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The Effects of Androgens on the Mechanical Properties of Primate Bone

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Feral adult female cynomolgus monkeys were divided into three groups and treated for two years: (1) normal controls; (2) weak androgenic treatment (androstenedione + estrone); and (3) strong androgenic treatment (testosterone). The tibiae and the trabecular bone of femoral head from each group were tested mechanically. There were no significant changes in the elastic modulus and shear modulus of the tibiae, measured by three point bending and torsion tests, among the three groups. Significant increases in energy absorption capacity (+45% for testosterone) and maximum shear stress (+19.4% for androstenedione and +39% for testosterone) of the tibiae, measured by torsion tests, and the cortical bone density (+5.5% for androstenedione and +8.7% for testosterone), were observed. Testosterone treatment significantly increased torsional rigidity (+23%) and bending stiffness (+15%) of the tibiae while androstenedione did not change any of these structural properties. The results of compression tests of the trabecular bone samples indicated significant increases in their elastic modulus after androstenedione (+88%) or testosterone (+107%) treatment. The maximum compressive stress of the testosterone treated samples was significantly higher than those of both normal (+28%) and androstenedione treated groups (+26%). The trabecular bone density increased after both androgenic treatments. This increase was significant for the testosterone treated group (+8.6%). We conclude that in the young cynomolgus monkey, long-term androgenic treatment significantly improves some of the mechanical properties of both cortical and trabecular bones, increases bone density, and the stronger the androgen, likely, the more pronounced is the effect. (*Bone 17:265–270; 1995*)

Introduction

Sex steroids are known to profoundly influence bone mass and bone turnover. Women are at a greater risk of developing osteoporosis than men,[30,37] and women's risk increases if they are surgically or naturally postmenopausal.[31–33] Many studies have shown the effect of estrogen deprivation on skeletal mass[5,26] and bone mechanical properties[22] in monkeys. It is also known that sex is a major determinant of skeletal mass in humans[37] and in primates,[34] with males having a higher bone mass than females. The direct effects of androgen on bone cell metabolism have been observed with androgen receptors identified in cultured human osteoblast-like cells from both males and females.[11,21] It has also been shown that testosterone deficiency in men reduces bone density,[15] whereas androgen excess in women can increase or maintain normal bone mass in the face of low or undetectable estradiol levels.[6,12]

The effects of androgen deficiency and androgen treatments on bone loss, body weight, and growth rate[20,38,41,42] as well as changes in mechanical properties due to sex differences[30] have frequently been investigated in rat models. However, rats cycle more frequently than humans and their cortical bone does not remodel like that of humans. Other animal models such as rabbit,[17] dogs,[24] and guinea pig[40] have also been used to study the effects of androgens. Nonhuman primates are good animal models for skeletal physiology due to their taxonomic affinities to humans. However, they have not been used to study the effects of androgens. There have been few studies investigating sexual dimorphism[34] in these animals.

While it is known that estrogen is necessary for maintaining bone density and mechanical properties in premenopausal woman, the role of the androgens in changing bone mechanical properties still needs to be clarified. The female cynomolgus macaque (Macaca fascicularis) has been a useful animal model to study estrogen-deficient osteopenia (osteoporosis).[5,18,23,27,43] It has a reproductive physiology that closely resembles that of the human female in terms of ovarian derived sex-steroid hormone milieu, particularly estradiol and progesterone. It also shares a pattern of similar periodicity and hormonal surges with the cycling human female.[4,23] Therefore, this animal is expected to be a good model for studying the effects of androgens on bone mechanical properties.

In this study, mechanical properties of cortical and trabecular bones of a young but fully grown female macaque were assessed after a two-year (equivalent to six human years) androgenic treatment and compared to controls. Considering the lifespan of macaques (about 25–30 years), in biologic terms, each macaque years is equal to three human years.[35]

The objectives of the present study were to compare the mechanical properties of cortical and trabecular bones of the control and androgen treated primates and to evaluate the long-term effects of androstenedione or testosterone. Cortical bone and trabecular bone were represented by the cortical shaft of the tibia and the trabecular bone of the femoral head region, respectively.

