Original Article

Long-term effects of aging and orchidectomy on bone and body composition in rapidly growing male rats

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Abstract

The purpose of this study was to assess the long-term effects of aging and sex hormone deficiency on skeletal metabolism and body composition in rapidly growing male rats. Sprague-Dawley male rats were sham-operated (sham) or orchidectomized (ORX) at 3 months of age. Eight sham rats and eight ORX rats at each time point were serially sacrificed at 3, 4, 8, 12, 15, and 23 months of age. Bone mass in sham rats rapidly increased until 8 months of age, then slightly increased between 8 to 12 months of age; thereafter, an age-related decrease in bone mass was found between 12 to 23 months of age. In sham rats, bone formation parameters decreased between 3 and 8 months, and maintained at the lower level between 8 and 23 months of age, while bone resorption parameters decreased between 3 and 12 months, and thereafter, increased with age between 12 and 23 months of age. ORX significantly inhibited age-related gain in body weight, lean body mass, and cancellous and cortical bone mass and decreased peak bone mass (approximately 20% less versus sham). Further, we found that the lower bone and lean body mass in ORX rats was due to the lack of age-related gain rather than the net loss from basal controls. These data suggest that sex hormones are important factors for the accumulation of peak bone and lean body mass in male rats.

Keywords: Orchidectomy, Aging, Peak Bone Mass, Osteopenia, Body Composition

Introduction

The rat is widely used as an animal model for skeletal research[1-4]. Aging and sex hormones play important roles in skeletal development and maintenance in both females and males[2,5-9]. The effects of aging and sex hormone deficiency on skeletal metabolism have been studied in great detail in female rats[1,2,4,10]. Characterizing the changes in the female rat skeleton with age and sex hormone deficiency have provided a significant amount of information to our understanding of female skeletal metabolism and its disorders. This information has been very useful for developing treatment strategies for postmenopausal osteoporosis[11].

The orchidectomized (ORX) rat model has been used to study the effects of androgen deficiency on the male skeleton[3,9,12-14]. Furthermore, the ORX rat model has been used to test the effects of therapeutic agents in the male skeleton[15-17]. Although the effects of androgen deficiency on the male rat skeleton have been investigated in previous studies, most of the studies were performed in young or aged rats for a relatively short duration[3,9,13-15] or in aged rats for a long duration[12]. The long-term effect of aging and androgen deficiency in rapidly growing male rats has not been well documented.

The purposes of our studies were to characterize the age-related changes in bone mass, bone formation and bone resorption, and to determine the long-term (20 months) effects of orchidectomy on bone and body composition starting in rapidly growing, 3-month-old male rats. Dual energy X-ray absorptiometry (DEXA), peripheral quantitative computerized tomography (pQCT), and standard static and dynamic histomorphometric techniques were employed to determine the changes in bone mass, structure, bone formation, bone resorption, and body composition.

Materials and methods

Animals and study protocols

Sprague-Dawley male rats (Charles River Co., Wilmington, MA) starting at 3 months of age were used in these studies.

The rats were housed singly in 20 cm x 32 cm x 20 cm cages at local vivarium conditions (24 °C and 12 h/12 h light-dark cycle) and allowed to acclimate for 2 weeks before the beginning of the studies. All rats were allowed free access to water and a pelleted commercial diet (Agway ProLab 3000, Agway County Food, Inc., Syracuse, NY) containing 0.97% calcium, 0.85% phosphorus and 1.05 IU/g of Vitamin D3. The experiments were conducted according to Pfizer Animal Care approved protocols and animals were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

In the first study, Sprague-Dawley male rats at 3 months of age were randomized into groups (n=8 per group) according to their body weight. Therefore, the body weight at the beginning of the study did not differ. The rats were sham-operated (sham) or orchidectomized (ORX) at 3 months of age (n=8 per group per time point). Sham and ORX rats were serially sacrificed at 3, 4, 8, 12, 15, and 23 months of age. All rats were given subcutaneous injections of 10 mg/kg calcein (Sigma Chemical Co., St. Louis, MO), a fluorochrome bone marker, at 12 and 2 days before sacrifice in order to determine dynamic changes in bone tissues[18].

In the second study, Sprague-Dawley male rats were sham (n=10) or ORX (n=10) at 3 months of age. Whole body DEXA was performed at 12 months of age. Total body weight, total body bone area, areal bone mineral content and density, fat and lean body mass were determined.

Femoral areal bone mineral measurements

The right femur from each rat in the first study was removed during necropsy and scanned ex vivo using dual-energy X-ray absorptiometry (DEXA, QDR-1000/W, Hologic Inc., Waltham, MA) equipped with "Regional High Resolution Scan" software. The scan field size was 5.08 x 1.902 cm, resolution was 0.0254 x 0.0127 cm and scan speed was 7.25 mm/second. The femoral scan images were analyzed and total femoral bone area, areal bone mineral content (aBMC), and areal bone mineral density (aBMD) were determined as described previously[19].

Peripheral quantitative computerized tomography (pQCT). Analyses of proximal tibiae

Computerized tomographic measurements of proximal tibiae from the first study were performed by pQCT using a Stratec XCT-960M equipped with "XMICE v5.1" software (Norland Medical Systems, Ft. Atkinson, WI) specifically modified for use on small bone specimens[20]. The XCT-960M was calibrated daily with a standard of hydroxyapatite embedded in acrylic plastic. Attenuation thresholds for cortical and trabecular bone were set at 2200 and 1450 mg/cm^3 respectively. Cortical bone was defined and analyzed using Cortical Mode 1, while trabecular bone was determined using Peel Mode 3. The coefficient of variation for the measurements of volumetric total bone mineral content (vTot.C) was 1.9%,

volumetric total bone mineral density (vTot.D) was 1.1%, volumetric cortical bone mineral content (vCt.C) was 1.5%, volumetric cortical bone mineral density (vCt.D) was 1.1%, volumetric trabecular bone mineral content (vTb.C) was 4.3%, and volumetric trabecular bone mineral density (vTb.D) was 1.5% in our laboratory. In this study, a scan encompassing a 1 mm-thick slice was performed 6 mm from the proximal end of tibia with a voxel size of 0.175 mm. As illustrated by other investigators[21], this area was chosen as the growth plate is approximately 3 mm from the proximal end of the tibia, and thus the area scanned corresponds approximately to the section of the tibia that histomorphometric measurements were performed.

