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Evaluation of Long-term Vitamin E Insufficiency or Excess on Bone Mass, Density and Microarchitecture in Rodents

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Abstract

High dietary α-tocopherol levels reportedly result in osteopenia in growing rats, while α-tocopherol deficiency in α-tocopherol transfer protein knockout (α-TTP KO) mice results in increased cancellous bone mass. Since osteoporosis is a disease associated primarily with aging, we hypothesized that age-related bone loss would be attenuated in α-TTP KO mice. Cancellous and cortical bone mass and microarchitecture were assessed using dual energy x-ray absorptiometry and micro-computed tomography in 2-year-old α-TTP KO and wildtype (WT) male and female mice fed DL-α-tocopherol acetate. In contrast to our expectations, differences in cancellous bone were not detected between WT and α-TTP KO mice in either gender and α-TTP KO males had *lower* ($p < 0.05$) cortical bone mass than WT males. We therefore evaluated bone mass, density and microarchitecture in proximal femur of skeletally mature (8.5-months-old) male Sprague-Dawley rats fed diets containing low (15 IU/kg), adequate (75 IU/kg), or high (500 IU/kg diet) DL-α-tocopherol acetate for 13 weeks. Low dietary α-tocopherol did not increase bone mass. Furthermore, no reductions in cancellous or cortical bone mass were detected with high dietary α-tocopherol. Failure to detect increased bone mass in aged α-TTP KO mice or bone changes in skeletally mature rats fed either low or high levels of α-tocopherol does not support the hypothesis that α-tocopherol has a negative impact on bone mass, density or microarchitecture in rodents.

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Keywords

Vitamin E; osteoporosis; rodent; skeleton; oxidative stress

Introduction

The redox status of bone cells may be critical for optimal bone health, especially during aging. Reactive oxygen species play an important role in normal osteoclast function and are greatly increased following a fracture, thereby facilitating fracture repair (1). However, in addition to these physiological actions, reactive oxygen species have been implicated as contributing factors in the etiology of a variety of age-related skeletal pathologies, including osteoporosis (2–5).

Antioxidants, such as vitamin E (α-tocopherol), may play a role in facilitating the delicate balance between bone formation and resorption required to maintain bone integrity. α-Tocopherol concentrations are regulated in the liver by 1) the α-tocopherol transfer protein (α-TTP), a protein that facilitates α-tocopherol secretion into plasma for transport to extrahepatic tissues by lipoproteins, and 2) by vitamin E degradation via its metabolism (6). Vitamin E prevents the chain reaction of lipid peroxidation by intercepting peroxyl radicals and preventing them from damaging polyunsaturated fatty acids in membranes and lipoproteins (7). The precise role of α-TTP has been clarified using genetic models, including α-TTP knock out (KO) mice (8). Tissue levels of α-tocopherol are reported to be normal in heterozygote α-TTP−/+ mice. In contrast, mice with a homozygous deletion in α-TTP (α-TTP−/− mice) have dramatically reduced blood and tissue levels of α-tocopherol (9) and exhibit increased oxidative stress as they age (10).

Increased production of reactive oxygen species may contribute to the etiology of bone loss associated with chronic diseases, gonadal insufficiency and aging (11–16). Dietary supplementation with α-tocopherol above normal dietary requirements has been reported to slow bone loss associated with aging, gonadal hormone insufficiency, and disuse in preclinical rodent models (17–20). However, it was noted that chronic consumption of high levels of dietary α-tocopherol may negatively impact bone architecture (20). Furthermore, a recent study by Fujita et al. (21) reported that α-TTP KO mice have high bone mass resulting from decreased bone resorption and that administration of high dietary α-tocopherol to rats induces bone loss. Since osteoporosis is a disease most often associated with aging, we hypothesized that age-related bone loss would be attenuated in α-TTP KO mice. We therefore assessed cancellous and cortical bone mass and architecture using dual energy X-ray absorptiometry (DXA) and micro-computed tomography (μCT) in aged (22 to 24-month-old) α-TTP KO and wildtype (WT) male and female mice. Additionally, we evaluated the long-duration (13 weeks) effects of low, normal, and high levels of dietary α-tocopherol on bone of skeletally mature male rats. We hypothesized that α-tocopherol insufficiency would result in increased bone mass whereas α-tocopherol excess would result in bone loss.