Materials and Methods

Animal and Specimen Preparation

A group of 36 feral young adult female cynomolgus macaques (M. fascicularis) (mean age 7.4 years as estimated by dentition;

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age range 4–11 years) imported from Indonesia (Charles River Research Primates, Port Washington, NY, USA) were used in the experiment.[19] All the animal experiments were conducted at the primate colony in the Department of Comparative Medicine, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC, USA. The animals were randomly selected from a group of monkeys involved in a large two-year experiment designed to study the inhibition of diet-induced coronary artery atherosclerosis by different hormonal therapies.[19] The animals were randomized into experimental groups based on their lipid profiles and body weight: Group 1 were intact monkeys who served as normal controls (N = 12). Group 2 (androstenedione + estrone) were implanted subcutaneously (s.c.) with silastic tubing delivering 4-androsten-3,17-dione and 1,3,5(10)-estratrien-3-OL-17-ONE (estrone) continuously (tube length 3.0/2.5 cm; amount of hormone delivered 2.5/2.0 cm respectively) (N = 12). Group 3 (testosterone) were implanted subcutaneously (s.c.) with silastic tubing delivering continuous testosterone (4-ANDROSTEN-17B-OL-3-ONE) (4-cm silastic tubing delivering 3.5 cm hormone) (N = 12). The animals were observed and cared for as outlined by Carlson et al.[9] At the end of the experimental period (24 months) the animals were sacrificed. At sacrifice, right femurs and right tibiae were removed, frozen immediately (−20°C), bagged, labeled, and sent on dry ice to our laboratory for mechanical testing. Upon arrival, bones were stored at −70°C. Before testing, specimens were removed from the freezer, thawed at room temperature, and cleaned of all soft tissue. The specimens were kept moist during the preparation and the testing procedure using saline solution.

Mechanical Testing

From each group, both cortical and trabecular bones were tested using a screwdriven mechanical testing machine (Floor Model TT-CM, Instron Corp, Canton, MA, USA) capable of performing both torsion and tension-compression tests. For better understanding of the method used for measurements of bone mechanical properties, the reader may refer to many articles reporting on the basic biomechanical measurements of bone.[8,13,38]

In testing of cortical bone, 33 tibiae, 11 from each group, were first tested by nondestructive three-point bending in order to measure their bending stiffness. These tests were then followed by destructive torsion tests to measure torsional stiffness and failure torque of the bones. The remaining three tibia, one from each group, were used as trial specimens for the three-point bending test.

In three-point bending tests, each tibia was placed on two supports that were 60 mm apart and the load was applied at the midpoint of the bone between two supports at a deformation rate of 0.2 cm/min. The tibiae were positioned so that bending occurred about the medial-lateral axis. The three extra tibiae were first tested to failure, in order to assure that the bending tests were non-destructive. Considering the failure load (yield point) of the tibiae (about 65 kg), a nondestructive load magnitude of about 15 kg was chosen in order to have a measurable linear response. At this load magnitude and for only one loading cycle, it was not expected that any significant microdamage be created. In this case, many cycles are needed to cause any significant increase in the bone crack density.[25,28] From the recorded load-displacement data, the bending stiffness K of the bone was calculated.

$$K = \frac{P}{\delta}, \tag{1}$$

where P is the applied load and δ is the midpoint displacement. This was then normalized to find the elastic modulus (E) of the bone using the following equation:

$$E = \frac{PL^3}{48I\delta}, \tag{2}$$

where L is the length of the bone between two supports and I is the moment of inertia. In calculating the moment of inertia, the tibial cross sections were assumed to be tubular:

$$I = \frac{\Pi(d_e^4 - d_i^4)}{64}, \tag{3}$$

where d_e and d_i are the tibial external and internal diameters, respectively. The values chosen for d_e and d_i were the average values of the external and internal diameters at three different cross sections (at the midpoint and at the two supports). At each cross section, average values of external and internal diameters were measured with a pair of digital callipers. This was done after the fractured bones were glued back together with Crazy Glue, and were cut perpendicular to the tibial longitudinal axis at the proximal and distal regions (at the two supports) and at the midshaft (midpoint between the two supports). The values of the internal and external diameters at the midshaft cross section of each tibia were used to calculate the midshaft cross-sectional area (A) and polar moment of inertia (J).