Proximal tibial cancellous bone and tibial shaft cortical bone histomorphometry

Static and dynamic cancellous and cortical bone histo-morphometric analyses were performed on proximal tibial metaphyses and tibial shafts, respectively, for the first study. Undecalcified, methyl methacrylate embedded frontal longitudinal sections of the proximal tibial metaphysis at 4 and 10 μm thickness, and cross-sections of tibial shafts (just proximal to the tibiofibular junction) at 20 μm thickness were prepared for histomorphometry as described previously[22,23]. The 4 μm proximal tibial sections were stained with modified Masson's trichrome stain, while the 10 μm proximal tibial sections and the 20 μm tibial shaft sections remained unstained. A Bioquant OS/2 histomorphometry system (R&M Biometrics, Nashville, TN) was used for the static and dynamic histomorphometric measurements of cancellous bone of the proximal tibial metaphysis (the area between 1 and 4 mm distal to the growth plate) and cortical bone of the tibial shafts. Trabecular bone volume (TBV), trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular
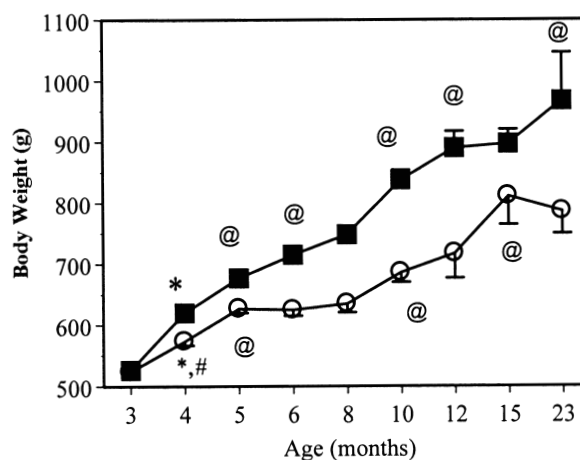
Figure 1. Age-related changes in body weight in sham-operated rats (filled square) and orchidectomized rats (open circle) between 3 to 23 months of age. *: p < 0.05 vs. basal started from this time point; #: p < 0.05 vs. sham at the same age started from this time point; @: p < 0.05 vs. the previous time point in the same group. Error bar represents standard error of mean.
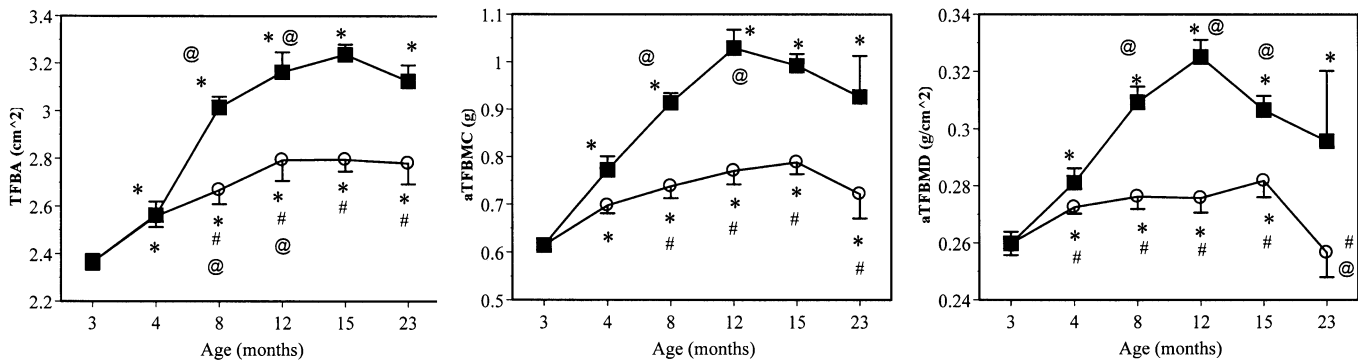
Figure 2. Age-related changes in total femoral bone area (TFBA), areal total femoral bone mineral content (aTFBMC) and areal total femoral bone mineral density (aTFBMD) in sham-operated rats (filled square) and orchidectomized rats (open circle) between 3 to 23 months of age. *: p < 0.05 vs. basal controls; #: p < 0.05 vs. sham at the same age; @: p < 0.05 vs. the previous time point in the same group. Error bar represents standard error of mean.

separation (Tb.Sp), osteoclast number per mm bone perimeter (Oc.N/BS), percent osteoclast surface (Oc.S/BS), percent mineralizing surface (%MS), mineral apposition rate (MAR), bone formation rates (BFR) were determined as described previously[23,24] for the proximal tibial metaphysis. Total tissue area, cortical bone area, marrow cavity area, periosteal and endocortical percent mineralizing surface (P-MS and E-MS), mineral apposition rate (P-MAR and E-MAR), and bone formation rate-surface referent (P-BFR/BS and E-BFR/BS) were determined for the tibial shaft cortical bone[23].

A fixed (non-growth adjusted) sampling site of the proximal tibial metaphysis was chosen for the measurements of pQCT and cancellous bone histomorphometry in the current study, since there is very little change in the length of the cancellous bone metaphysis with time[25]. An early investigation by Turner found that cancellous bone turnover in growing rats represents a maturation process that differs fundamentally from bone turnover in the adult[25]. Therefore, the pQCT and histomorphometric data from the rapid growing phase of male rats may represent the maturation of the metaphysis while the data from the adult and aged phases of male rats may represent the true cancellous bone turnover. Nevertheless, the pQCT and histomorphometric data of proximal tibial metaphysis provide the information of bone mass and structures at different ages, and more importantly, provide the information on the difference between sham and ORX rats in the same age group.

