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Effects of Hypertrophy Exercise in Bone Turnover Markers and Structure in Growing Male Rats

Authors
Elena Nebot[1, 2], Virginia A. Aparicio[1, 3], Peter Pietschmann[2], Daniel Camiletti-Moirón[1, 4], Garyfallia Kapravelou[1], Reinhold G. Erben[5], Rosario Martínez[1], Cristina Sánchez-González[1], Jesús M. Porres[1], Juan Llopis[1], María López-Jurado[1], Pilar Aranda[1]

Affiliations
1 CIBM, IMUDS, Department of Physiology, School of Pharmacy, University of Granada, Spain
2 Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Austria
3 Department of Public and Occupational Health, EMGO+ Institute for Health and Care Research, VU University Medical Centre, Amsterdam, The Netherlands
4 Department of Physical Education, Faculty of Education Sciences, University of Cádiz, Cádiz, Spain
5 Department of Biomedical Sciences, Institute of Physiology, Pathophysiology, and Biophysics, University of Veterinary Medicine, Vienna, Austria

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Correspondence
Elena Nebot, PhD
Department of Physiology, School of Pharmacy
University of Granada
Campus universitario de Cartuja s/n
18071 Granada
Spain
Tel.: +34/958/243 882, Fax: +34/958/248 959
enebot@ugr.es

ABSTRACT

The benefits of exercise on bone density, structure and turnover markers are rather controversial. The present study aimed to examine the effects of hypertrophy exercise (HE) on bone. 20 male Wistar rats were randomly distributed in 2 experimental groups, one performing HE and the other untrained over 12 weeks. Plasma parameters, bone mineral content, bone mineral density (BMD), structure, and trabecular and cortical microarchitecture were measured. Femur Mg content was 12 % higher ($p < 0.001$), whereas femur length, dry weight, P content, and aminoterminal propeptides of type I procollagen were lower in the HE group (all, $p < 0.05$). Total BMD and cortical/subcortical BMD were higher (both, $p < 0.01$), whereas total cross-sectional and trabecular areas were lower (both, $p < 0.001$), and cortical area and thickness were lower in the HE (both, $p < 0.05$). Trabecular connectivity density, number, mean density of total and bone volume were higher in the HE (all, $p < 0.05$). Cortical volume fraction and the mean density of total volume of the diaphysis were lower, whereas the cortical volume density was higher in the HE (all, $p < 0.05$). This HE protocol may have beneficial effect on cancellous bone microarchitecture, but it induces low bone formation and is associated with hypogonadism in growing male rats. However, this type of training might be inefficient to maintain appropriate cortical thickness.

Introduction

The final effect of exercise, and more specifically its type, intensity and duration of the stimulus on bone density, structure and turnover markers is rather controversial. The benefits of exercise on bone health are highly discussed by numerous studies in humans and animals [15]. Moreover, benefits of hypertrophy exercise (HE) on both bone mineral content (BMC) and bone mineral density (BMD) have been largely demonstrated [7]. In fact, physical activity has a positive effect on bone mass and microstructure in adolescents [25], adults [14], and the elderly [36]. In animal models, forced exercise or jumping have also shown a positive effect on bone strength [19], as well as some beneficial effects of swim exercise on bone structure, turnover, and strength [21].

Bone adapts to the level of exercise intensity required [27] depending on the mechanical stress generated by exercise. Different exercise modes have different effects on growth and bone mass [24]. Although the most suitable types of sporting activities remain unknown, participation in weight-bearing activities generating high ground reaction forces, mainly if they include jumps, sprints, and rapid changes of directions, seem to have the most evident osteogenic effects during growth [42]. Body mass and exercise act on bone through altered mechanical loading, which modifies bone structure or density by stimulating signaling pathways that regulate bone formation and resorption [31].

Studies on BMC are mainly focused on calcium (Ca) due to the interest on osteoporosis in aging. There are only few studies concerning magnesium (Mg) content of bone [33, 35], and one study addresses zinc (Zn) content in growing mice under exercise [16]. However, Mg and Zn play an important role since the cofactors of enzymes are involved in the energy production [26].