In torsional testing, about 3 cm of the proximal end and 3 cm of the distal end of each tibia were positioned in rectangular cavities of two brass cups clamped to the torsion apparatus using set screws. Using visual inspection, this was done in such a manner that the axis of the bone was as close to the axis of rotation as possible. The length of the specimens between the fixed ends was about 65–75 mm. The cup cavities were then filled with polymethyl methacrylate (PMMA) and allowed to set for 10 min. The proximal cup was fixed at the top in a chuck attached to a reaction torque cell and the distal chuck at the bottom was fixed in a chuck attached to the actuator. The torque was applied in the same direction and a rate of 38.4° per min for all the bones. From the slope of the linear region of the recorded torque-angular displacement data normalized against the test length (L), the torsional stiffness (D) of bones was calculated:

$$D = \frac{TL}{\theta}, \tag{4}$$

where T is the applied torque and θ is the angular displacement. This was then divided by the polar moment of inertia (J) to find the shear modulus (G) of the bones.

$$G = \frac{TL}{J\theta}. \tag{5}$$

The polar moment of inertia J is twice the value of moment of inertia I calculated for the three point bending test. Maximum shear stress (τ_{max}) was calculated by normalizing the failure torque data from the formula:

$$\tau_{max} = \frac{T_{max} r_e}{J}, \tag{6}$$

where T_{max} is the maximum torque or the torque at failure, r_e is the external radius, and J is the polar moment of inertia of the cross-section fractured. Maximum shear stress was at the cross

section with minimum diameter (distal region) where fracture, with a spiral configuration, occurred. Maximum shear strain (γ_{max} was obtained by normalizing the maximum rotation (θ_{max}) from the formula:

$$\gamma_{max} = \frac{r_e \theta_{max}}{L}. \quad (7)$$

The energy absorbed in the bone up to the point of failure (U) was obtained by measuring the area under the torque-twist curve divided by the bone volume.

In compressional testing of trabecular bone, 33 cylindrical specimens (11 from each group), with a diameter of 5 mm and a length of 6 mm, were cut from the femoral head region (**Figure 1**) using a diamond core drill under continuous irrigation. The specimens were then placed unsupported in the testing machine and the compressive force was applied in the axial direction at a deformation rate of 0.025 cm/min. The position of the endplates of the cylinders during compression were corrected by an interposed steel ball bearing.[29] The load-deformation curves recorded consisted of an initial linear portion from which the bone stiffness was calculated and a plateau of almost constant force was chosen to be the maximum compressive force.[16] These were then normalized to find elastic modulus and maximum compressive stress of the trabecular bone samples.

Density Measurements

The density of the cortical bone samples was calculated as:

$$\text{density} = \frac{\text{wet weight}}{\text{bone volume}}.$$

Bone volume was measured using Archimedes's principle:

$$\text{volume} = \frac{(\text{wet weight} - \text{submerged weight})}{\text{water density}}.$$

The submerged weight was measured by immersing the bone samples in water and weighing them while suspended from an analytical balance. After removing the samples from the water and drying the excess water on the bone, they were weighed in air (wet weight).

For measuring the density of trabecular bone samples, the bone marrow was pumped out by compressing the samples up to about half of their lengths. The marrow was then washed away from the bone with tap water, and the same procedure as that of cortical bone was used to measure the trabecular bone material density.

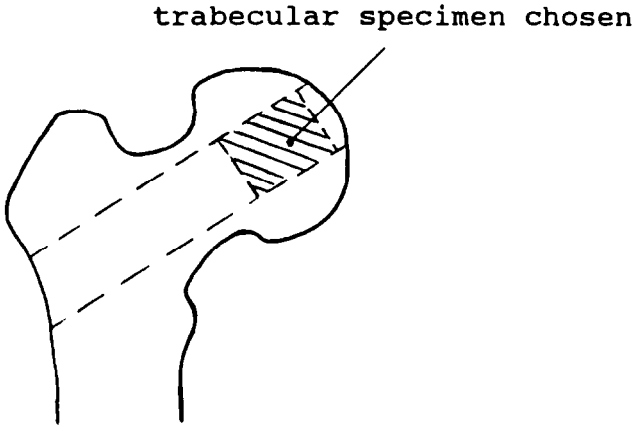

Figure 1. The region where the trabecular bone samples were chosen.