Whole body DEXA analyses

In the second study, all rats at 12 months of age, under ketamine/xylazine anesthesia, underwent dual-energy X-ray absorptiometry (DEXA, QDR-1000/W, Hologic Inc., Waltham, MA) equipped with Rat Whole Body Scan software. The rat whole body scan images were analyzed and the total body bone area, areal bone mineral content, areal bone mineral density, lean and fat body mass were determined as described previously[26].

Statistical analysis

Data are expressed as mean±SEM. Statistics were calculated using StatView 4.0 package (Abacus Concepts, Inc., Berkeley, CA). The analysis of variance (ANOVA) test followed by Fisher's protected least significant difference (PLSD) was used to compare the differences between each group[27]. A value of p<0.05 was considered a significant difference.

Results

Body weight (Fig. 1)

In the first study, the body weight was recorded for all of the remaining sham and ORX rats at 3, 4, 5, 6, 8, 10, 12, 15, and 23 months of age. Body weight increased gradually from 526±5 grams at 3 months of age to 967±79 grams at 23 months of age in sham controls (Fig. 1). ORX significantly inhibited weight gain beginning 1 month post-surgery and the body weight was maintained lower than sham-operated controls throughout the study. At 4, 5, 6, 8, 10, 12, 15, and 23 months of age, body weight in ORX rats was significantly lower by 8%, 8%, 13%, 15%, 18%, 19%, 10%, and 19%, respectively, as compared with sham controls (Fig. 1).

Femoral areal bone mineral content and density by DEXA (Fig. 2)

Total femoral bone area (TFBA) in sham rats increased until 15 months of age. This parameter peaked at 15 months of age (+37% greater than that at 3 months of age) and maintained at this level throughout the study. Similarly, areal total femoral bone mineral content (aTFBMC) and density (aTFBMD) increased with age until 12 months of age. At this age, aTFBMC increased significantly by 67.5% and aTFBMD by 25.1% as compared with those at 3 months of age. Thereafter, age-related decreases in aTFBMC and aTFBMD were found between 12 and 23 months of age (-16.3% and -13.8%, respectively).
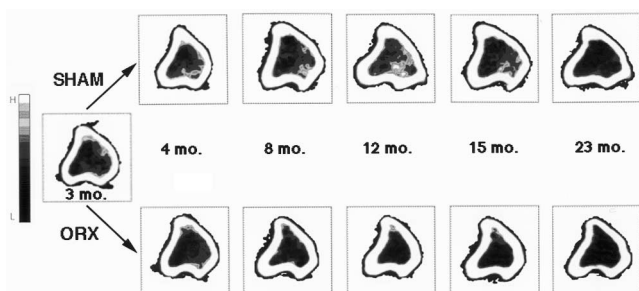
ORX significantly inhibited the age-related gain in TFBA, aTFBMC and aTFBMD throughout the study period. At 12 months of age, when the sham rats reached peak bone mass, TFBA, aTFBMC and aTFBMD in ORX rats were significantly lower by 11.7%, 25.4% and 15.2% as compared with sham controls. The differences in TFBA, aTFBMC and aTFBMD between sham and ORX remained throughout the study.

pQCT parameters of proximal tibiae (Figs. 3-5)

As illustrated in Figure 3, the trabecular and cortical bone of sham rats increased until 12 months of age; thereafter, both decreased with age. ORX partially inhibited the cortical bone gain and completely inhibited the trabecular bone gain with age.

Volumetric total bone mineral content (vTot.C) increased between 3 and 12 months of age in sham rats (Fig. 4). At 12 months of age, sham rats had 40% higher vTot.C than at 3 months of age. Between 12 to 23 months of age, vTot.C decreased with age. There was 13% less vTot.C at 23 months of age compared with 12 months of age. Similarly, volumetric total bone mineral density (vTot.D) increased until 12 months of age (+36% vs. 3 months old). Thereafter, vTot.D decreased with age (-10% at 23 months old vs. 12 months old). ORX partially inhibited the age-related increase in vTot.C, while it did not affect vTot.D as compared with sham controls. vTot.C in ORX rats was significantly increased with age at 8, 12 and 15 months as compared with basal controls. However, ORX rats had significantly lower vTot.C at 8, 12 and 15 months of age as compared with sham controls (-16%, -16% and -12%, respectively). There was no significant difference in vTot.D between sham and ORX throughout the study (Fig. 4).

Both volumetric trabecular bone mineral content (vTb.C) and density (vTb.D) increased significantly at 8 and 12 months as compared with basal controls (3 months old) in sham rats (Figs. 5). vTb.C increased significantly by 70% and vTb.D by 64% at 12 months of age as compared with those at 3 months old in sham rats. These parameters returned to the basal levels (3 months old) at 15 and 23 months of age. ORX completely inhibited the age-related increases in vTb.C and

vTb.D. In all age groups, vTb.C and vTb.D did not differ from basal controls in ORX rats. At 8, 12, and 15 months of age, ORX rats had significantly less vTb.C compared with sham controls. Similarly, ORX rats had significantly less vTb.D at 8 and 12 months of age compared with sham controls.

