of the ash content and Ca and P per mg bone, rather than per bone, demonstrated estradiol and testosterone alter net bone formation, but not the amount of mineral per unit bone. Ovariectomy increased hypertrophic cartilage. While estradiol did not alter tibial area in ovariectomized mice, it caused an increase in intact females. The total amount of growth plate cartilage in ovariectomized animals was decreased by estradiol to levels typical of intact animals due to a greater decrease in the hypertrophic cartilage in the ovariectomized mice, as well as a greater increase in metaphyseal bone area. Testosterone had no effect on these parameters in the females. Orchiectomy decreased the amount of growth plate cartilage, but increased the hypertrophic zone. Estradiol increased growth plate cartilage in intact male mice, but decreased it in orchiectomized mice. This difference was also seen in the hypertrophic zone. Total growth plate cartilage and hypertrophic cartilage were increased by testosterone in intact males, whereas metaphyseal and epiphyseal bone area were decreased. The results show for the first time that there is a gender-specific response in both male and female mice to both estradiol and testosterone, whether or not the animals have been gonadectomized. For many parameters, orchiectomized mice behave like females in response to both sex steroids, indicating that the male gonad is needed for mouse bone to exhibit the male phenotypic response to estradiol and testosterone.

Key words: Testosterone; Estradiol; Bone growth; Bone modelling; Mice

1. Introduction

It has been known for many years that treatment of growing animals with sex hormones induces specific skeletal changes. Mice treated with hydroxyestrin benzoate had more trabecular bone, and the growth plates decreased in height or disappeared [1,2]. Estradiol valerianate administered to pregnant rats during the third week of pregnancy resulted in the persistence of endochondral bone trabeculae and calcified cartilage in the medullary cavities of fetal long bones [3]. In young rats, long bone growth was inhibited [4].

Testosterone has been shown to enhance endochondral bone formation, including proliferation of chondrocytes in the growth plate cartilage [5] and the incorporation of $^{35}SO_4$ in cartilage of growing rabbits at puberty [6,7]. Direct intraepiphyseal injections of testosterone in rats increased the height of growth plate cartilage by 15% [7,8]. Testosterone was also found to enhance the proliferation of mouse-derived osteoblasts in vitro in a dose-dependent fashion [9].

Several studies point to the possibility that, in animals and perhaps in man, there is a selective, sex-dependent response to estrogens, as well as to androgens. Moritake et al. [10] have found that treatment with estrogens and testosterone in gonadectomized Ishibashi rats with congenital kyphosis is sex-dependent. Somjen et al. [11] recently found an elevation of creatine kinase and DNA synthesis in cartilage and bone of 20-day-old female rats treated with 17β-estradiol. However, estradiol had no effect on creatine kinase activity and DNA synthesis in the diaphyseal bone of

male rats and only slightly raised creatine kinase activity levels in the epiphyseal cartilage of males. This sex-dependent response disappeared after gonadectomy [12].

We have recently shown a sex-related effect of estradiol and testosterone on fetal mouse radii and ulnae in culture [13]. Estradiol enhanced bone growth, mineralization and collagen synthesis in bones from female mouse fetuses, but not from males. In contrast, testosterone enhanced bone formation in male fetal bones and not in females, suggesting that there may be a specific sex-related effect of these hormones in the mouse in vivo.

The purpose of the present study was, therefore, to examine the effects of estrogen and testosterone on the growth of long bones in male and female mice, with and without gonadectomy, in order to see whether there is a sex-related effect in mice in vivo and whether it changes after gonadectomy. The results show for the first time that there is a gender-specific response of both male and female mice to both estradiol and testosterone, whether or not the animals have been gonadectomized. Moreoever, orchiectomized mice behave like females in response to both sex steroids, indicating that the male gonad is needed for mouse bone to exhibit the masculine phenotypic response to estradiol and testosterone.

2. Materials and methods

Male and female albino mice from the Hebrew University Sabra strain were used for the study. When they were 3 weeks old, male and female mice were weighed (mean ± S.E.M., 11.04 ± 0.50 g for males; 12.27 ± 0.82 g for females), and their nose-to-tail lengths were determined (7.25 ± 0.25 cm for males and 6.89 ± 0.31 cm for females). Animals whose weight differed from the mean by two or more standard deviations were deleted from the experiment. We chose 128 males and 128 females for the study; gonads were removed from 64 males and from 64 females. Females had ovariectomies using a posterior abdominal approach, and orchiectomies were performed in the males without damaging the epididymis or the vas deferens. The animals were anesthetized with 10% chloralhydrate (0.4 ml/100 g body wt.). Following surgery, the weaned gonadectomized and intact animals were divided into single-sex groups of eight animals each. Beginning 1 week after surgery, the animals were randomly separated into different treatment groups, and every second day thereafter animals received intramuscular injections of either 17β-estradiol valerianate (0.1, 0.5, or 1.0 μg/g) or testosterone in similar doses. Hormone was dissolved in Arachis Oil prior to injection. 17β-Estradiol, testosterone, and Arachis Oil were purchased from Sigma, St. Louis, MO. Treatment continued for a period of 4 weeks. During treatment, animals were weighed three times per week and their nose-to-tail length measured.

At the end of the experiment, animals were euthanised by ether inhalation. Blood was removed for calcium determination by atomic absorption spectroscopy (Synchron Beckman MG 1000, Technicon). Both tibial bones were removed. The right tibia was X-rayed (Electron Ray, 15 mA, 0.1 s, 60 kV), and the X-ray was analysed by computerized morphometry, measuring length, width, and surface area (Galai Electra Optical Inspection and Diagnosis Laboratories, Migdal Haemeck, Israel, computer program Cue 2). The same tibia was kept for 4 h at 37°C, weighed (i.e.

bone weight), and then ashed at 850°C for 12 h. The ash weight was measured, and the ash was dissolved in 0.1M HCl. After dilution to 10 ml, the bone calcium and phosphate contents were determined. Calcium content was quantitated by atomic absorption spectroscopy and the phosphate content by a colorimetric method [14].