Statistical Analysis

The results are indicated as mean ± standard deviation (SD). Tukey's studentized range test was used for paired comparison among the three groups. Changes were considered significant for p values less than 0.05.

Results

General

The monkeys receiving the androstenedione/estrone supplementation demonstrated an incompletely suppressed ovulatory pattern (oligomenorrhea). Plasma estrone concentrations in this group of monkeys ranged from 50–100 pg/mL (similar to what is found at the early follicular phase) and androstenedione levels were in the range of 1.5–3.0 ng/mL, values that are twice the normal serum levels in an intact cycling female cynomolgus monkey.[3] Testosterone and LH values were not assessed. There was a general suppression of progesterone values to approximately one-half the normal levels, while estradiol levels remained relatively normal (mean value range 60.4 to 122.3 pg/mL).[2]

Testosterone levels from the experimental female monkeys achieved and sustained a normal–high range for *male* age-matched monkeys (12–20 ng/mL) throughout the two-year study period. Estradiol levels were severely suppressed to a mean value range of 13.1–17.1 pg/mL as were progesterone values to a mean value range of 0.46–0.64 ng/mL. This represents a reduction in both hormones to 25% of the normal values, creating a hormonally induced menopause. There was complete arrest of cycling activity (anovulation) in the experimental group (Adams, private communication). The initial and final body weight of the various groups of monkeys are given in **Table 1.** Only the testosterone treated group show a significant increase in body weight after two years of treatment.

Tibia

The means and standard deviations for cortical bone mechanical properties, structural properties, geometrical properties, and cortical bone density of the tibiae from each group are listed in Table 2. Tibial cortical bone mechanical properties are the elastic modulus (E) and shear modulus (G) representing the intrinsic

Table 1. Initial and final body weight of normal and androgen treated primates (mean ± SD)

	Normal	Androstenedione	Testosterone
Initial body weight (kg)	3.35 ± 0.40	3.75 ± 0.93	3.67 ± 0.56
Final body weight (kg)	3.58 ± 0.64	3.90 ± 1.07	4.91 ± 0.74
Significance	NS	NS	$p < 0.0001$

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stiffness of the tibiae, maximum shear strain (γ_{max}) representing bone ductility, energy per unit volume absorbed up to the point of failure (U) representing bone toughness, and the maximum shear stress (τ_{max}). Torsional rigidity (D) and bending stiffness (K) are the measured structural properties representing the extrinsic stiffness of the tibiae. Midshaft external diameter (d_e), midshaft internal diameter (d_i), midshaft polar moment of inertia (J), and midshaft cross-sectional area (A) are the geometrical properties.

The results show no significant changes in the elastic and shear moduli (E and G) and maximum shear strain (γ_{max}) of the tibiae among the three groups. Significant increases in maximum shear stress (τ_{max}) and cortical bone density (ρ) of the tibiae were observed after androstenedione or testosterone treatments. Treatment with testosterone also significantly increased the energy absorption capacity (U) of tibiae. Although no significant differences in these properties were observed between the androstenedione and testosterone treated groups, with the exception of the elastic moduli, we observed an increasing trend in the mechanical properties and bone density with the strength of androgen. Torsional rigidity (D) and bending stiffness (K) of the tibiae of the testosterone treated group were significantly higher than those of the normal and the androstenedione treated group. Midshaft external diameter (d_e), cross-sectional area (A), and polar moment of inertia (J) of the testosterone treated group were not significantly higher than those of the control group and were significantly higher than those of the androstenedione-treated group. There were no significant differences among the internal diameters (d_i) of the three groups.