Materials and Methods

Experimental Design

Two separate experiments were conducted. Experiment 1 was designed to evaluate the effects of α-TTP deficiency on cancellous and cortical bone mass and architecture in aged α-TTP KO mice. Experiment 2 was designed to evaluate the long-term effect of altered vitamin E status on bone mass and architecture in skeletally mature male rats. The experimental protocols were approved by the Institutional Animal Care and Use Committee

at the University of California, Davis (Experiment 1) and Oklahoma State University (Experiment 2). For both experiments, the animals were housed in temperature- and humidity-controlled rooms on a 12-hour light-dark cycle and maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Experiment 1: Effects of α-tocopherol transfer protein deficiency on bone—α-TTP KO mice were generated as described (22). The mice were back-crossed onto a C57BL6 genetic background and maintained on this background at an in-house colony at the University of California, Davis. The experimental protocol has been described in detail elsewhere (23). In brief, α-TTP KO mice and their WT littermates were fed a custom diet containing 35 IU α-tocopherol acetate/kg diet (Mouse Diet 5015) for the first 8 months of study (24). The mice were then switched to the standard mouse chow with 66 IU α-tocopherol acetate/kg diet (PicoLab Mouse Diet 5058) until 22 to 24 month of age (24); α-TTP KO mice are severely vitamin E deficient on either diet (25). Diets were purchased from Purina Test Diets (Richmond, PA, USA). The composition of diets used in these experiments followed the recommendation for rodent diets by the American Institute of Nutrition. Six male and 11 female α-TTP KO and 10 male and 3 female WT mice were available for skeletal evaluation. Following necropsy, femora and lumbar vertebrae were excised and placed in 70% ethanol and shipped to Oregon State University for DXA and μCT analysis.

Experiment 2: Effects of vitamin E on bone in male Sprague-Dawley rats—The experimental design has been described in detail elsewhere (20). In brief, skeletally mature 8.5-month-old male Sprague-Dawley rats (Harlan, Indianapolis, IN) were randomly assigned into one of 3 treatment groups; 1) low-dietary vitamin E (15 IU/kg diet, n=7), 2) adequate-dietary vitamin E (75 IU/kg diet, n=8), or 3) high-dietary vitamin E (500 IU/kg diet, n= 8), resulting in progressive increases in serum levels of vitamin E (20). The rats were housed individually and maintained on their respective diets for 13 weeks. Following necropsy, femora were excised and placed in 70% ethanol solution and the proximal half of the femur was evaluated using DXA and μCT.

DXA Analysis

Bone mineral content (BMC, mg) and area (cm^2) were measured in the proximal half of the femur using DXA (Piximus, Lunar Corp., Madison, WI, USA). Bone mineral density (BMD) was calculated as BMC/area (mg/cm^2).

μCT Analysis

μCT was used for nondestructive three-dimensional evaluation of bone volume and bone architecture. In Experiment 1, femora and 2nd lumbar vertebrae were scanned using a Scanco μCT40 scanner (Scanco Medical AG, Basserdorf, Switzerland) at a voxel size of 12μm × 12μm × 12μm. The threshold value for evaluation was determined empirically and set at 265 (gray scale, 0-1000). Entire femora (cancellous + cortical bone) were evaluated followed by evaluation of cortical bone in the femur diaphysis and cancellous bone in the distal femur metaphysis. For the femoral diaphysis, 20 slices (240 μm in length) of bone were evaluated and total cross-sectional volume (cortical and marrow volume, mm^3), cortical volume (mm^3), marrow volume (mm^3), cortical thickness (μm), and I_{Polar} (a surrogate index of bone strength in torsion, mm^4) were determined. For the distal femoral metaphysis, 50 slices (600 μm in length) of cancellous bone (secondary spongiosa only) were measured starting 500 μm proximal to the growth plate. Analysis of the lumbar vertebra included the entire region of secondary spongiosa between the cranial and caudal growth plates. Direct cancellous bone measurements in the distal femoral metaphysis and lumbar vertebra included cancellous bone volume fraction (bone volume/tissue volume or

volume of total tissue occupied by cancellous bone, %), trabecular thickness (mean thickness of individual trabeculae, μm), trabecular number (number of trabecular intersects per unit distance, mm^{-1}), and trabecular spacing (the distance between trabeculae, μm).