The left tibia was fixed in 4% buffered formalin and partially decalcified in 5% formic acid for 24 h. Mid-longitudinally sectioned half bones were embedded in glycol methacrylate, 3-mm thick longitudinal sections were stained by toluidine blue, and morphometric analysis at the light microscopic level was performed using a 'Galai's' computerized system (Migdal Haemeck, Cue 3 System) to measure the epiphysis, the growth plate, and the metaphysis. In the epiphysis and metaphysis, the trabecular bone volume, bone marrow volume, total bone volume, and the volume of calcified cartilage were measured. In the growth plate, the area occupied by each one of the zones of the plate (i.e. reserve zone, proliferating and hypertrophic zones, as well as the total growth plate volume) was also analysed.

2.1. Statistical analysis

Differences among groups were analysed using analysis of variance (ANOVA), and, for comparison of the differences between treated and control animals, Bonferroni's *t*-test was performed. Significance of the results was determined by using 95% confidence limits ($P < 0.05$).

3. Results

3.1. Animal weight

At 3 weeks, all animals weighed approximately the same and exhibited comparable nose-to-tail lengths. However, by the fourth week (1 week following gonadectomy), there were distinct differences in weight between intact females and ovariectomized females or between intact males and orchiectomized males (Table 1),

Table 1
Weight and nose-to-tail length of male and female mice, 7 days post gonadectomy

Gender	Gonadectomy	Experiment	Weight (g)	Length (cm)
Female	–	1	16.04 ± 0.50	8.75 ± 0.15
		2	16.09 ± 1.02	8.56 ± 0.13
Female	+	1	21.40 ± 0.70[a]	8.90 ± 0.12
		2	22.40 ± 1.10[a]	9.00 ± 0.10
Male	–	1	13.05 ± 0.92[c]	9.00 ± 0.27
		2	13.89 ± 0.53[c]	8.84 ± 0.32
Male	+	1	28.26 ± 0.92[a,b]	9.88 ± 0.23
		2	28.76 ± 0.92[a,b]	9.88 ± 0.23

[a] $P < 0.05$, gonadectomized vs. non-gonadectomized.
[b] $P < 0.05$, orchiectomized male vs. normal and ovariectomized females.
[c] $P < 0.05$, ovariectomized females vs. normal males.

with the gonadectomized mice showing enhanced weight gains. In contrast, nose-to-tail length was comparable for the intact and gonadectomized animals.

Administration of estradiol for 19–21 days to non-ovariectomized female mice enhanced weight gain in a dose-dependent manner (Table 2); peak weight gain occurred from day 10 to day 17 (data not shown). However, by 28 days of estradiol administration, no significant effects on weight gain were seen. In the ovariectomized females, the addition of estradiol for 19–21 days significantly inhibited weight gain, and this was true at 28 days as well. In contrast, in the intact males, estradiol reduced weight gain. This effect was even more pronounced at 28 days. In gonadectomized males treated with estradiol, weight gain was increased at lower doses; at the highest concentration of estradiol tested, weight at 19–21 days was comparable with that of animals which received vehicle only. No significant effect of estradiol was observed in orchiectomized males at 28 days of estradiol treatment.

Testosterone increased weight gain in non-ovariectomized mice, but had no effect on the ovariectomized animals at 19–21 days and at 28 days (Table 3). In higher doses, testosterone enhanced weight gain in the intact males but had no effect in the orchiectomized animals.

3.2. Animal length

Ovariectomy or orchiectomy alone did not affect length. Estradiol had no effect on the length of the orchiectomized male mice, but at high doses, a decreased length of the intact male animals was found (data not shown). Estradiol caused an increase in nose-to-tail length in intact female mice from days 5–7. However, with ovariectomized females, a slight inhibition of nose-to-tail length was found at later time points (data not shown).

Table 2
Effects of 17β-estradiol on weight gain in female and male mice with and without gonadectomy

Gender	Gonadectomy	μg Estradiol/g weight			
		0	0.1	0.5	1.0
19–21 Days					
F	−Ovariectomy	25.65 ± 0.70	29.75 ± 0.82[a]	30.90 ± 0.61[a]	30.23 ± 0.70[a]
F	+Ovariectomy	31.10 ± 0.80	29.06 ± 0.60	29.80 ± 1.00	28.70 ± 0.40[b]
M	−Orchiectomy	33.31 ± 0.85	33.90 ± 0.32	30.44 ± 0.60[b]	31.53 ± 0.58[b]
M	+Orchiectomy	34.71 ± 1.39	38.45 ± 1.58[a]	38.04 ± 2.02[a]	34.67 ± 1.51
28 Days					
F	−Ovariectomy	29.09 ± 0.90	28.99 ± 0.75	29.35 ± 0.83[a]	28.68 ± 1.07
F	+Ovariectomy	31.70 ± 0.80	30.64 ± 0.65	29.83 ± 1.12	28.81 ± 0.58[b]
M	−Orchiectomy	37.99 ± 0.90	32.11 ± 0.48[a]	30.87 ± 1.20[b]	32.52 ± 0.67[b]
M	+Orchiectomy	36.56 ± 1.04	38.86 ± 1.58	38.46 ± 1.96	34.79 ± 1.68

Data represent the mean ± S.E.M. for eight mice and are from a single experiment. Animals were weighed after 19–21 and 28 days of hormone therapy.
[a] $P < 0.05$, treatment vs. control, where treatment values are greater than control values.
[b] $P < 0.05$, treatment vs. control, where treatment values are less than control values.