The extrapolation of rodent studies to humans regarding bone status is widely found in the literature due to similar patterns of bone structure and metabolism [1, 4]. The use of rodent experimental models is especially useful on bone metabolism because years, not weeks, would be required to assess BMD changes in humans. The present study aimed to examine the effects of a hypertrophy protocol based on strength training on bone properties in rats, analyzing plasma, urinary and structural bone parameters and specific markers. To the best of our knowledge, this is the first comprehensive study concomitantly analyzing the effects of HE on bone status in rats by BMC, specific bone markers of formation and resorption, parameters of bone density and structure by peripheral quantitative computed tomography (pQCT), and 3D outcomes for trabecular and cortical bone microarchitecture by micro-computed tomography (μCT).

Materials and Methods

Animals and experimental design

A total of 20 albino male Wistar rats were allocated into 2 groups (n = 10 each), one performing the HE protocol and the other sedentary for 12 weeks. The animals, aged 5 weeks and with an initial body weight of 150 ± 8 g, were located in a well-ventilated thermostatically controlled room (21 ± 2 °C), with relative humidity ranging from 40 to 60 %. Animals had free access to distilled water and consumed an AIN-93M diet ad libitum [38].

Body weight was measured weekly in all animals on the same day and hour and after a fasting period of 8 h, and the amount of food consumed by each rat was registered daily. On day 74, a 12-h urine sample from each animal was collected for biochemical analysis.

At the end of the experimental period, and after 8 h of fasting, the animals were anaesthetized with ketamine-xylazine and sacrificed by cannulation of the abdominal aorta. Blood was collected, and the hematocrit was measured with heparin as an anticoagulant using an automated Sysmex KX-21 hematology analyzer (Sysmex Corporation, Kobe, Japan), and centrifuged at 3 500 rpm for 25 min to separate plasma, which was frozen in liquid nitrogen (N) and stored at − 80 °C for subsequent biochemical analysis. The carcass weights were recorded. Carcass weight is the weight of the slaughtered animal's cold body after being skinned, bled and eviscerated and following removal the head, the tail and the feet. Femurs were defleshed. The left femur was fixed in formalin and stored in 70 % ethanol for BMD analysis, and the right femur was frozen for femur ash analysis.

All experiments were undertaken in accordance with the Ethical Standards in Sport and Exercise Science Research [20] as well as the Directional Guides Related to Animal Housing and Care [13]. All procedures were approved by the Animal Experimentation Ethics Committee of the University of Granada (2011-343).

Hypertrophy exercise

The experimental group was trained following a strength training protocol in a motorized treadmill (Panlab Treadmills for 5 rats, LE 8710R) with weights in a bag tied with a cord to the tail. This type of training was chosen in order to reproduce the type of exercise performed by young men interested in gaining muscle mass and strength who are common users of gyms. Our training protocol follows the established principles for human strength training, involving weights, repetitions and sets to maximize muscle gains [12].

The training group exercised on alternate days (3–4 sessions/week). The animals ran at a constant speed of 35 cm/s during the whole experimental period in their dark phase. The training protocol used in the present study has been previously developed and described in depth by Aparicio et al. [3].

Untrained animals were managed identically to trained animals, with the exception of exercise training.

Chemical analyses

The total N content of the quadriceps was determined according to Kjeldahl's method. Crude protein amounts were calculated as N × 6.25. Femurs were weighed after drying them. Bone and feces ashes were prepared by calcination at 500 °C to a constant weight. Ca, Mg and Zn content in bone, and Ca content in feces, were determined by atomic absorption spectrophotometry using a Perkin-Elmer Analyst 300 spectrophotometer (PerkinElmer, Wellesley, MA, USA). Analytical results were validated by standard reference material (Community Bureau of Reference, Geel, Belgium). Phosphorus (P) content was determined using the methodology described by Chen, Toribara and Warner [10]. Urinary pH was analyzed using a bench pH-meter (Crison, Barcelona, Spain). Urinary citrate was measured using a commercial kit (Spinreact, Gerona, Spain). Plasma urea, Ca and alkaline phosphatase were measured using an autoanalyzer (Hitachi-Roche p800, Hoffmann-La Roche, Switzerland). Plasma testosterone concentrations were measured by radioimmunoassay using the Testo-CTK I-125 Kit (DiaSorin, Italy).