Volumetric cortical bone mineral content (vCt.C) and density (vCt.D) significantly increased with age until 12 months, thereafter, both decreased with age in sham rats (Fig. 5). vCt.C significantly increased by 60% and vCt.D by 22% in sham rats at 12 months as compared with 3 months old. At 23 months, vCt.C decreased by 12% and vCt.D decreased by 8% as compared with those at 12 months of age. In ORX rats, significantly lower vCt.C was found at 8 (-10%), 12 (-7%) and 15 (-9%) months of age as compared with sham rats.
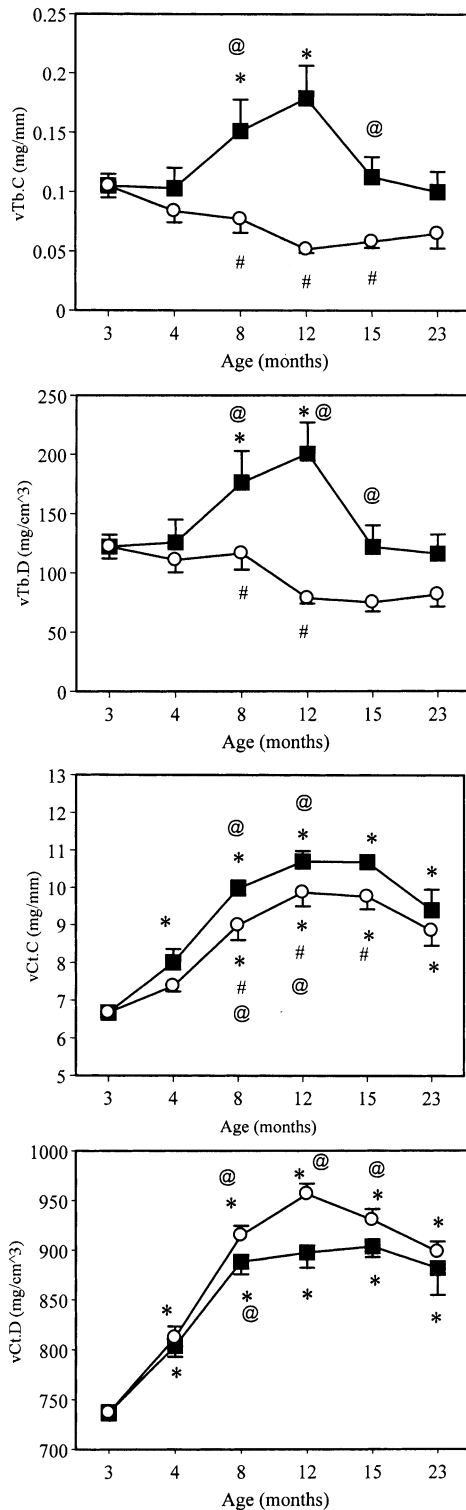
Proximal tibial cancellous bone histomorphometry (Fig. 6 and Table 1)

Effects of aging

An age-related increase in trabecular bone volume (TBV) and trabecular thickness (Tb.Th) was found until 8 months of age in sham rats (Fig. 6 and Table 1). At this age, sham
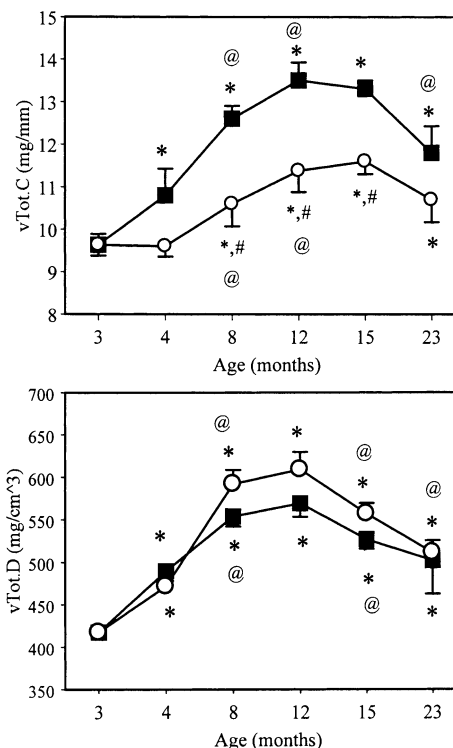
Figure 4. Age-related changes in volumetric total bone mineral content (vTot.C) and volumetric total bone mineral density (vTot.D) of proximal tibial metaphysis determined by pQCT in sham-operated rats (filled square) and orchidectomized rats (open circle) between 3 to 23 months of age. *: $p < 0.05$ vs. basal controls; #: $p < 0.05$ vs. sham at the same age; @: $p < 0.05$ vs. the previous time point in the same group. Error bar represents standard error of mean.

Figure 3. pQCT images of proximal tibial metaphysis from sham-operated (SHAM) and orchidectomized (ORX) rats from 3, 4, 8, 12, 15, and 23 months of age.

Figure 5. Age-related changes in volumetric trabecular bone mineral content (vTb.C), volumetric trabecular bone mineral density (vTb.D), volumetric cortical bone mineral content (vCt.C) and volumetric cortical bone mineral density (vCt.D) of proximal tibial metaphysis determined by pQCT in sham-operated rats (filled square) and orchidectomized rats (open circle) between 3 to 23 months of age. *: $p < 0.05$ vs. basal controls; #: $p < 0.05$ vs. sham at the same age; @: $p < 0.05$ vs. the previous time point in the same group. Error bar represents standard error of mean.

rats had 32% higher TBV and 32% higher Tb.Th than sham rats at 3 months of age. There were no significant differences in TBV or Tb.Th between 8 and 12 months of age in sham rats. In sham rats, TBV and Tb.Th was significantly decreased at both 15 and 23 months of age as compared with 12 months of age. No significant difference was found in trabecular number (Tb.N) and separation (Tb.Sp) between 3 and 12 months of age in sham rats. In sham rats, Tb.N decreased significantly at 15 and 23 months of age, and Tb.Sp increased significantly at 23 months of age as compared with the basal controls (Table 1).

Osteoclast number per mm bone perimeter (Oc.N/BS) decreased significantly at 8 and 12 months of age and increased to the level of basal controls (3 months of age) at 15 and 23 months of age in sham rats. Compared with basal controls, osteoclast surface (Oc.S/BS) decreased at 12 months of age and increased to the level of basal controls (3 months of age) at 15 and 23 months of age (Fig. 6 & Table 1).

Percent mineralizing surface (MS), mineral apposition rate (MAR), bone formation rate-bone volume referent (BFR/BV), bone formation rate-bone surface referent (BFR/BS) and bone formation rate-tissue volume referent (BFR/TV) decreased sharply with age until 8 months of age and maintained at the lower levels until 23 months of age (Fig. 6 and Table 1).