Table 3
Effects of testosterone on weight gain in female and male mice with and without gonadectomy

Gender	Gonadectomy	μg Testosterone/g weight			
		0	0.1	0.5	1.0
19–21 Days					
F	–Ovariectomy	25.89 ± 0.77	28.24 ± 0.53[a]	28.84 ± 0.81[a]	29.84 ± 1.03[a]
F	+Ovariectomy	30.60 ± 1.10	31.20 ± 0.90	30.30 ± 1.00	31.50 ± 0.90
M	–Orchiectomy	34.60 ± 1.55	37.61 ± 1.00	38.90 ± 1.91[a]	38.44 ± 0.90[a]
M	+Orchiectomy	34.59 ± 1.48	29.45 ± 1.89	33.17 ± 0.91	35.11 ± 1.07
28 Days					
F	–Ovariectomy	29.31 ± 0.89	33.79 ± 0.39[a]	32.31 ± 0.88[a]	33.97 ± 0.98[a]
F	+Ovariectomy	32.93 ± 1.45	33.83 ± 1.22	32.01 ± 1.01	33.05 ± 0.74
M	–Orchiectomy	36.87 ± 1.59	39.32 ± 0.96	40.59 ± 2.03	48.82 ± 0.92[a]
M	+Orchiectomy	36.56 ± 1.04	39.20 ± 0.94	36.79 ± 1.06	38.13 ± 1.24

Data represent the mean ± S.E.M. for eight mice and are from a single experiment. Animals were weighed after 19–21 days and 28 days of hormone therapy.
[a] $P < 0.05$, Treatment vs. control.

Testosterone increased nose-to-tail length in the normal female mice, but not in the ovariectomized mice. Higher doses of testosterone increased length from 2–10 days in the normal males, but had no effect on length in the orchiectomized animals.

3.3. Serum calcium

Serum Ca was unaffected by ovariectomy, whereas orchiectomy resulted in decreased serum Ca levels. Estradiol treatment reduced serum Ca in gonadectomized male and female mice, with the greatest reduction at 0.5 μg estradiol/g weight. In the ovariectomized females, serum Ca was decreased from 2.2 mM to 2.0 mM; and in the orchiectomized males, serum Ca was reduced from 1.8 mM to levels as low as 1.3 mM (results not shown). In the intact female mice, estradiol treatment increased serum Ca, with a peak effect at 0.5 μg estradiol/g weight (from 2.3 mM to 2.8 mM).

3.4. Bone characteristics

Tibial bone characteristics were also altered. Bone weight in ovariectomized female mice was greater than that observed in the intact animals (Fig. 1A). Estradiol caused a dose-dependent increase in bone weight in both intact and ovariectomized animals. The relative fractional increase was unaffected by ovariectomy.

The ash weight of the tibiae from female mice was comparable in the ovariectomized and intact mice (Fig. 1B). In both groups of females, ash weight increased in response to estradiol treatment. Maximal response was seen in the intact mice at 0.5 μg estradiol/g weight. The ovariectomized mice showed increased tibial ash content at lower estradiol concentrations and exhibited maximal increases at 1.0 μg estradiol/g weight. Ovariectomy did not affect the estradiol-induced fractional increase in ash content.

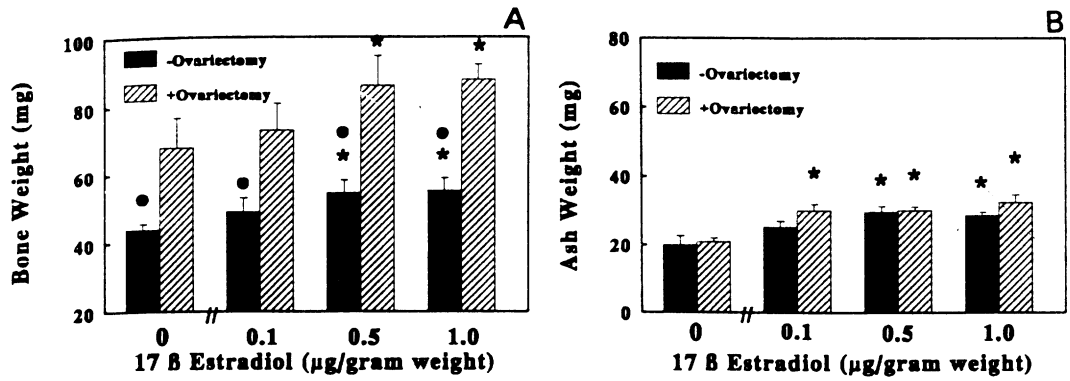

Fig. 1. The effects of 17β-estradiol on (A) total weight and (B) ash weight per bone of tibiae from ovariectomized and intact female mice. Values are the mean ± S.E.M., $n = 8$. ●$P < 0.05$, normal mice vs. ovariectomized mice; *$P < 0.05$, treatment vs. control.

The mineral content of the bones was sensitive to estradiol treatment. Ovariectomy resulted in increased Ca (Fig. 2A) and P (Fig. 2B) content in female mice. Treatment of intact mice with 0.1 µg estradiol/g weight caused an increase in the Ca content of the bone. Higher doses of estradiol resulted in no further increase (Fig. 2A). Phosphorus content was increased in animals treated with 0.5 µg estradiol/g weight, with no further increase at 1.0 µg estradiol/g weight (Fig. 2B). In contrast, ovariectomized mice exhibited a dose-dependent increase in Ca content, which was seen at 0.1 µg estradiol/g weight and was maximal in animals treated with 1.0 µg estradiol/g weight. Similarly, estradiol treatment caused an increase in bone P in the ovariectomized mice; maximal P content was achieved with 0.1 µg estradiol/g weight and remained constant over the doses of estradiol tested (Fig. 2B).