Bone turnover markers

Plasma bone markers were determined by enzyme immunoassays. Osteocalcin was determined in the Rat-MID™ Osteocalcin, aminoterminal propeptides of type I procollagen (PINP) in the Rat/Mouse PINP, degradation products from C-terminal telopeptides of type I collagen using a RatLaps™, and tartrate-resistant acid phosphatase (TRACP 5b) was measured using the RatTRAP™ assay (Immunodiagnostics System Ltd., Boldon, UK).

Bone mineral density and structure

Volumetric BMD was measured by pQCT using an XCT Research M + pQCT machine (Stratec Medizintechnik, Pforzheim, Germany). One slice (0.2 mm thick) in the mid-diaphysis of the femur was measured as a cortical bone site, and 3 slices in the distal femoral metaphysis located 1.5, 2, and 2.5 mm proximal to the articular surface of the knee joint were measured as a site rich in trabecular bone. Bone mineral density values of the distal femoral metaphysis were calculated as the mean over 3 slices. A voxel size of 0.070 mm and a threshold of 710 mg/cm^3 were used for calculation of cortical BMD. Trabecular BMD was calculated by using a threshold of 450 mg/cm^3.

μCT analyses

Bone microarchitecture parameters of the distal femora were analyzed by μCT using a μCT-35 device (Scanco Medical AG, Switzerland). The microtomographic imaging system was equipped with

a 0.5 mm focal spot X-ray tube as a source. The long axis of the biopsies was oriented along the rotation axis of the scanner. The X-ray tube was operated at 55 kVp with an intensity of 145 µA. Scan parameters were set with a voxel size of 3.5 µm and a 7.2 mm field of view resulting in, at best, a 5.2 µm spatial resolution at 10 % modulation transfer function (manufacturer specifications). A total of 345 slices in the mid-diaphysis were obtained as a cortical bone site located by selecting the reference line in the mid-diaphysis (50 % of the length of the femur) and then analyzed 4.14 mm distal. For the trabecular parameters in the metaphysis, 231 slices were obtained by selecting the knee joint as a reference line; the relative position of the first slice to the reference line was calculated at 1.5 mm and then analyzed 1.39 mm distal.

Statistical analysis

The results are presented as mean and standard deviation. Differences between untrained and HE groups were analyzed using the Student's t-test with the final body weight, food intake, urine, plasma and bone parameters as dependent variables. The level of statistical significance was set at 0.05.

Results

Body weight, blood and urinary parameters

The effect of HE on final body weight, blood and urinary parameters is presented in ▶ **Table 1**. Final body weight, carcass weight and food intake were lower (9, 7 and 10 %, respectively) (all, $p < 0.01$), and quadriceps N content was 15 % higher in the HE group ($p < 0.01$). Plasma Ca, iron (Fe) and testosterone concentration were lower (14, 15 and 65 %, respectively), and total proteins, alkaline phosphatase, corticosterone and hematocrit were higher in the HE (4, 39, 20 and 4 %, respectively) (all, $p < 0.05$). Urinary citrate excretion was 31 % lower in the HE ($p < 0.05$).

Femur weight, length, mineral content and bone turnover markers

The effect of the HE on femur dry and ash weight and length of femur, BMC and turnover markers is presented in ▶ **Fig. 1** and ▶ **Table 2**, respectively. Femur length and dry weight were lower in the HE group (1 and 3 %, respectively) (both, $p < 0.05$). Femur Mg content was 12 % higher ($p < 0.001$), and P content 7 % lower in the HE ($p < 0.05$), whereas PINP was 28 % lower ($p < 0.05$).

Bone density and area of femur metaphysis and diaphysis

The effect of HE on parameters of femur metaphysis density and area is presented in ▶ **Fig. 2** and ▶ **Table 3**, respectively. Total BMD and cortical/subcortical BMD were higher (3 % for both) (all, $p < 0.01$), whereas total cross-sectional and trabecular areas were lower in the HE group (7 and 10 %, respectively) (both, $p < 0.001$).