In Experiment 2, the proximal half of the femur was available for evaluation. Each femur was scanned using a Scanco μCT40 scanner (Scanco Medical AG, Basserdorf, Switzerland) at a voxel size of 16μm × 16μm × 16μm. The threshold value for evaluation was determined empirically and set at 245 (gray scale, 0-1000). Cancellous bone was evaluated in the femoral head followed by evaluation of cortical bone in the femoral diaphysis. Direct cancellous bone measurements included cancellous bone volume fraction (%), trabecular thickness (μm), trabecular number (mm^{-1}), and trabecular spacing (μm). For the femoral diaphysis, 20 slices (320 μm in length) of bone were evaluated (21 mm distal to top of the femoral head) and total cross-sectional volume (mm^3), cortical volume (mm^3), marrow volume (mm^3), cortical thickness (μm), and I_{Polar} (mm^4) were determined.

Statistical Analysis

T-tests were used to evaluate differences between WT and α-TTP KO mice within each gender in Experiment 1. A one-way ANOVA was used to evaluate differences among treatment groups in Experiment 2. Statistical analysis was performed using R 2.14.1 (The R Foundation for Statistical Computing, Vienna, Austria). Differences were considered significant at $p<0.05$. All data are expressed as mean ± SE.

Results

Experiment 1: Effects of α-tocopherol transfer protein deficiency on bone

The effects of chronic α-tocopherol deficiency on body weight and bone mass, density, and microarchitecture in aged male and female mice are presented in Table 1. Terminal body weight did not differ between WT and α-TTP KO male mice. Total femur BMC was lower ($p < 0.05$) and there was a tendency for femur BMD ($p = 0.07$) and total femur bone volume ($p = 0.1$) to be lower in the α-TTP KO males compared to WT littermates. Cross-sectional volume ($p < 0.01$), cortical volume ($p < 0.01$), cortical thickness ($p < 0.04$) and I_{polar} ($p < .01$) in the femur diaphysis were lower in the male α-TTP KO mice compared with WT mice. No significant differences in cancellous bone volume fraction, trabecular number, trabecular thickness or trabecular spacing were detected between WT and α-TTP KO male mice in either the distal femur metaphysis or the lumbar vertebra. However, there was a tendency ($p = 0.1$) for trabecular thickness in the distal femur metaphysis to be lower in the male α-TTP KO mice compared to WT mice.

Terminal body weight did not differ between WT and α-TTP KO female mice. No differences were detected in femur cortical bone mass, density or microarchitecture between female WT and α-TTP KO mice. Similarly, no differences in cancellous bone microarchitecture were detected in either femur or lumbar vertebra between female WT and α-TTP KO mice.

Experiment 2: Effects of α-tocopherol on bone in skeletally mature male Sprague-Dawley rats

The effects of three different dietary α-tocopherol intakes on bone mass, density and microarchitecture in skeletally mature male Sprague-Dawley rats are presented in Table 2. No significant differences in proximal femur BMC and BMD, femoral head cancellous bone volume fraction and trabecular number, trabecular thickness and trabecular spacing, or femur diaphysis cross-sectional volume, cortical volume, marrow volume, cortical thickness,

and I_{Polar} were detected among the rats fed low, adequate, or high α-tocopherol for 13 weeks.

Discussion

Bone mass and architecture were evaluated in aged (22–25-month-old) male and female α-tocopherol-deficient mice and in skeletally mature (8.5-month-old) male rats supplemented with three different levels of dietary α-tocopherol for 13 weeks. Differences in cancellous bone were not detected between WT and α-TTP KO mice in either gender whereas male α-TTP KO mice exhibited *lower* cortical bone size, mass, density and thickness compared to WT males. Differences in cancellous and cortical bone mass and architecture were not detected among rats supplemented with low, adequate, or high levels of α-tocopherol.

In stark contrast to our results in aged mice, Fujita and colleagues (21) reported that skeletally immature α-TTP homozygote and heterozygote KO mice have a high cancellous bone mass phenotype. In addition, high levels of dietary α-tocopherol were reported to reduce cancellous bone mass in 3-month-old α-TTP KO mice and to reduce cancellous and cortical bone mass in 3-month-old rats. Although no intergroup differences in bone formation or osteoclast number were detected, osteoclast size was decreased in the α-TTP KO mice. In contrast, high concentrations of α-tocopherol increased osteoclast differentiation *in vitro*. Based on these findings, Fujita and colleagues concluded that bone mass is regulated by circulating α-tocopherol and that α-tocopherol acts to lower bone mass exclusively by increasing osteoclast function. As discussed below, several lines of evidence lead us to question the above interpretation.