Orchiectomy alone in male mice had little effect on tibial weight (Fig. 3A), ash weight (Fig. 3B), or calcium (Fig. 4A) and phosphorus (Fig. 4B) content. Estradiol treatment increased all parameters in the orchiectomized mice in a dose-dependent

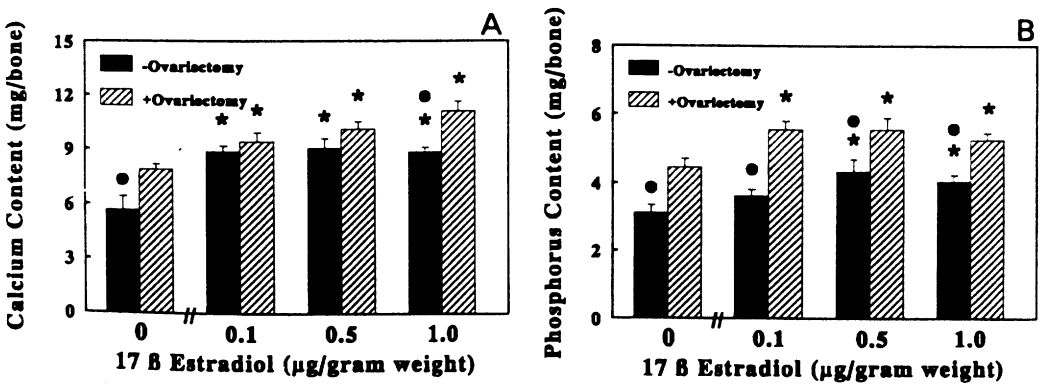

Fig. 2. The effects of 17β-estradiol on tibial (A) calcium and (B) phosphorus content in ovariectomized and intact female mice. Values are the mean ± S.E.M., $n = 8$. ●$P < 0.05$, normal mice vs. ovariectomized mice; *$P < 0.05$, treatment vs. control.

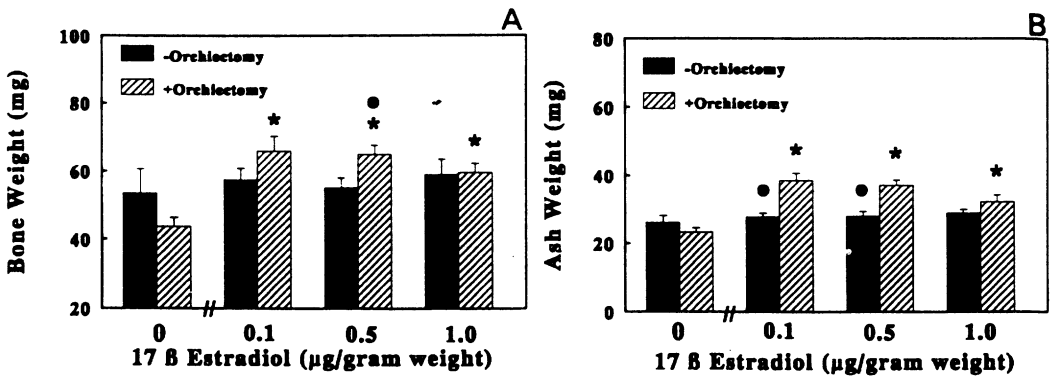

Fig. 3. The effects of 17β-estradiol on (A) total weight and (B) ash weight per bone of tibiae from orchiectomized and intact male mice. Values are the mean ± S.E.M., $n = 8$. $^{\bullet}P < 0.05$, intact vs. orchiectomized mice; $^{*}P < 0.05$, treatment vs. control.

manner. However, estradiol treatment had no effect on the normal male tibiae. Testosterone at doses of 1.0 µg estradiol/g body weight decreased tibial weight (Fig. 5A), ash weight (Fig. 5B), and calcium (Fig. 6A) and phosphate (Fig. 6B) content only in intact males.

When the Ca and P contents of the bone were calculated as a function of bone weight, rather than per bone, no effects of either estradiol or testosterone were observed in intact or gonadectomized mice (data not shown).

3.5. Morphometrics

Ovariectomy resulted in an increase in the amount of growth plate cartilage (Fig. 7A) due to the increase in the hypertrophic zone (Fig. 7B). There was only a slight increase in the size of the tibia, whereas metaphyseal bone area was slightly decreased (Fig. 8A), as was the epiphyseal bone area (Fig. 8B). X-ray morphometry showed that estradiol had no effect on tibial size in the ovariectomized mice, but it caused an increase in the intact animals at 0.5 and 0.1 µg estradiol/g body weight (results

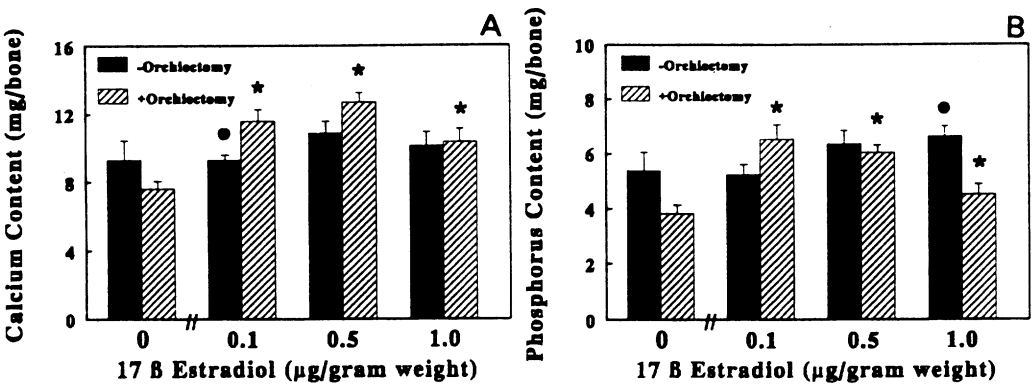

Fig. 4. The effects of 17β-estradiol on tibial (A) calcium and (B) phosphorus content in orchiectomized and intact male mice. Values are the mean ± S.E.M., $n = 8$. $^{\bullet}P < 0.05$, intact vs. orchiectomized mice; $^{*}P < 0.05$, treatment vs. control.