Trabecular Bone

Table 3 shows the mean and standard deviations for the elastic modulus (E), maximum compressive stress (σ_{max}) tested under compression, and density (ρ) of the trabecular bone samples of the normal and the androgen-treated groups. The results show a significant increase in the elastic modulus (E) after androstenedione or testosterone treatment compared to control, but no significant changes between the two treatment groups. The maximum compressive stress (σ_{max}) of the testosterone-treated samples was significantly higher than that of both normal and androstenedione-treated groups, which were not significantly different. The trabecular bone density (ρ) of the normal group was nonsignificantly lower than that of the androstenedione-treated group and significantly lower than that of the testosterone-treated group. We observed an increasing trend in the mechanical property parameters as well as bone density of the trabecular bone with the strength of the androgen.

Discussion

This study was performed in order to investigate the effect of different androgenic stimuli on the mechanical properties of both cortical and trabecular bone, using the young cynomolgus (M. fascicularis) monkey as the animal model. The usefulness of the macaque as a model for studies in skeletal biology has been shown in the literature.[18,23]

The mature rat has been frequently used as a model to investigate the effect of androgen treatment on bone quality in humans.[36,41,42] It has been reported that androgen treatment stimulated mineralization and bone formation[41,42] and prevented loss of cancellous bone in castrated rats, whereas it suppressed bone formation in ovariectomized rats.[36] However, these results may not predict the cortical bone properties in animals with Haversian remodeling, such as guinea pig, dog, and primate, that display androgen-related changes in cortical bone. Martin et al.[24] studied the influence of age, weight, and sex on bone loss in the beagle tibia and showed that, when compared on the basis of age, males had more mineral, a wider tibia, and a greater mineral-to-width ratio than females. Vanderschueren et al.[40] performed single-photon absorptiometry on the tibia in adult male guinea pigs and found that androgen deficiency resulted in a decrease in tibial cortical bone density, while leaving tibial trabecular bone density unchanged.

Considering the lack of studies on the effects of androgens on bone mechanical properties, we performed mechanical testing on both tibial cortical and femoral head trabecular bones. In testing of the tibiae, three point bending was used to measure their bending stiffness, and the bones were loaded to failure only in torsion. In order to subject the bone to equally severe loading conditions at every section along its length, enabling identification of a weak cross section, a torsion test is required.[8] We found that androgen treatment significantly increases density, maximum shear stress, and energy absorption capacity of the tibia. No change in the elastic modulus of the tibial cortical shaft was observed after treatment with androgens (**Table 2**). It has been reported that cortical bone strength[1] and modulus[10] increase with increasing mineral density. However, the distribution pattern of bone mineral crystals in the organic phase as well as variations in the organic, fat, and water fractions of bone can change bone strength or stiffness as predicted by mineral density measurement alone.[7] In our study, we measured the real density of the bone including both mineral and organic phases. As shown in Table 2,

Table 2. Mechanical, structural, and geometric properties, and density of the tibiae of normal and androgen treated primates

	Normal	Androstenedione	Testosterone
Elastic modulus E (MPa)	8418 ± 1606	8353 ± 1436	8041 ± 1163
Shear modulus G (MPa)	2807 ± 510	2825 ± 407	2876 ± 368
Maximum shear strain γ_{max} (%)	1.86 ± 0.38	1.96 ± 0.26	2.15 ± 0.33
Energy U (J/cm^3)	0.2427 ± 0.0830*	0.3063 ± 0.0792	0.352 ± 0.0773*
Maximum shear stress τ_{max} (MPa)	46 ± 8.7*†	54.9 ± 11†	63.9 ± 12.4*
Torsional rigidity D (N cm^2/rad)	10557.2 ± 2361.4*	9367.2 ± 922.9†	12989.3 ± 2164.7*†
Bending stiffness K (N/mm)	348.2 ± 55.7*	321.6 ± 49.1†	401.1 ± 53.5*†
Midshaft external diameter d_e (mm)	7.89 ± 0.71	7.54 ± 0.52*	8.22 ± 0.38*
Midshaft internal diameter d_i (mm)	3.54 ± 0.69	3.55 ± 0.56	3.49 ± 0.66
Polar moment of inertia J (mm^4)	379.1 ± 129.8	307.4 ± 89.2*	435.3 ± 78.9*
Cross-sectional area A (mm^2)	39.1 ± 6.8	34.6 ± 4.4*	43.2 ± 4.1*
Density ρ (g/cm^3)	1.64 ± 0.096*†	1.729 ± 0.095†	1.781 ± 0.052*

*†In each row, numbers with any of these symbols in common are significantly different ($p < 0.05$).