The effect of HE on parameters of femur diaphysis density and area is presented in ▶ **Table 3**. Cortical area and thickness were 3 % lower in the HE (both, $p < 0.05$). Bone morphology is presented in ▶ **Fig. 3** as a general overview of the femurs.

Trabecular and cortical bone microarchitecture

The effect of HE on trabecular and cortical bone microarchitecture in femur metaphysis and diaphysis is presented in ▶ **Fig. 4,5**, and ▶ **Table 4**. Trabecular connectivity density, number, and mean density of total and bone volume were higher in the HE group (30, 12, 5 and 2 %, respectively) (all, $p < 0.05$). Cortical bone volume fraction was 3 % lower, the mean density of total volume of the diaphysis 2 % lower, whereas the cortical bone volume density was 0.5 % higher in the HE (all, $p < 0.05$).

Discussion

The main findings of this study were: i) HE had differential effects on cortical and trabecular compartments. ii) HE reduced bone length development, but increased the BMD in the cortical/subcortical area of the metaphysis. Moreover, a decrease in total cross-sectional area was detected, generating a smaller trabecular area. iii) HE decreased cortical area and thickness in bone diaphysis, associated with low bone formation. Therefore, HE might be inefficient to maintain appropriate cortical thickness.

The HE program promotes lower daily food intake and final body weight [23, 43]. The weight loss induced by the HE might be due to the high levels of corticosterone, promoted by the strong stress caused by long-term training [34]. Corticosterone could act by decreasing the food intake and by the lipolytic effect on the adipose tissue [2]. Testosterone was reduced after the HE showing a deficiency in anabolic process, which shows an impaired anabolic condition [28].

Rats were 5 weeks old at the beginning of the experimental period, being in the exponential growth phase. The shorter bone length could be due to the premature epiphyseal closure, with the exercise protocol having started at a development stage in which growth had still not completed. Skeletal growth ends when rats are around 120 days old [11]. Kiiskinen [29] described a delay in the growth of long bones in rats trained with a prolonged exercise protocol, however, Borer and Kelch [8] found mismatched results, showing increases in size with the practice of less intense exercise. The effect of training on growth depends on the intensity and duration of the protocol. If the bone is subjected to strong tension, it sacrifices its potential for growth to maintain its configuration and stability [18].

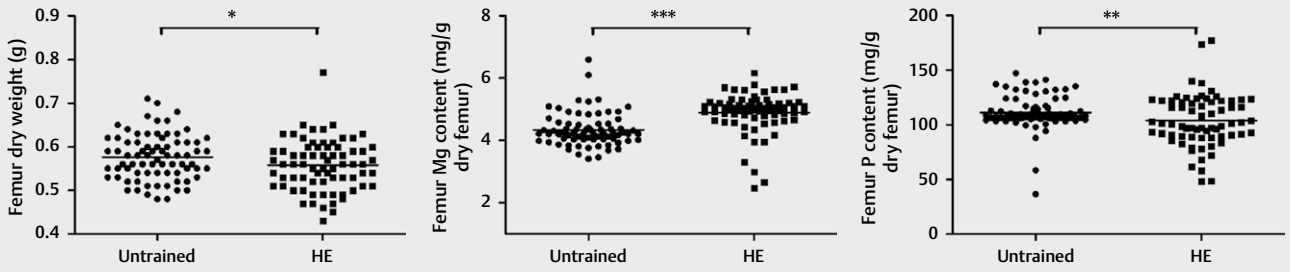
Although trained rats had shorter bones, femur ash weight of the trained rats was similar to the untrained ones. Nonetheless, regarding the changes at the plasma level, HE avoided bone Ca release while maintaining the same levels of this mineral as the untrained group. Aparicio et al. [3] observed that resistance training was effective at enhancing BMC mid- to long-term. However, Bennell et al. [6] did not appreciate differences in the bone of rats developing a similar training protocol.

We observed an increase of the total BMD in the metaphysis, especially at the cortical/subcortical level, but the trabecular BMD did not change. Moreover, the low total cross-sectional area also reduced the trabecular area. Exercise during growth seems to increase the BMD peak in the loading bones of active adolescents compared with sedentary controls [5]. During that period, exercise

▶ **Table 1** Effects of hypertrophy strength exercise on final body weight, blood, urinary and fecal parameters.