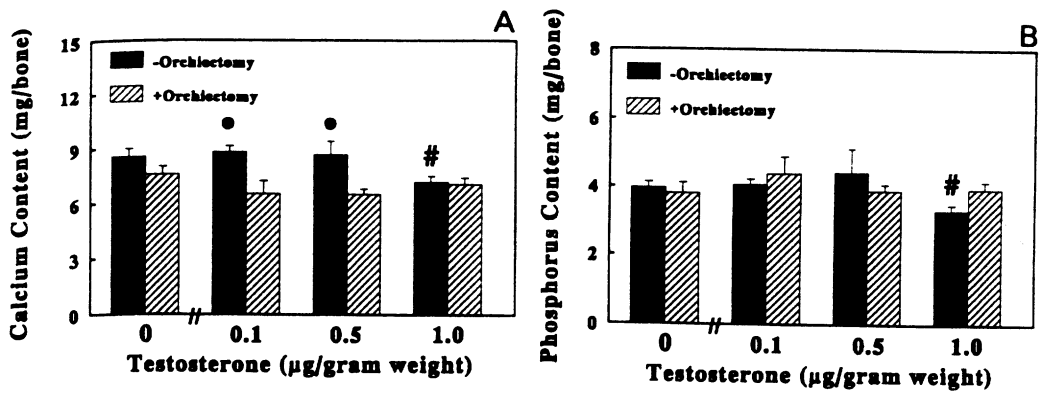

Fig. 5. The effects of testosterone on (A) total weight and (B) ash weight per bone of tibiae from orchiectomized and intact male mice. Values are the mean ± S.E.M., $n = 8$. ● $P < 0.05$, intact vs. orchiectomized mice; * $P < 0.05$, treatment vs. control.

not shown). Estradiol had no effect on the total amount of growth plate cartilage in the intact animals, but at doses of 0.1 and 0.5 μg estradiol/g body weight, it decreased the amount of growth plate cartilage in the ovariectomized females to levels comparable with that in the intact female mice (Fig. 7). The hypertrophic zone was decreased by estradiol in both intact and ovariectomized females. The percent decrease was less in the intact mice, was maximal at 0.1 μg estradiol/g body weight, and remained at decreased levels over the dose range tested (Fig. 7B). In the ovariectomized mice, decreased hypertrophic cartilage was seen at 0.1 and 0.5 μg estradiol/g weight only (Fig. 7B). The metaphyseal bone area was increased in both intact and ovariectomized mice (Fig. 8A). The fractional increase was greatest in the ovariectomized females and was maximal at 0.5 μg estradiol/g body weight. In the intact animals, the increase was the same with all the doses tested (Fig. 8A). Epiphyseal bone area also increased in response to all doses of estradiol. In the intact mice, maximal increases were seen at 1.0 μg estradiol/g body weight, whereas in the ovariectomized animals, maximum increases were detected at 0.1 μg estradiol (Fig. 8B).

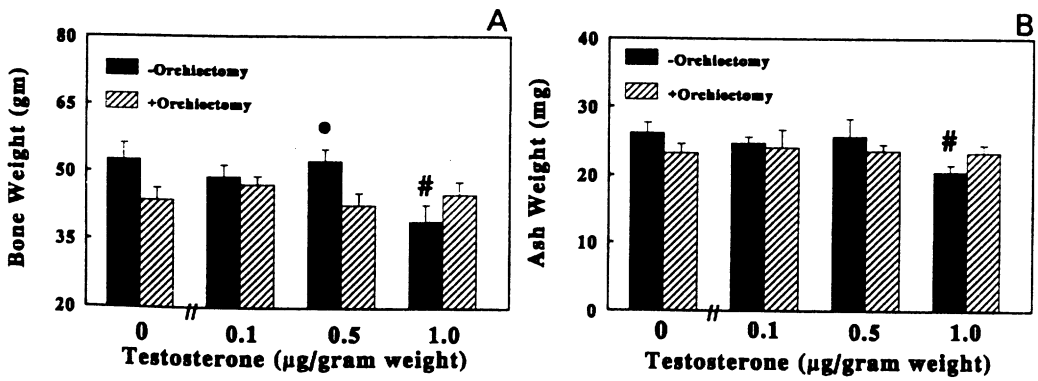

Fig. 6. The effects of testosterone on (A) tibial calcium and (B) phosphorus content in orchiectomized and intact male mice. Values are the mean ± S.E.M., $n = 8$. ● $P < 0.05$, intact vs. orchiectomized mice; * $P < 0.05$, treatment vs. control.

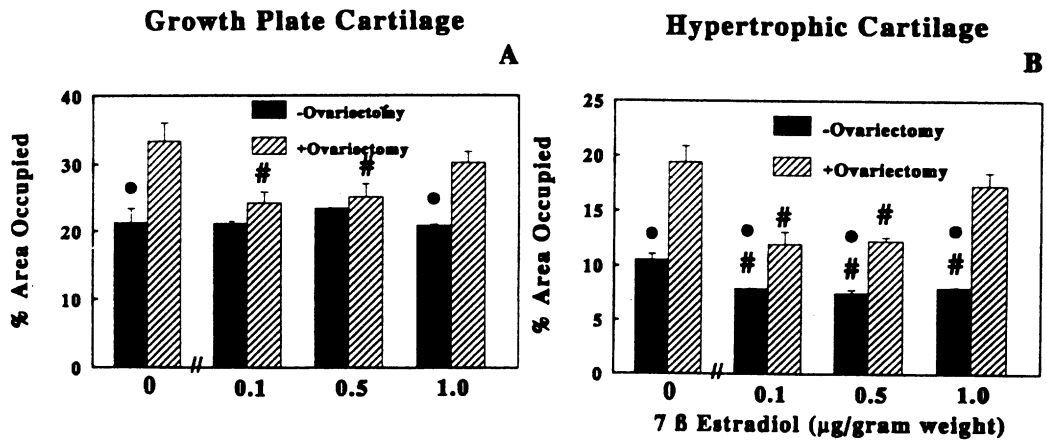

Fig. 7. Computerized histomorphometry (A) of the proximal tibial growth plate and (B) of the hypertrophic zone in ovariectomized and intact female mice after treatment with 17β-estradiol. Values are the mean ± S.E.M., $n = 8$. ● $P < 0.05$, intact (without ovariectomy) vs. ovariectomy; @ $P < 0.05$, treatment vs. control.