Table 3. Mechanical properties and density of the trabecular bone of normal and androgen-treated primates

	Normal	Androstenedione	Testosterone
Elastic modulus E (MPa)	392 ± 70*†	737 ± 152†	812 ± 123*
Maximum stress σ_{max} (MPa)	23.6 ± 4.8*	23.19 ± 2.6†	29.8 ± 5.4*†
Density ρ (g/cm^3)	1.326 ± 0.089*	1.389 ± 0.045	1.440 ± 0.058*

*†In each row, numbers with any of these symbols in common are significantly different ($p < 0.05$).

no change in the value of tibial midshaft internal diameter was found among the three groups while the value of external diameter (bone width) of the testosterone treated group was nonsignificantly higher than that of the normals and significantly higher than that of the androstenedione treated group. This resulted in a higher polar moment of inertia for the testosterone treated group and a further improvement of its tibial structural properties, such as its torsional rigidity and bending stiffness which were significantly higher than both the normal and the androstenedione treated group. In a cross-sectional study of the beagle tibia, Martin et al.[24] reported that males had a larger bone width than females. As mentioned before, in the testosterone-treated group, we have also found an increase, although not significant, in tibial external diameter (bone width) but no change in its internal diameter. This suggests that treatment with testosterone may likely promote periosteal formation.

In our study, density and mechanical properties (elastic modulus and maximum compressive stress) of trabecular bone samples increased significantly with increasing strength of androgen treatment **(Table 3).** In our study, most of the monkeys had their long bone growth plate closed but had not reached their peak bone mass as reported by Jerome et al.[19] Mosekilde[30] reported the effect of sex on age-related loss of vertebral trabecular mass and structure, where she demonstrated a sex-related difference in the change in vertebral trabecular architecture with age, with a higher tendency to perforation of the horizontal supporting struts in females than in males. Although the increase in trabecular bone density may have contributed to the improvement of mechanical properties in our study,[7] the geometry of trabecular bone at a microstructural level may also have had a role in increasing its mechanical properties.[30]

Eventov et al.[14] have reported that cancellous bone remodeling and microanatomy are not necessarily the same at different skeletal sites. The femoral head trabecular bone samples used in this study may then not necessarily reflect trabecular bone mechanical properties or density in the vertebral region. It is also worth noting that the monkeys used in this study were young, with an average age of 7.4 years equivalent to a 22-year-old human female[35] which is lower than the age of a postmenopausal woman.

In conclusion, in a young female cynomolgus monkey, androgenic treatment for a period of two years corresponding to six years in human terms[35] increases the mechanical properties and densities of tibial cortical bone and femoral head trabecular bone. This increase becomes more significant as the androgen becomes stronger. Only testosterone treatment significantly increases tibial structural stiffness, whereas androstenedione treatment does not change tibial structural properties. Finally, treatment with testosterone makes the tibia stronger, tougher, and stiffer.