	Untrained	Hypertrophy exercise	%	P-value
Final body weight (g)	340.6 (3.8)	310.6 (4.2)	−8.8	**<0.001**
Carcass weight (g)	176.5 (2.0)	164.9 (2.1)	−6.6	**<0.001**
Food intake (g/day)	16.2 (0.2)	14.6 (0.3)	−9.9	**<0.001**
Quadriceps N content (g/100 g DM)	13.1 (0.7)	15.1 (0.8)	15.3	**<0.001**
Blood parameters				
Plasma Ca (mg/dL)	12.3 (0.7)	10.5 (0.6)	−14.4	**0.046**
Plasma Mg (mg/dL)	2.7 (0.3)	2.2 (0.1)	−19.3	0.096
Plasma P (mg/dL)	6.9 (0.3)	6.3 (0.3)	−9.2	0.110
Urea (mg/dL)	29.9 (1.6)	32.2 (1.0)	7.8	0.210
Total proteins (g/dL)	5.4 (0.1)	5.6 (0.1)	3.7	**0.037**
Plasma corticosterone (ng/mL)	806.1 (34.1)	970.6 (35.3)	20.4	**0.004**
Plasma testosterone (ng/mL)	2.7 (0.3)	1.0 (0.2)	−64.7	**<0.001**
Plasma Fe (µg/dL)	176.0 (5.5)	149.7 (6.7)	−15.0	**0.003**
Hematocrit (%)	45.7 (0.7)	47.3 (0.4)	3.5	**0.043**
Urinary parameters				
Ca (mg/L)	2.3 (0.2)	2.3 (0.2)	0.9	0.914
Ca (mg/day)	0.6 (0.1)	0.8 (0.2)	42.4	0.064
Citrate (g/L)	1.6 (0.6)	1.1 (0.3)	−30.9	**0.040**
pH	7.3 (0.2)	6.8 (0.2)	−5.7	0.084
Fecal parameters				
Ca (mg/day)	58.1 (3.2)	60.0 (2.7)	3.3	0.649
P (mg/day)	26.6 (1.5)	28.0 (2.0)	5.2	0.575

Values expressed as mean (standard deviation). N, nitrogen; DM, dry matter



▶ **Fig. 1** Effects of hypertrophy strength exercise (HE) on bone weight and bone mineral content. * $p<0.05$ * * $p<0.01$ * * * $p<0.001$.

acts in a synergic way, with the growth-related bone development leading to a higher bone mass at the end of the pubertal period [17]. The higher bone Mg content could have contributed to the increase of the BMD, but P content did not contribute to the increase of the metaphysis BMD. We would suggest that some amount of the P mobilized from the bone was excreted in urine, because it is known that the parathyroid hormone increases during training [40].

The HE decreased the cortical area and thickness in the diaphysis, which might indicate that the amount of bone formed was not enough to maintain cortical thickness as it adapted to the mechanical load related to the training protocol. However, Bradney, Pearce, Naughton, et al. [9] observed increased femoral cortical thickness in pre-pubertal boys practicing moderate exercise 3 times per week. They described that the growing skeleton is sensitive to exercise and the moderate exercise undertaken before puberty may increase femoral BMD by increasing cortical thickness. In our study, the small cortical area and thickness are in relation to the testosterone level. In a population of young men, testosterone was a positive predictor of cortical bone size at both the radius and tibia, but they might not yet have attained cortical peak bone mass [30], which could explain the positive association of plasma testosterone level with bone size.