Effects of ORX

ORX completely inhibited the age-related increase in TBV by partially inhibiting an age-related increase in Tb.Th and by decreasing Tb.N (Fig. 6 and Table 1). There was significantly lower TBV at 4 (-21%), 8 (-29%) and 12 (-69%) months of age, and Tb.N at 8 (-27%) and 12 (-62%) months of age in ORX rats as compared with their age-related sham controls. Tb.Sp was significantly higher in ORX than in sham at 8 and 12 months of age.

Compared with sham controls, ORX rats had significantly higher Oc.N/BS (+74%) and Oc.S/BS (+76%) at 4 months of age (1 month post-surgery). Thereafter, no significant difference was found in these parameters between sham and ORX rats (Fig. 6 and Table 1).

BFR/TV was significantly lower at 8 (-42%) and 12 (-62%) months of age in ORX rats as compared with sham controls. No significant difference was found in MS, MAR, BFR/BV and BFR/BS between ORX and sham rats.

Tibial shaft cortical bone histomorphometry (Figs. 7 & 8)

Effects of aging

Total tissue area increased until 15 months of age and was maintained at this level at 23 months of age. At 15 months of age, total tissue area increased by 33% as compared with that at 3 months of age (Fig. 7). Cortical bone area rapidly increased with age until 12 months of age (+24%), and maintained at this level until 15 months of age; thereafter, a

slight and non-significant decrease occurred with age at 23 months of age (Fig. 7). Marrow cavity area continuously increased with age throughout the study (Fig. 7).

Periosteal and endocortical mineralizing surface (P-MS and E-MS), mineral apposition rate (P-MAR and E-MAR), and bone formation rate (P-BFR/BS and E-BFR/BS) decreased sharply with age until 8 months of age. Thereafter, P-MS decreased significantly at 23 months of age as compared with 15 months of age. E-MAR and E-BFR/BS increased significantly at 12 months compared with 8 months (Fig. 8).
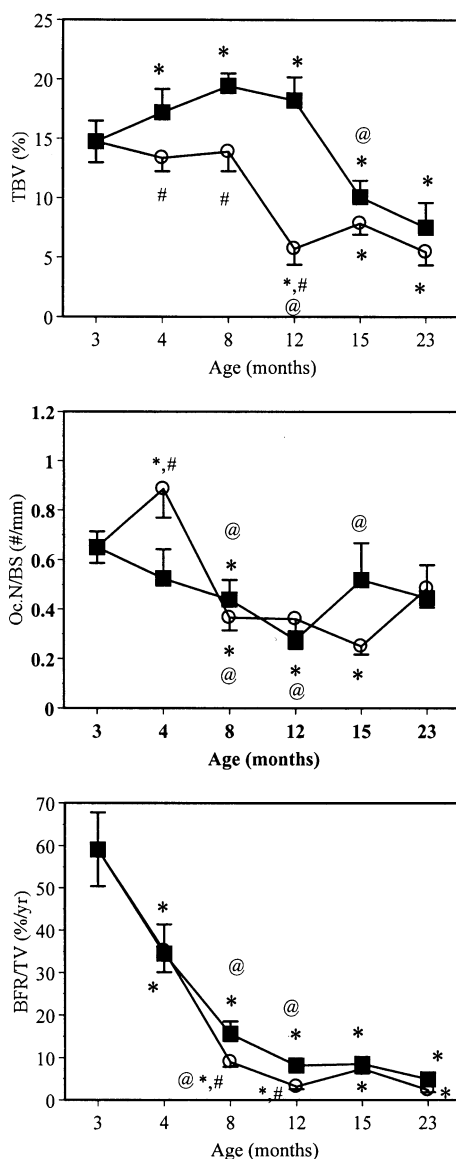
Figure 6. Age-related changes in trabecular bone volume (TBV), osteoclast number per mm bone surface (Oc.N/BS) and bone formation rate/tissue volume referent (BFR/TV) of proximal tibial metaphyseal cancellous bone in sham-operated rats (filled square) and orchidectomized rats (open circle) between 3 to 23 months of age. *: p < 0.05 vs. basal controls; #: p < 0.05 vs. sham at the same age; @: p < 0.05 vs. the previous time point in the same group. Error bar represents standard error of mean.

Effects of ORX

ORX partially inhibited the age-related increase in total tissue area and completely inhibited the age-related increase in cortical bone area (Fig. 7). Total tissue area was significantly lower at 15 months of age (-15%) and cortical bone area was significantly lower at 8 (-10%), 12 (-7%) and 15 (-18%) months of ages in ORX rats as compared with sham controls. Marrow cavity area was significantly higher at 8 and 12 months of age in ORX rats compared with sham rats.

In ORX rats, P-MS and P-BFR/BS were significantly decreased at 4 and 8 months of age and P-MAR was significantly decreased at 4 months of age as compared with sham controls. Similarly, E-MS and E-BFR/BS were significantly decreased at 4 months of age in ORX rats when compared with sham controls (Fig. 8). E-MS increased significantly in 15 months of age compared with 12 months of age in ORX rats.

Effects of ORX on body composition and total body areal bone mineral (Fig. 9)

In the second study, total body bone area, areal bone mineral content and density were significantly lower by 9%, 20% and 12% in ORX as compared with the sham controls at 12 months of age (9 months post-surgery). Total body weight was significantly lower by 19%, lean body mass by 30%, and fat body mass was non-significantly lower by 9%, in ORX rats compared with sham-operated rats (Fig. 9).

Discussion

We have demonstrated that male rats underwent significant age-related changes in bone mass and structure between 3 and 23 months of age. Sham rats continuously gained body weight throughout the study. The results from this study show that the age-related changes in bone mass between 3 and 23 months of age can be separated into three phases: increase, plateau, and decrease. Before 12 months of age, bone mass increased with age. Peak bone mass was achieved by 12 months of age. Age-related bone loss was found in cancellous bone after 12 months of age, and in cortical bone after 15 months of age.