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References

1. Amtmann, E. Mechanical stress, functional adaption and the variation structure of the human femur diaphysis. Ergeb. der Anat. und Entwickl. Berlin: Springer; 1971; 44.
2. Barbieri, R. Polycystic ovarian disease. Ann Rev Med 42:199–204; 1991.
3. Billiar, R., Richardson, D., Andersoon, E., Mahajan, D., and Little, B. The effect of chronic and acyclic elevation of circulating androstenedione or estrone concentrations on ovarian function in the rhesus monkey. Endocrinology 116:2209–2220; 1985.
4. Bourget, C., Femino, A., Franz, C., and Longcope, C. Estrogen and androgen dynamics in the cynomolgus monkey. Endocrinology 122:202–206; 1988.
5. Bowles, E. A., Weaver, D. S., Telewski, F. W., Wakefield, A. H., Jaffe, M. J., and Miller, L. C. Bone measurement by enhanced contrast image analysis: Ovariectomized and intact Macaca fascicularis as a model for human osteoporosis. Amer J Phys Anthropol 67:99–103; 1985.
6. Buchanan, J. R., Hospodar, P., Myers, C., Leuenberger, P., and Demers, L. M. Effect of excess endogenous androgens on bone density in young women. J Clin Endocrinol Metab 67:937–943; 1988.
7. Burr, D. B. The relationships among physical, geometrical and mechanical properties of bone, with a note on the properties of nonhuman primate bone. Yearbook Phys Anthropol 23:109–146; 1980.
8. Burstein, A. H. and Frankel, V. H. A standard test for laboratory animal bone. J Biomech 4:155–158; 1971.
9. Carlson, C., Tulli, H., Jayo, M., Loeser, R., Tracy, R., Mann, K., and Adams, M. Immunolocalization of noncollagenous bone matrix proteins in lumbar vertebrae from intact and surgically menopausal cynomolgus monkeys. J Bone Miner Res 8:71–81; 1993.
10. Choi, K., Kuhn, J. L., Ciarelli, M. J., and Goldstein, S. A. The elastic moduli of human subchondral, trabecular, and cortical bone tissue and the size-dependency of cortical bone modulus. J Biomech 23:1103–1114; 1990.
11. Colvard, D. S., Eriksen, E. E., Keeting, P. E., Wilson, E. M., Lubahn, D. B., French, F. S., Riggs, B. L., and Spelsberg, T. C. Identification of androgen receptors in normal human osteoblast-like cells. Proc Natl Acad Sci USA 86:854–857; 1989.
12. Dixon, J. E., Rodin, A., Murby, B., Chapman, M. G., and Fogelman, I. Bone mass in hirsute women with androgen excess. Clin Endocrinol 30:271–277; 1989.
13. Einhorn, T. A. Bone strength: The bottom line. Calcif Tissue Int 51:333–339; 1992.
14. Eventov, I., Frisch, B., Cohen, Z., and Hammel, I. Osteopenia, hematopoiesis, and bone remodeling in iliac crest and femoral biopsies: A prospective study of 102 cases of femoral neck fractures. Bone 12:1–6; 1991.
15. Finkelstein, J. S., Klibanski, A., Neer, R. M., Greenspan, S. L., Rosenthal, D. I., and Crowley, W. F. Osteoporosis in men with idiopathic hypogonadotropic hypogonadism. Ann Intern Med 196:354–361; 1987.
16. Gibson, L. J. The mechanical behaviour of cancellous bone. J Biomech 18: 317–328; 1985.
17. Gilsanz, V., Roe, T. F., Gibbens, D. T., Schulz, E. E., Carlson, M. E., Gonzalez, O., and Boechat, M. I. Effect of sex steroids on peak bone density of growing rabbits. Amer J Physiol 255:E416–E421; 1988.
18. Jayo, M. J., Weaver, D. S., Adams, M. R., and Rankin, S. E. Effects on bone of surgical menopause and estrogen therapy with or without progesterone replacement in cynomolgus monkeys. Am J Obstet Gynecol 163:614–618; 1990.
19. Jerome, C. P., Carlson, C. S., Register, T. C., Bain, F. T., Jayo, M. J., Weaver, D. S., and Adams, M. R. Bone functional changes in intact, ovariectomized, and ovariectomized, hormone-supplemented adult cynomolgus monkeys (Macaca fascicularis) evaluated by serum markers and dynamic histomorphometry. J Bone Miner Res 9:527–540; 1994.
20. Kapur, S. P. and Reddi, A. H. Influence of testosterone and dihydrotestoster-