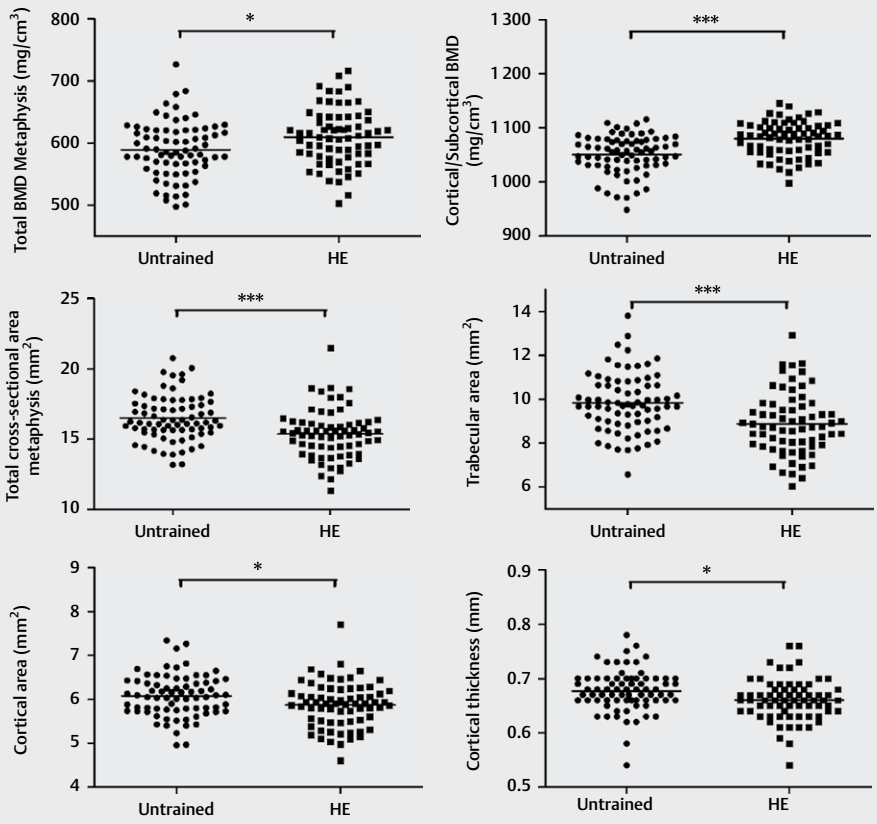
In our study, the specific bone formation marker PINP was lower in the trained rats. Sipos, Rauner, Skalicky, et al. [39] also found decreased osteoblast activity in running rats, whereas the osteoclast activity was elevated. This finding is in line with the increased osteoclast activity and thus diminished BMD of the appendicular skeleton in subjects doing hard exercise [22]. The comparably high osteoclast activity in aged running rats is linked to an age-related pronounced bone resorption [37].

▶ **Table 2** Effects of hypertrophy strength exercise on bone mineral content and turnover markers.

	Untrained	Hypertrophy exercise	%	P-value
Femur length (cm)	3.50 (0.03)	3.46 (0.03)	−1.14	**0.016**
Femur ash weight (g)	0.3779 (0.0042)	0.3680 (0.0046)	−2.6	0.112
Bone mineral content				
Ca (mg/g dry femur)	232.2 (2.1)	232.6 (3.1)	0.2	0.580
Ca (mg/g ash)	355.9 (2.8)	352.5 (4.4)	−1.0	0.775
Mg (mg/g ash)	6.6 (0.1)	7.4 (0.1)	12.1	**<0.001**
P (mg/g ash)	170.2 (2.9)	158.0 (4.6)	−7.1	**0.028**
Zn (µg/g dry femur)	321.0 (8.7)	303.5 (8.4)	−5.4	0.147
Zn (µg/g ash)	489.7 (13.5)	461.5 (13.5)	−5.8	0.141
Specific bone turnover markers				
Osteocalcin (ng/mL)	212.8 (14.7)	184.7 (13.5)	−13.2	0.167
PINP (ng/mL)	17.9 (2.1)	12.8 (1.5)	−28.3	**0.050**
C-terminal telopeptides of type I collagen (ng/mL)	14.2 (1.1)	13.0 (0.7)	−8.5	0.352
TRACP 5b (UI/L)	2.99 (0.01)	2.97 (0.01)	−0.7	0.466

Values expressed as mean (standard deviation). PINP, aminoterminal propeptides of type I procollagen; TRACP 5b, tartrate-resistant acid phosphatase

Additional parameters are shown in ▶ **Fig. 1**



▶ **Fig. 2** Effects of hypertrophy strength exercise (HE) on selected pQCT parameters of bone density and area in femur (metaphysis and diaphysis). * $p<0.05$ * * * $p<0.001$.