Similar to humans, rodents experience pronounced cancellous bone loss as they age (26–31). Our finding of a lack of differences in cancellous bone volume fraction or architectural endpoints (trabecular number, thickness and spacing) between aged WT and α-TTP KO mice in the present study does not support the hypothesis that normal levels of α-tocopherol contribute to age-related loss of cancellous bone. Moreover, circulating α-tocopherol levels are normal in α-TTP−/+ mice; thus, the high cancellous bone mass phenotype that Fujita and colleagues reported in the heterozygote mice are probably not due to reduced α-tocopherol levels. Indeed, there is evidence for a beneficial effect of increased α-tocopherol on the aging skeleton. Arjmandi and colleagues fed 6- and 24-month-old male mice a diet containing either adequate (30 mg/kg diet) or high (500 mg/kg diet) levels of α-tocopherol for 30 days (17). Treatment had no effect on bone in 6-month-old mice. In the 22 to 24-month-old mice, high α-tocopherol intake enhanced bone quality as evidenced by improved material and structural properties and resulted in increased bone dry weight, protein, and mRNA transcripts for the bone matrix proteins osteocalcin and type Iα-collagen. The authors suggested that α-tocopherol attenuates age-related increases in oxidative stress in aged mice. The Arjmandi et al. animal study is potentially relevant to humans; Ostman and colleagues reported that in elderly men, low serum levels of vitamin E are associated with lower BMD (32), a finding that has also been described recently in postmenopausal women (33). Fujita and colleagues (21) hypothesize that α-tocopherol influences bone mass by increasing osteoclast differentiation (fusion of osteoclasts). Interestingly, vitamin E has also been shown to enhance multinucleated giant cell formation *in vitro* (34). However, although relatively few human studies have investigated the effects of α-tocopherol and/or other forms of vitamin E on bone metabolism (35–37), none have shown a negative effect on bone mass or demonstrated an association of vitamin E with bone resorption (36, 38).

In the present study, we focused on skeletally mature rats and aged mice. Our rationale for using older rodents is that they are better models than growing animals for age-related skeletal diseases such as osteoporosis (39, 40) as well as better models for the populations

more likely to be consuming vitamin E supplements. Bone loss occurs in adults as a result of a remodeling imbalance in which bone resorption predominates over bone formation (39). Cancellous bone remodeling has been identified on endocortical and cancellous bone surfaces in skeletally mature rodents but little or no bone remodeling occurs in rodents during growth (41). The slightly higher cancellous bone volume fraction in the 3-month-old α-TTP KO mice reported by Fujita and colleagues (21) may represent a transient net accumulation of bone in the growing KO mice associated with an alteration in bone growth. The stochastics of changes in cancellous bone architecture over time can be readily modeled (42). Due to the extraordinarily rapid rate of cancellous bone turnover in normal young mice (600–1200 % per year) (43), even a transient perturbation in bone turnover during growth could account for the reported 20% difference in trabecular bone volume fraction between WT and α-TTP KO mice. Testing this possibility would require evaluation of α-TTP KO mice across their lifespan. Whatever the precise explanation for the differences in bone volume fraction at 3 months of age observed by Fujita and colleagues (21), the α-TTP KO mice evaluated in this study were clearly not protected from age-related cancellous bone loss.

The serum vitamin E levels in rats fed low, adequate and high levels of vitamin E were reported previously (20). Although low dietary vitamin E did not impact bone architecture in normal weight-bearing rats, it did accentuate the detrimental skeletal effects of hindlimb unloading, a model for skeletal disuse. Compared to adult rats fed low levels of vitamin E, blood levels of vitamin E are even lower in α-TTP KO mice (25). Typical plasma α-tocopherol concentrations in chow-fed α-TTP KO male mice are 0.2 compared to 8 nmol/ml in WT mice. The lower blood levels during growth and longer duration of lower plasma α-tocopherol concentrations could potentially explain the osteopenia observed in mice but not rats.