Testosterone had no effect on any of the bone parameters in either intact or ovariectomized females (data not shown).

Orchiectomy decreased the amount of growth plate cartilage (Fig. 9A), but the size of the hypertrophic zone was increased (Fig. 9B). Castration did not significantly affect the metaphyseal bone area (Fig. 10A), but epiphyseal bone area was decreased (Fig. 10B). The total tibial area (based on X-rays) was also decreased (data not shown).

When the male mice were treated with estradiol, the amount of growth plate cartilage was differentially altered in the intact and orchiectomized animals (Fig. 9A). There was an increase in the intact mice, significant at 0.5 and 1.0 µg estradiol/g body weight. In the orchiectomized mice, growth plate cartilage was decreased at all doses

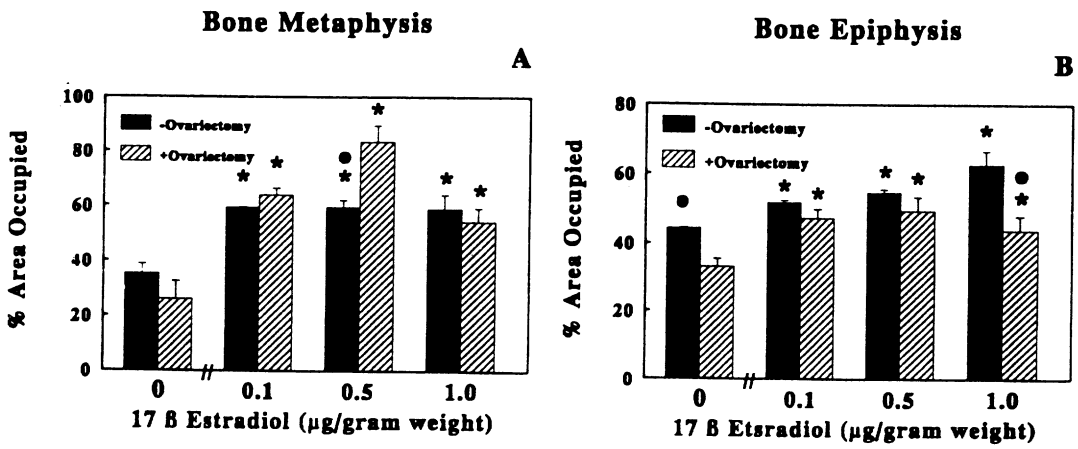

Fig. 8. Computerized histomorphometry of proximal tibial (A) metaphysis and (B) epiphysis in ovariectomized and intact female mice after treatment with 17β-estradiol. Values are the mean ± S.E.M., $n = 8$. ● $P < 0.05$, intact (without ovariectomy) vs. ovariectomized mice; * $P < 0.05$, treatment vs. control.

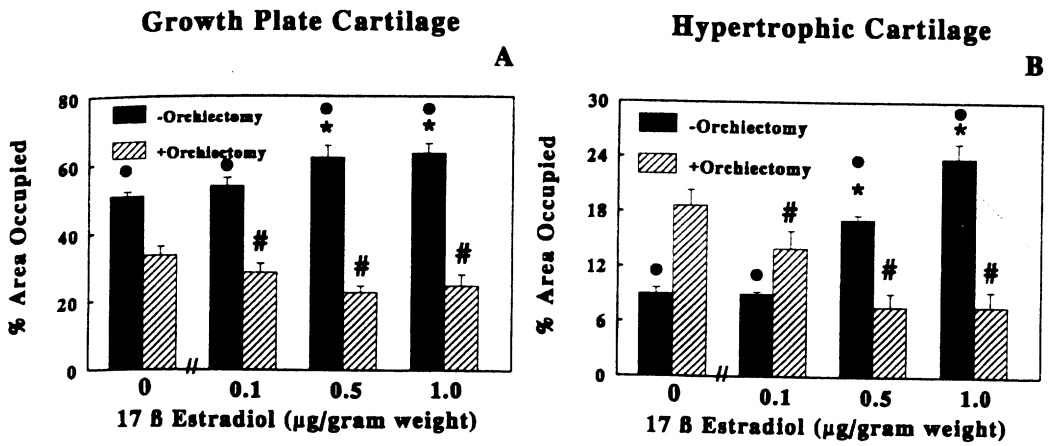

Fig. 9. Computerized histomorphometry of (A) the proximal tibial growth plate and (B) its hypertrophic zone in orchiectomized and intact male mice after treatment with 17β-estradiol. Values are the mean ± S.E.M., $n = 8$. $^{\bullet}P < 0.05$, intact (without orchiectomy) vs. orchiectomized mice; $^{*}P < 0.05$, treatment vs. control, where treatment values are greater than control values; $^{@}P < 0.05$, treatment vs. control, where treatment values are less than control values.

tested, with maximal effects seen at 0.5 µg estradiol. The hypertrophic cartilage was similarly affected (Fig. 9B). Intact mice showed a dose-dependent increase, maximal at 1.0 µg estradiol, whereas orchiectomized mice showed a dose-dependent decrease maximal at 0.5 µg estradiol/g body weight. Total bone size (based on X-rays) was unaffected by estradiol in intact and orchiectomized mice (results not shown). In the intact males, neither metaphyseal bone area nor epiphyseal bone area were affected by estradiol (Fig. 10). However, in the castrated males, estradiol caused a dose-dependent increase in both parameters, significant at all doses tested and maximal at 0.5 µg estradiol/g body weight.