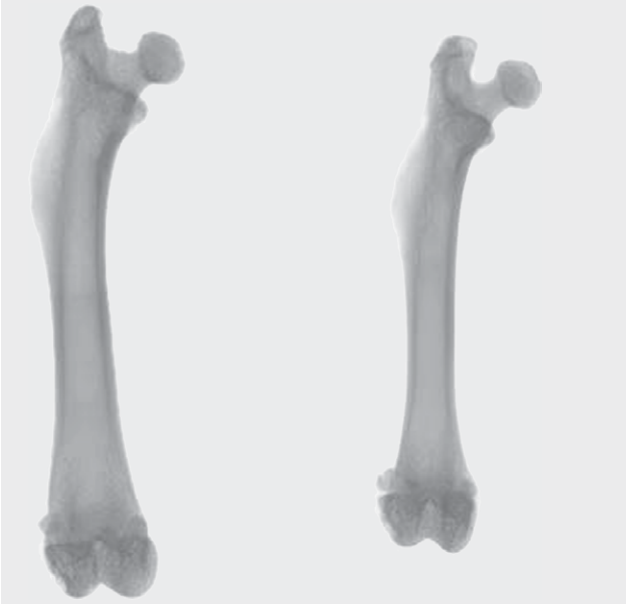
Despite the low levels of the specific bone formation marker, we found higher trabecular connectivity density, number, and greater mean density of total and bone volume in the metaphysis. Ma, Turpeinen, Silvennoinen, et al. [32] also described higher trabecular microarchitectural parameters in voluntary wheel-running obese mice. This type of exercise decreased all the cortical bone

▶ **Table 3** Effects of hypertrophy strength exercise on selected pQCT parameters of bone density and area in femur (metaphysis and diaphysis).

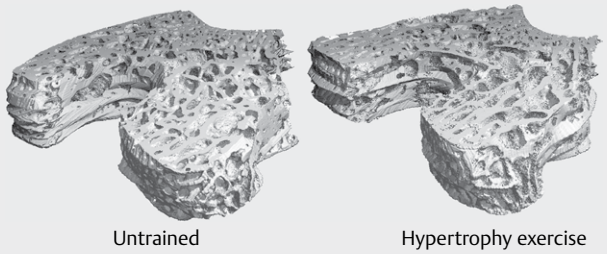
	Untrained	Hypertrophy exercise	%	P-value
Metaphysis				
Trabecular BMD (mg/cm^3)	256.1 (7.4)	243.1 (7.5)	−5.1	0.219
Cortical/Subcortical area (mm^2)	6.6 (0.1)	6.5 (0.1)	−2.7	0.089
Diaphysis				
Total BMD Diaphysis (mg/cm^3)	905.3 (5.0)	896.0 (7.1)	−1.0	0.288
Total cross-sectional area (mm^2)	9.8 (0.1)	9.6 (0.1)	−2.2	0.110
Cortical BMD (mg/cm^3)	1388.4 (1.5)	1389.9 (1.6)	0.1	0.477
Periosteal perimeter (mm)	11.1 (0.1)	11.0 (0.7)	−1.3	0.115
Endocortical perimeter (mm)	6.9 (0.04)	6.8 (0.06)	−0.4	0.657

Values expressed as mean (standard deviation). pQCT, peripheral quantitative computed tomography; BMD, bone mineral density

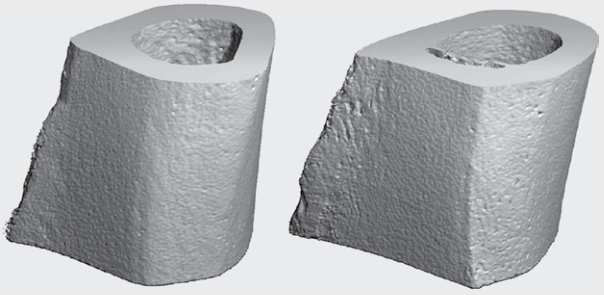
Additional parameters are shown in ▶ **Fig. 2**



▶ **Fig. 3** Representative radiographs of femurs of untrained **a** rats and rats that performed hypertrophy strength exercise **b**.