Dynamic cancellous bone histomorphometric analyses of the proximal tibiae show that there are age-related decreases in both the bone resorption and formation activities in sham rats until 12 months of age. Thereafter, bone formation remained at a lower level, and bone resorption activity increased significantly between 12 and 15 months of age. These results indicate that age-related cancellous bone loss in male rats was due to the increased bone resorption, while bone formation remained at a lower level. Both trabecular number and trabecular thickness decreased significantly, which led to a significant decrease in trabecular bone volume at 15 months of age as compared with that at 12 months of age. Total tissue area in the tibial shaft cortical bone increased with age until 15 months. However, at the same
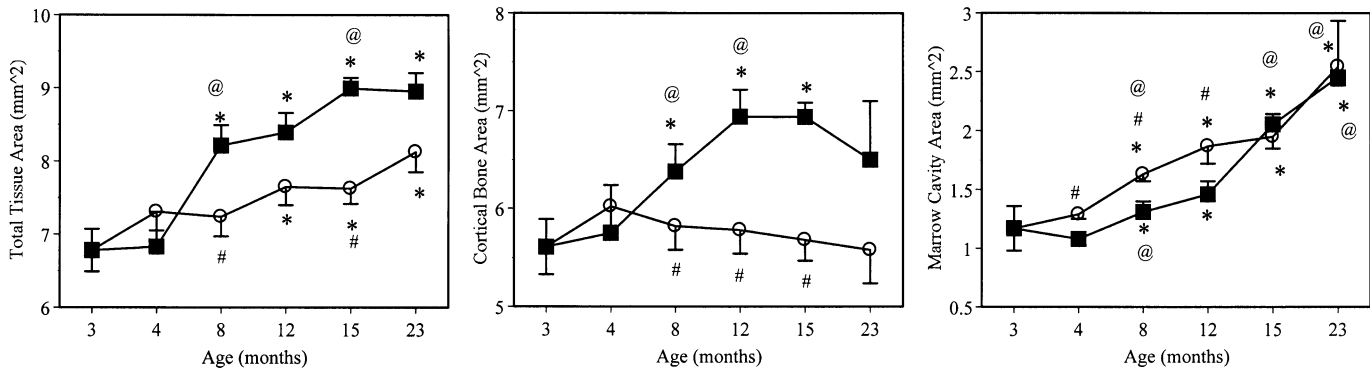
Figure 7. Age-related changes in total tissue area, cortical bone area and marrow cavity area of tibial shaft cortical bone in sham-operated rats (filled square) and orchidectomized rats (open circle) between 3 to 23 months of age. *: p<0.05 vs. basal controls; #: p<0.05 vs. sham at the same age; @: p<0.05 vs. the previous time point in the same group. Error bar represents standard error of mean.

time, the marrow cavity area also increased with age. These changes led to the plateau of cortical bone area at 12 months of age and maintained at this level until 15 months of age. These results suggest that the age-related bone loss is much slower in cortical bone than in trabecular bone due to the continuous bone formation on the periosteal surface. Between 15 and 23 months of age, bone loss on the endocortical surface (expansion of marrow cavity) is greater than bone deposition on the periosteal surface (increase in total tissue area), leading to a slight decrease in cortical bone area with age. Similar to that observed with cancellous, periosteal and endocortical bone formation sharply decreased until 8 months of age, and maintained at these levels.

In this study, we have also demonstrated that androgen deficiency induced by ORX in rapidly growing male rats inhibited age-related gain in body weight, lean body mass, and bone mass. Age-related gain in body weight, lean body mass, total bone mass, and cortical bone mass was partially inhibited, while age-related gain in cancellous bone mass was completely inhibited by androgen deficiency. Beginning at 4 months of age, ORX rats had lower body weight and bone mass. At 12 months of age, when the sham-operated rats reached peak bone mass, the ORX rats had 19% less body weight, 20% less total body areal bone mineral content, 30% less lean body mass, 25% less total femoral areal bone mineral content and 69% less trabecular bone volume in the

Age (months)		3 (basal)	4	8	12	15	23
Trabecular Thickness (µm)	SHAM	63.2±2.9	76.9±6.2	83.2±3.3 *	87.6±5.9 *	64.7±2.9 @	63.7±6.7
	ORX		65.8±2.5	80.1±4.1 *	71.7±4.7 *	79.1±4.2 *,#	73.9±3.9
Trabecular Number (#/mm)	SHAM	2.28±0.21	2.24±0.15	2.35±0.15	2.07±0.17	1.02±0.2 *,@	1.14±0.23 *
	ORX		2.07±0.19	1.71±0.14 *,#	0.78±0.16 *,#,@	1.54±0.18 *	0.70±0.13 *
Trabecular Separation (µm)	SHAM	429±85	386±37	353±25	415±37	669±103	1050±276 *
	ORX		460±72	531±50 #	2034±745 *,#,@	1063±175 *	2554±797 *,@
Osteoclast Surface/Bone Surface (%)	SHAM	0.65±0.06	0.53±0.12	0.44±0.08	0.28±0.04 *,@	0.52±0.15 @	1.13±0.34 *,@
	ORX		0.89±0.12 #	0.37±0.05 *	0.36±0.01 *	0.25±0.03 *	1.14±0.21 *,@
Mineralizing surface/Bone Surface (%)	SHAM	37.2±1.73	27.4±3.5 *	25.9±2.7 *	10.5±1.5 *,@	21.1±2.2 *,@	16.6±5.2 *
	ORX		27.7±2.2 *	21.0±2.8 *	13.3±1.0 *	23.6±2.8 *	12.4±1.8 *
Mineral Apposition Rate (µm/day)	SHAM	1.12±0.08	0.86±0.09 *	0.42±0.05 *,@	0.63±0.07 *	0.47±0.06 *	0.50±0.07 *
	ORX		0.99±0.04	0.44±0.04 *	0.49±0.03 *	0.54±0.04 *	0.48±0.06 *
Bone Formation Rate/BV (%/year)	SHAM	406±33	196±32 *	81±16 *,@	49±13 *	100±22 *	99±34 *
	ORX		256±23 *	75±18 *	55±3.4 *	99±13 *	56±12 *
Bone Formation Rate/BS (µm³/µm²/d)	SHAM	0.42±0.03	0.25±0.04 *	0.11±0.03 *,@	0.07±0.01 *	0.10±0.02 *	0.09±0.03 *
	ORX		0.27±0.02 *	0.10±0.02 *	0.06±0.003 *	0.13±0.02 *	0.06±0.01 *

Mean±SEM; SHAM: sham-operated rats; ORX: Orchidectomized rats; *: p < 0.05 vs. Basal (3 months); #: p < 0.05 vs. Sham controls; @: p < 0.05 vs. the previous time point in the same group.