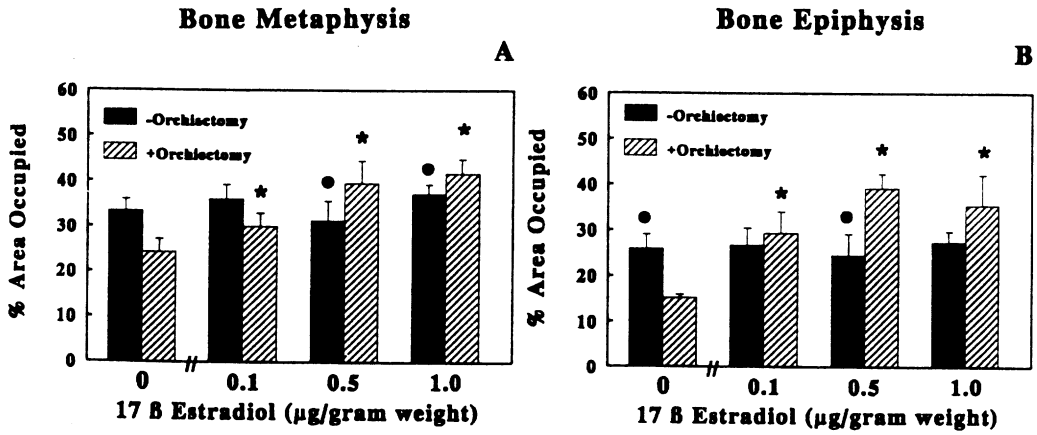

Fig. 10. Computerized histomorphometry of the proximal tibial (A) metaphysis and (B) epiphysis of orchiectomized and intact male mice after treatment with 17β-estradiol. Values are the mean ± S.E.M., $n = 8$. $^{\bullet}P < 0.05$, intact (without orchiectomy) vs. orchiectomized mice; $^{*}P < 0.05$, treatment vs. control.

The size of the tibia (based on X-rays) was slightly decreased by testosterone in intact and orchiectomized mice (data not shown). The amount of growth plate cartilage was affected by testosterone in the intact male mice only. When treated with 0.5 or 1.0 μg testosterone/g body weight, the intact males exhibited an increase in total growth plate cartilage, as well as in the amount of the hypertrophic zone (data not shown). Castration significantly reduced metaphyseal and epiphyseal bone area (Fig. 11). Testosterone had no effect on metaphyseal or epiphyseal bone area in the orchiectomized males, but caused a dose-dependent decrease in both parameters in the intact animals, maximal at 1.0 μg testosterone/g body weight.

Table 4 presents a summary of the effects of estradiol and testosterone on female and male mice.

4. Discussion

Estradiol had a sex-related effect on the skeleton of young growing mice. Whether or not female mice are ovariectomized, it enhanced bone formation with a concomitant reduction in the size of the growth plate. In normal males, however, estradiol had limited effects on the skeleton, whereas for a number of parameters, orchiectomized male mice were affected in a way similar to that seen in female mice. Testosterone mainly affected normal male mice, reducing the amount of metaphyseal and epiphyseal bone, and increasing the size of the growth plate. These effects were lost following orchiectomy. Furthermore, testosterone did not affect the skeleton of either normal or ovariectomized female mice.

These results support our previous observation that there is a sex-related effect of estradiol and testosterone on cartilage and bone. In these earlier studies, estradiol enhanced bone formation in 16-day-old female mouse fetal radii and ulnae in culture, but not in radii and ulnae obtained from male fetuses. Similarly, testosterone enhanced bone formation in male fetal bones, but not in those in female

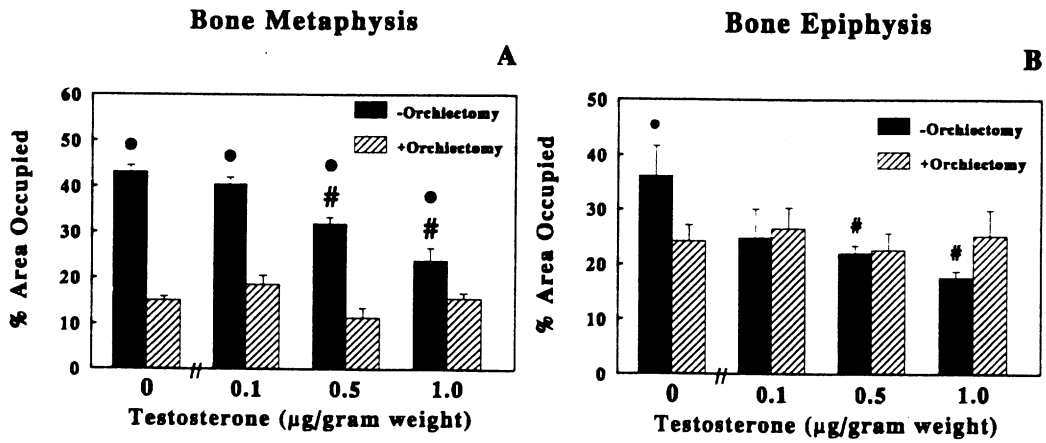

Fig. 11. Computerized histomorphometry of the proximal tibial (A) metaphysis and (B) epiphysis in orchiectomized and intact male mice after treatment with testosterone. Values are means ± S.E.M., $n = 8$. ●$P < 0.05$, intact (without orchiectomy) vs. orchiectomized mice; @$P < 0.05$, treatment vs. control.