▶ **Fig. 4** Representative micro-CT images of bone metaphysis in untrained **a** rats and rats that performed hypertrophy strength exercise **b**.



▶ **Fig. 5** Representative micro-CT images of bone diaphysis in untrained **a** rats and rats that performed hypertrophy strength exercise **b**.

parameters but increased the trabecular mineral density. At the femoral diaphysis, our HE program led to a lower bone volume fraction and mean density of total volume, whereas we observed a higher bone volume density in the trained rats.

The results of our investigation in rodents may be of relevance for the understanding of both beneficial and/or adverse effects of HE on bone health in humans. When extrapolating these findings to humans we would predict a beneficial effect on cancellous bone microarchitecture, biochemical evidence of hypogonadism and lower bone formation in sporty male adolescents and adults [41].

Conclusion

Our training protocol has a beneficial effect on cancellous bone compartment, induces a generalized low bone turnover and it is associated with hypogonadism. Thus, this type of training might be inefficient to maintain appropriate cortical thickness.

Limitations and strengths

The present study has some limitations that need to be mentioned. First, the current physiological results obtained in growing rodents must be confirmed in young adult human subjects. Second, an al-

▶ **Table 4** Effects of hypertrophy strength exercise on 3D outcomes for trabecular and cortical bone microarchitecture.

	Untrained	Hypertrophy exercise	%	P-value
Metaphysis				
TV (mm^3)	29.0 (0.3)	28.8 (0.5)	−0.7	0.761
BV (mm^3)	11.8 (0.2)	12.0 (0.2)	2.0	0.436
BV/TV (1)	0.4 (0.005)	0.4 (0.006)	2.4	0.098
Conn. D ($1/mm^3$)	426.2 (24.9)	552.4 (48.6)	29.6	**0.025**
SMI (1)	3.1 (0.2)	3.6 (0.3)	14.5	0.196
Tb.N (1/mm)	10.2 (0.4)	11.4 (0.4)	12.5	**0.020**
Tb.Th (mm)	0.087 (0.001)	0.086 (0.001)	−1.3	0.436
Tb.Sp (mm)	0.118 (0.005)	0.103 (0.006)	−12.7	0.051
Mean density of TV (mg HA/ccm)	369.2 (5.2)	387.4 (7.2)	4.9	**0.042**
Mean density of BV (mg HA/ccm)	860.9 (3.6)	874.8 (3.8)	1.6	**0.010**
Diaphysis				
TV (mm^3)	40.3 (0.5)	40.3 (0.7)	−0.2	0.923
BV (mm^3)	27.0 (0.4)	26.3 (0.5)	−2.5	0.244
BV/TV (1)	0.67 (0.004)	0.65 (0.004)	−3.0	**0.013**
Mean density of TV (mg HA/ccm)	716.5 (4.6)	701.6 (5.8)	−2.1	**0.047**
Mean density of BV (mg HA/ccm)	1065.4 (1.5)	1070.6 (1.7)	0.5	**0.023**

Values expressed as mean (standard deviation). TV, total volume; BV, bone volume; BV/TV, bone volume density; Conn. D, connectivity density; SMI, structure model index; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular spacing; HA, hydroxyapatite

ternative approach to assess bone remodeling might have been dynamic histomorphometry or tartrate-resistant acid phosphatase (TRAP) or hematoxylin and eosin staining. Third, mechanistic studies were beyond the scope of the current manuscript. On the other hand, we employed several specific and independent markers of bone formation and resorption. Moreover, bone parameters were analyzed with state-of-the-art pQCT and μCT devices, and we have assessed a large number of structural parameters. It is important to highlight the great importance of these devices together with the different methodological approaches used to determine the bone markers and the structural properties. A further important advantage of this study is that the exact amount of Ca, P, Mg and Zn present in the samples was measured directly.

Furthermore, it should be emphasized that this study for the first time found beneficial effects of HE on trabecular microstructure, despite the smaller femoral bone size.

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Conflict of interest

The authors declare that they have no conflict of interest.

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