Table 1. Selective histomorphometric parameters of proximal tibial cancellous bone.
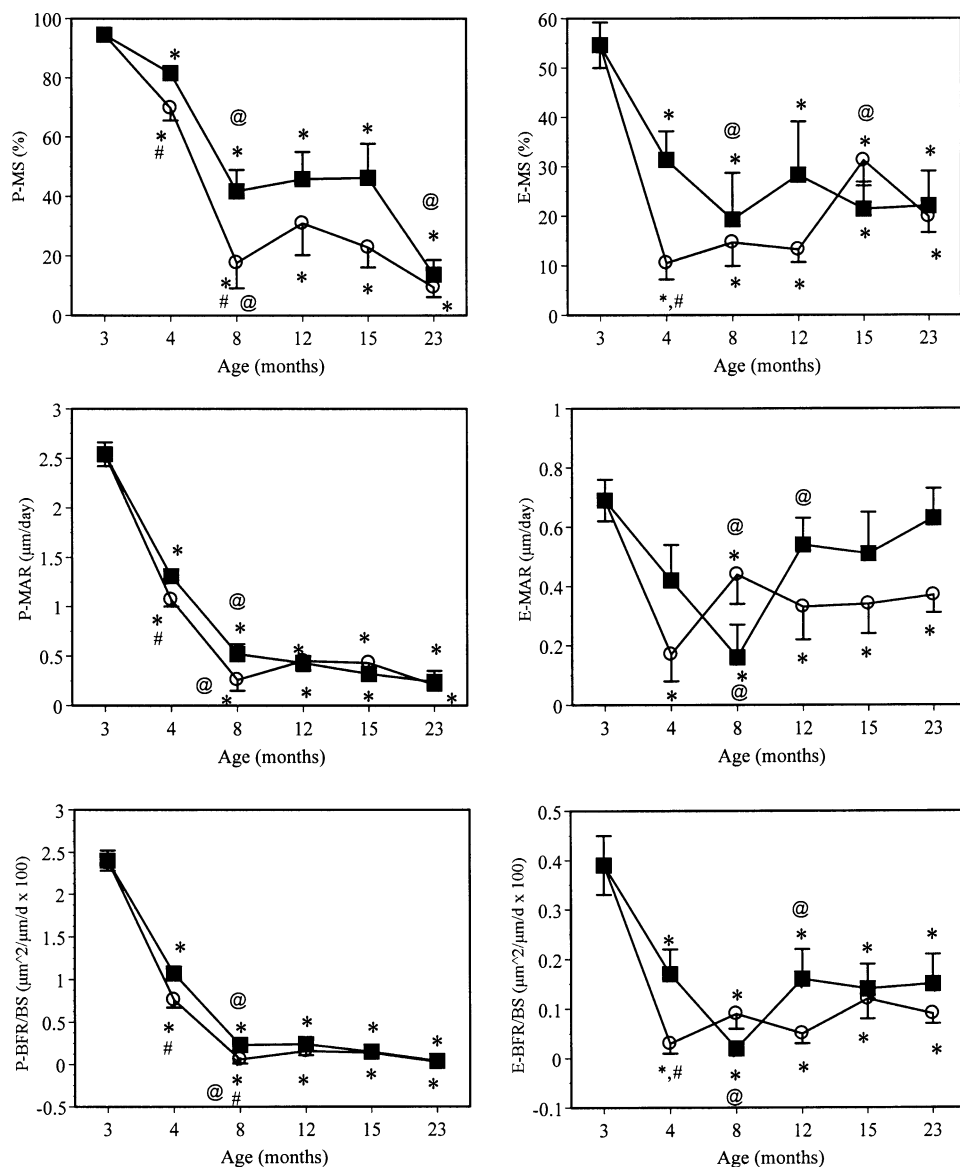
Figure 8. Age-related changes in periosteal mineralizing surface (P-MS), mineral apposition rate (P-MAR), bone formation rate/BS (P-BFR/BS), endocortical mineralizing surface (P-MS), mineral apposition rate (E-MAR), bone formation rate/BS (E-BFR/BS) of tibial shaft in sham-operated rats (filled square) and orchidectomized rats (open circle) between 3 to 23 months of age. *: $p < 0.05$ vs. basal controls; #: $p < 0.05$ vs. sham at the same age; @: $p < 0.05$ vs. the previous time point in the same group. Error bar represents standard error of mean.

related bone gain induced by androgen deficiency may be partially due to the indirect effect of decreasing food intake, and thus inhibiting body weight gain, in rapid growing ORX rats. The inhibition of body weight gain results in a decrease in mechanical loading on bone that could contribute to the inhibition of age-related bone gain in these growing ORX rats[28].