one on bone-matrix induced endochondral bone formation. Calcif Tissue Int 44:108–113; 1989.
21. Kasperk, C. H., Wegedal, J. E., Farley, J. R., Linkhart, T. A., Turner, R. T., and Baylink, D. J. Androgens directly stimulate proliferation of bone cells in vitro. Endocrinology 124:1576–1578; 1989.
22. Kasra, M. and Grynpas, M. D. Effect of long term ovariectomy on bone mechanical properties in young females cynomolgus monkeys. Bone 15:557–561; 1994.
23. Mann, D., Gould, K., and Collins, D. A. Potential primate model for bone loss resulting from medical oophorectomy or menopause. J Clin Endocrinol Metab 71:105–110; 1990.
24. Martin, R. K., Albright, J. P., Jee, W. S., Taylor, G. N., and Clarke, W. R. Bone loss in the beagle tibia: Influence of age, weight, and sex. Calcif Tissue Int 33:233–238; 1981.
25. Martin, B. A theory of fatigue damage accumulation and repair in cortical bone. J Orthop Res 10:818–825; 1992.
26. Mazess, B., Vetter, J., and Weaver, D. S. Bone changes in oophorectomized monkeys: CT findings. J Comput Assist Tomogr 11:302–305; 1978.
27. Miller, L. C., Weaver, D. S., McAlister, J. A., and Koritnik, D. R. Effects of ovariectomy on vertebral trabecular bone in the cynomolgus monkey (Macaca fascicularis). Calcif Tissue Int 38:62–65; 1986.
28. Mori, S. and Burr, D. B. Increased intracortical remodelling following fatigue damage. Bone 14:103–109; 1993.
29. Mosekilde, L., Viidik, A., and Mosekilde, L. Correlation between the compressive strength of iliac vertebral trabecular bone in normal individuals. Bone 6:291–295; 1985.
30. Mosekilde, L. Sex differences in age-related loss of vertebral trabecular bone mass and structure-biomechanical consequences. Bone 10:425–432; 1989.
31. National Institute of Arthritis and Musculoskeletal and Skin Disorders. Osteoporosis: cause, treatment, prevention. Bethesda: NIH publication No. 86-2226; 1986; 4; 29.
32. Peck, W. A., Riggs, B. L., and Bell, N. H. In: National Osteoporosis Foundation Physician's Resource Manual in Osteoporosis. Washington, DC: National Osteoporosis Foundation; 1987; 1; 33.
33. Phillips, S., Fox, N., Jacobs, J., and Wright, W. E. The direct costs of osteoporosis for American women aged 45 and older. Bone 9:271–279; 1986.
34. Pope, N. S., Gould, G., Anderson, C., and Mann, D. R. Effects of age and sex on bone densities in the Rhesus Monkey. Bone 10:109–112; 1989.
35. Rawlins, G. and Kessler, M. J. The Cayo Santiago Macaques: History, behaviour & biology. Albany: New York Press; 1986.
36. Russell, T. T., Wakley, G. K., and Hannon, K. S. Differential effects of androgens on cortical bone histomorphometry in gonadectomized male and female rats. J Orthop Res 8:612–617; 1990.
37. Seeman, E., Melton, L. J., O'Fallon, W. M., and Riggs, B. L. Risk factors for spinal osteoporosis in men. Amer J Med 75:977–983; 1983.
38. Turner, C. H. and Burr, D. B. Basic biomechanical measurements of bone: A tutorial. Bone 14:595–608; 1992.
39. Turner, R. T., Wakley, G. K., and Hannon, K. S. Differential effects of androgens on cortical bone histomorphometry in gonadectomized male and female rats. J Orthop Res 8:612–617; 1990.
40. Vanderschueren, D., Van Herck, E., Suiker, A. M., Allewart, K., Visser, W. J., Geusens, P., and Bouillon, R. Bone and mineral metabolism in the adult guinea pig: Long-term effects of estrogen and androgen deficiency. J Bone Miner Res 7:1407–1414; 1992.
41. Wakley, G. K., Schutte, D. H., Hannon, K. S., and Turner, R. T. Androgen treatment prevents loss of cancellous bone in the orchidectomized rat. J Bone Miner Res 6:325–330; 1991.
42. Wakley, G. K., Scott, V. S., and Turner, R. T. Sex and site-specific effects of the anabolic steroid clostebol acetate on bone in rats. Orthop Trans 19:78; 1994.
43. Welles, E. G., Jayo, M. J., Weaver, D. S., Carlson, C. S., and Rankin, S. E. Characterization of serum chemistry of bone turnover in surgically postmenopausal cynomolgus macaques. J Bone Miner Res 5:S182 (432); 1990.

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