Compared to WT males, femora from our aged α-TTP KO male mice exhibited lower BMC that is, at least in part, due to reductions in cross-sectional bone volume and cortical bone volume. The normal marrow volume in the KO mice excludes a net increase in bone resorption as an explanation. Instead, these architectural changes are most likely due to reduced radial bone growth in the α-TTP KO mice. Importantly, the lower polar moment of inertia measured in male α-TTP KO mice suggests that instead of being beneficial, severe chronic α-tocopherol deficiency results in decreased bone strength. In contrast to the aged males, no significant differences in femoral BMC or cortical bone microarchitecture were detected in our aged female α-TTP KO mice. Unfortunately, only 3 WT females were available for analysis, precluding definitive conclusions regarding the effect of α-tocopherol deficiency on cortical bone in female mice. Therefore, further research will be required to confirm our preliminary conclusion that lower cortical bone mass in aged α-TTP KO mice is gender-specific.

Severe α-tocopherol deficiency in humans and in mice is associated with ataxia and disturbed neural function (23). Therefore, we cannot rule out the possibility that neuromuscular decline influences the skeletal effects of α-tocopherol deficiency in either growing or aged α-TTP KO mice. However, manipulation of dietary α-tocopherol had no significant effect on bone mass, density, or architecture in adult male rats.

Dietary supplementation with α-tocopherol has been reported to have a bone sparing effect in ovariectomized rodents and to improve fracture healing in rodents (18, 19, 44). Additionally, we have shown that α-tocopherol supplementation provides modest bone protective effects during skeletal unloading (20). However, the results of quantitative histomorphometric analysis in the same study suggested that high levels of α-tocopherol may negatively impact cancellous bone architecture in mature rats (20). This possibility was

not confirmed in the current study by either densitometry or high-resolution μCT. The present analysis shows that increases in dietary α-tocopherol from low to adequate to a level comparable to high-dose supplementation had no significant effects on femoral bone mass, density, cortical microarchitecture or cancellous microarchitecture. The reason for this apparent discrepancy between the initial histomorphometric analysis and the current analysis is not entirely clear. However, the histomorphometric analysis in which we observed lower cancellous bone surface and trabecular number with high vitamin E treatment was based on measurement of single 4 μm thick sections at a region of interest with low cancellous bone volume fraction. In the present analysis, we evaluated a cancellous compartment in 3-dimensions by μCT at a site rich in cancellous bone.

Increasing dietary α-tocopherol levels by 33-fold only increased serum levels of α-tocopherol by ~2.3 fold in rats (20), a finding that is in alignment with human studies evaluating the effects of high α-tocopherol supplementation on plasma α-tocopherol levels (45). Unlike other fat-soluble vitamins, vitamin E does not readily accumulate in the liver because metabolism and excretion are important hepatic regulatory pathways to limit vitamin E concentrations (46).

In summary, our studies in aged α-TTP KO mice with severe α-tocopherol deficiency do not support the hypothesis that α-tocopherol contributes to age-related decline in bone mass in mice. Indeed, sustained α-tocopherol deficiency may lead to a gender-specific decrease in cortical bone mass and strength in males. Furthermore, our studies in skeletally mature rats detect neither a positive nor a negative effect of either low or high dietary α-tocopherol on cortical or cancellous bone.

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Highlights

- High vitamin E status may have negative skeletal effects in growing mice and rats.
- Aged vitamin E deficient α-tocopherol transfer protein (α-TTP) KO males had *lower* cortical, but not cancellous bone mass.
- No differences in bone were detected in adult rats fed low, adequate or high vitamin E diets for 3 months.
- No evidence of negative impact of vitamin E status on bone was apparent in aging rodents.

Table 1

Effect of α-TTP KO on body weight and bone mass and architecture in the femur and lumbar vertebra of 22- to 24-month-old male and female mice.