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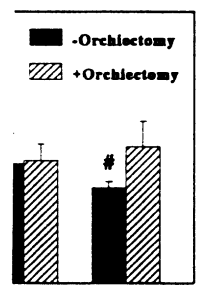

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Table 4
Summary of the effects of 17β-estradiol and testosterone on normal and gonadectomized female and male mice

Measured parameters	Females				Males			
	Normal		Ovariectomy		Normal		Orchiectomy	
	E_2	T	E_2	T	E_2	T	E_2	T
Body weight	↑	↑	↓	NC	↓	↑	NC	NC
Body length (cm)	↑	↑	↓	NC	↓	↑	NC	NC
Tibial weight (mg)	↑	NC	↑	NC	NC	↓	↑	NC
Ash weight (mg)	↑	NC	↑	NC	NC	NC	↑	NC
Calcium content (mg/bone)	↑	NC	↑	NC	NC	↓	↑	NC
Phosphate content (mg/bone)	↑	NC	↑	NC	NC	↓	↑	NC
Serum Ca (mM)	↑	NC	↓	NC	NC	NC	↓	NC
Tibial surface area (X-rays)	↑	NC	NC	NC	NC	↓	NC	NC
Metaphyseal bone area	↑	NC	↑	NC	NC	↓	↑	NC
Epiphyseal bone area	↑	NC	↑	NC	NC	↓	↑	NC
Growth plate area	NC	NC	↓	NC	↑	↑	↓	NC
Hypertrophic cartilage area	↑	NC	↓	NC	↑	↑	↓	NC

E_2, estradiol; T, testosterone; NC, no change.

mouse fetuses [13]. In culture, estradiol was found to enhance the differentiation and maturation of costal cartilage chondrocytes derived from female, but not from male, rats, while testosterone enhanced differentiation of cultured male-derived chondrocytes [15,16]. Others have also shown gender-dependent effects of sex hormones. Somjen et al. [12] have demonstrated a sex-related effect of estradiol on the bone of growing rats, which appeared to be vitamin D-related. The sex-related effect disappeared after gonadectomy or following prenatal or neonatal androgenisation of female rats [17].

The present study shows, for the first time, that orchiectomy of the male mice made their skeletons responsive to estradiol in a way similar to the response of normal or ovariectomized females. A similar finding was observed by us in male rats, where castration made them responsive to estradiol [18]. These results may point to a possible antagonistic effect of testosterone (or other testicular hormones) to the specific actions of estradiol on bone. Estradiol, on the other hand, does not seem to antagonise the effects of testosterone on bone because ovariectomy did not permit any specific effect of testosterone on the skeleton in female mice. The development of the genital organs in male fetuses in most species is dependent on androgen secretion by the fetal gonads [17]. Lack of androgens would result in the development of a female genital tract [19]. It is possible to compare the effects of sex hormones on cartilage and bone in a similar way. The specific effects of estradiol on bone are avoided by testosterone and, therefore, intact males do not respond to estradiol. In contrast, orchiectomy, which reduces androgen production, may be permissive for estradiol to act on growing bones in a way similar to that of female mice.

Why testosterone, the 'classic anabolic hormone', affected the skeleton of intact, and not of orchiectomized, male mice is not clear, since an increase in bone density was described in hypogonadic human males following testosterone treatment [20]. One possible explanation may be that, in mice, testosterone does not act alone and/or directly on the skeleton, but exerts its effects through other gonadal hormones. In spite of the fact that specific receptors for estradiol and testosterone were demonstrated in cartilage and bone by various investigators [21–23], pointing to a direct effect on bone, there is still substantial evidence that the effects may be indirect [5,24]. Furthermore, in man, testosterone was found to increase the amount of bone [20], while in mice, bone was decreased. Decreased amounts of metaphyseal bone following testosterone treatment were also recently observed in rats [20]. It is possible, however, that the high doses of testosterone induce maturational delay of the growth plate cartilage and, hence, a wider growth plate and less endochondral bone formation. Similar findings were observed in vitamin D-deficient rickets (i.e. wide, non-mineralized growth plate and less metaphyseal bone) [25]. The increase we found in the height of the growth plate in castrated male mice is in line with similar observations made by other investigators [5,7,8].

We do not have a good explanation for the hypocalcemia noted in the orchiectomized mice. It may be that existence of male sex hormones is needed to maintain normal Ca levels, and this may be indirectly mediated by $1,25\text{-}(OH)_2D_3$. Whether or not hypoproteinemia was present was not determined.

Some of the effects observed on bone may have been due to alterations in weight gain, either as a function of gonadectomy or due to hormone therapy. Changes in weight may have been related to differences in exercise and distribution of force on the growing bones. Gonadectomy is known to increase food intake above normal ad libitum controls, making pair feeding impractical [26].

The difference in doses of hormone used in the study prevent direct comparisons between estradiol and testosterone effects. Under normal physiologic conditions, serum testosterone is lower than serum estrogen. In addition, serum estrogen fluctuates with the phase of the estrus cycle. We selected concentrations that were at the high end of normal for testosterone, but normal for estrogen at the high peak of the cycle.

This study also clearly demonstrates the ambiguities that can result from in vivo studies. While ovariectomy resulted in increased bone weight and Ca and P content of the bone, ash weight was unaffected. One interpretation may be that the ash content reflects hormone-dependent changes other than Ca and P. The data also show that changes in Ca and P are due to net changes in the amount of bone and not in the amount of calcification per unit bone.

Differences in the response of cartilage and bone to various bone seeking hormones (such as glucocorticoids) were described by many investigators for mice, rats, rabbits, and man [27]. The existence of species variation in the response of the skeleton to sex hormones may explain differences between our results and those observed in other systems. The data reported in this study point to the complex relations that exist between sex steroids in normal animals. These undergo profound changes following gonadectomy.

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