The tissue level mechanism for ORX-induced inhibition of bone gain in these rapidly growing male rats may involve activation of bone resorption in the early phase and depression of bone formation in the later phase in cancellous bone. Proximal tibial cancellous bone osteoclast number and osteoclast surface increased significantly 1 month post-surgery, and BFR/TV decreased significantly 5 and 9 months post-surgery in ORX rats as compared to their sham controls. At the ages of 15 and 23 months, the bone resorption and bone formation parameters did not differ significantly between ORX and sham rats, indicating steady state effect of ORX in these rats. These results are in agreement with the report by Gunness and Orwoll[13] in young ORX rats in which they found that ORX induced a significant increase in osteoclast number and osteoclast surface at 4 weeks post-surgery.

proximal tibial metaphysis as compared with sham controls. However, body weight and bone mass in ORX rats at 12 months of age were not less than that of basal controls (3 months of age). Therefore, the lower body weight and bone mass in androgen deficient, rapidly growing male rats was due to the lack of age-related bone gain rather than the net loss from basal controls. These findings differ from those observed with aged (13 months old) male rats in which long-term androgen deficiency induced net loss in body weight and bone mass as reported by Erben et al.[12] and Ke et al.[15]

In the current study, the rats were not pair fed, and the food intake was not determined. The inhibition of the age-

However, the results from the current study differ from those observed with aged male rats[12]. Erben and co-workers[12] reported that ORX in 13-month-old male rats induced a significant increase in bone resorption as well as in bone formation parameters at 2 weeks post-surgery and both maintained at the elevated levels until the end of the study (9 months post-surgery).

Another possible mechanism for lower cancellous bone mass in the secondary spongiosa in ORX rats may be due to the effects of androgen deficiency on primary spongiosa in rapidly growing male rats. The effect of ORX on primary spongiosa in young rats has not been documented in detail,

and requires more investigation. However, the lower cancellous bone mass in secondary spongiosa could partially stem from a reduction of the volume of the primary spongiosa in these growing ORX rats.
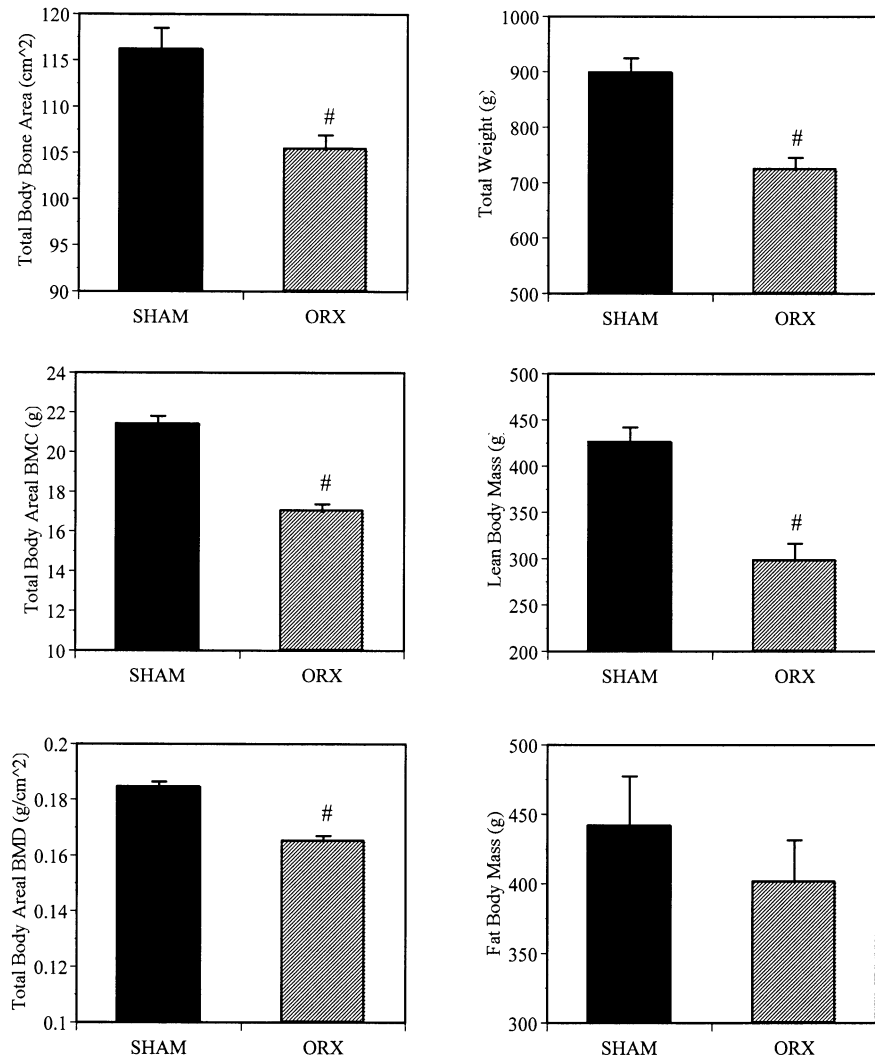
Inhibition of periosteal bone formation that reduces the expansion of total tissue area and stimulation of endocortical bone resorption that leads to the enlargement of the marrow cavity are the most important effects of ORX in cortical bone of tibial shaft in young rats. In ORX rats, the amount of new bone formation on the periosteal surface equals the amount of bone that has been removed by resorption on the endocortical surface (increased marrow cavity area). Thus, cortical bone area is maintained at a basal and consistent level throughout the study.

The whole body DEXA data confirmed our observation in the tibiae and femurs. The lower lean body mass and slightly lower fat body mass accounts for the lower body weight in ORX rats. At 12 months of age, total body bone area, bone mineral content and bone mineral density was lower in ORX than in sham rats.

In summary, our data demonstrate that male rats reach their peak bone mass at 12 months of age. Increased bone resorption leads to an age-related decrease in bone mass after 12 months of age. Furthermore, our results demonstrate that androgen deficiency in rapidly growing male rats results in lower body weight, lower bone and lean body mass by inhibiting the age-related gain of these parameters. Bone resorption was elevated in the early phase followed by a decreased bone formation in androgen-deficient rats. In conclusion, sex hormones are important factors for the accumulation of peak bone and lean body mass in male rats.

Figure 9. Effects of orchidectomy (ORX) in total body bone area, total body areal bone mineral content, total body areal bone mineral density, total body weight, lean body mass, and fat body mass at 9 months post-surgery (12 months of age) as compared with sham controls (SHAM). **#**: $p < 0.05$ vs. sham. Error bar represents standard error of mean.

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