	Males			Females		
Endpoint	**WT (n=10)**	**KO (n=6)**	**T-Test (P=)**	**WT (n=3)**	**KO (n=10)**	**T-Test (P=)**
Body weight (g)	33.3 ± 0.7	30.8 ± 1.6	0.13	27.8 ± 0.8	23.7 ± 1.2	0.18
Dual Energy X-ray Absorptiometry (DXA)						
Femur						
Bone area (cm^2)	0.509 ± 0.009	0.483 ± 0.010	0.11	0.447 ± 0.011	0.461 ± 0.012	0.54
BMC (mg)	0.025 ± 0.001	0.022 ± 0.001	**0.05**	0.019 ± 0.002	0.020 ± 0.001	0.66
BMD (mg/cm^2)	0.048 ± 0.001	0.045 ± 0.001	0.07	0.041 ± 0.003	0.042 ± 0.001	0.72
Micro-computed Tomography (μCT)						
Femur						
Bone volume (mm^3)	20.3 ± 0.8	18.5 ± 0.6	0.10	16.5 ± 1.4	16.7 ± 0.8	0.89
Femur Diaphysis (cortical bone)						
Cross-sectional volume (mm^3)	0.58 ± 0.01	0.54 ± 0.01	**0.01**	0.46 ± 0.01	0.46 ± 0.02	0.97
Cortical volume (mm^3)	0.23 ± 0.01	0.20 ± 0.01	**0.01**	0.16 ± 0.01	0.17 ± 0.01	0.83
Marrow volume (mm^3)	0.35 ± 0.01	0.34 ± 0.01	0.60	0.30 ± 0.00	0.30 ± 0.02	0.89
Cortical thickness (μm)	199 ± 7	178 ± 6	**0.04**	161 ± 11	166 ± 5	0.65
I_{polar} (mm)	0.73 ± 0.03	0.59 ± 0.02	**0.01**	0.40 ± 0.03	0.42 ± 0.04	0.87
Femur Metaphysis (cancellous bone)						
Bone volume/tissue volume (%)	4.4 ± 0.8	4.4 ± 0.8	0.99	0.5 ± 0.3	0.5 ± 0.1	0.94
Trabecular number (1/mm)	3.2 ± 0.1	3.2 ± 0.1	0.92	2.7 ± 0.1	2.8 ± 0.2	0.85
Trabecular thickness (μm)	45 ± 2	40 ± 1	0.10	31 ± 10	37 ± 2	0.37
Trabecular spacing (μm)	342 ± 9	319 ± 9	0.13	373 ± 16	380 ± 21	0.86
Lumbar Vertebra (cancellous)						
Bone volume/tissue volume (%)	14.9 ± 1.3	16.3 ± 1.5	0.51	10.7 ± 2.1	12.1 ± 1.4	0.61
Trabecular number (1/mm)	4.1 ± 0.2	4.1 ± 0.3	0.93	2.6 ± 0.2	2.6 ± 0.1	0.82
Trabecular thickness (μm)	41 ± 1	43 ± 1	0.41	40 ± 2	45 ± 1	0.14
Trabecular spacing (μm)	248 ± 15	242 ± 20	0.81	407 ± 32	398 ± 20	0.83

Data are mean ± SE

Table 2

Effects of vitamin E intake on femoral bone mass and architecture in skeletally mature male Sprague-Dawley rats.

Treatment	Low Vitamin E (15 IU/kg diet, n=7)	Adequate Vitamin (75 IU/kg diet, n=8)	High Vitamin E (500 IU/kg diet, n=8)	ANOVA (P=)
Dual Energy X-ray Absorptiometry (DXA)				
Proximal Femur (cancellous and cortical bone)				
Bone area (cm^2)	1.66 ± 0.03	1.72 ± 0.02	1.67 ± 0.03	0.12
BMC (mg)	0.364 ± 0.007	0.385 ± 0.009	0.362 ± 0.010	0.16
BMD (mg/cm^2)	0.220 ± 0.005	0.223 ± 0.004	0.217 ± 0.005	0.67
Micro-computed Tomography (μCT)				
Femoral Head (cancellous bone)				
Bone volume/Tissue volume (%)	36.6 ± 1.6	32.5 ± 2.4	34.2 ± 1.8	0.37
Trabecular number (1/mm)	7.6 ± 0.1	7.4 ± 0.1	8.0 ± 0.2	0.07
Trabecular thickness (μm)	91 ± 4	86 ± 4	88 ± 3	0.60
Trabecular spacing (μm)	173 ± 2	173 ± 3	171 ± 1	0.70
Femur Diaphysis (cortical bone)				
Cross-sectional volume (mm^3)	4.36 ± 0.15	4.80 ± 0.19	4.50 ± 0.07	0.12
Cortical volume (mm^3)	2.85 ± 0.09	3.02 ± 0.09	2.83 ± 0.09	0.26
Marrow volume (mm^3)	1.51 ± 0.07	1.78 ± 0.11	1.65 ± 0.06	0.13
Cortical thickness (μm)	813 ± 17	812 ± 16	777 ± 25	0.37
I_{polar} (mm)	26.3 ± 1.7	31.3 ± 2.3	27.2 ± 1.0	0.11