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PROLONGED PERFORMANCE OF A HIGH REPETITION LOW FORCE TASK INDUCES BONE ADAPTATION IN YOUNG ADULT RATS, BUT LOSS IN MATURE RATS

Vicky S Massicotte¹, Nagat Frara¹, Michele Y Harris¹, Mamta Amin¹, Christine K Wade², Steven N Popoff¹, and Mary F Barbe^{1,3,*}

¹Department of Anatomy and Cell Biology, Temple University School of Medicine, 3500 North Broad St., Philadelphia, PA 19140, USA

²Department of Physical Therapy, Thomas Jefferson University, Philadelphia, PA, 19107, USA

³Department of Physical Therapy, Temple University, Philadelphia, PA, 19140, USA

Abstract

We have shown that prolonged repetitive reaching and grasping tasks lead to exposure-dependent changes in bone microarchitecture and inflammatory cytokines in young adult rats. Since aging mammals show increased tissue inflammatory cytokines, we sought here to determine if aging, combined with prolonged performance of a repetitive upper extremity task, enhances bone loss. We examined the radius, forearm flexor muscles, and serum from 16 mature (14–18 mo of age) and 14 young adult (2.5–6.5 mo of age) female rats after performance of a high repetition low force (HRLF) reaching and grasping task for 12 weeks. Young adult HRLF rats showed enhanced radial bone growth (e.g., increased trabecular bone volume, osteoblast numbers, bone formation rate, and mid-diaphyseal periosteal perimeter), compared to age-matched controls. Mature HRLF rats showed several indices of radial bone loss (e.g., decreased trabecular bone volume, and increased cortical bone thinning, porosity, resorptive spaces and woven bone formation), increased osteoclast numbers and inflammatory cytokines, compared to age-matched controls and young adult HRLF rats. Mature rats weighed more yet had lower maximum reflexive grip strength, than young adult rats, although each age group was able to pull at the required reach rate (4 reaches/min) and required submaximal pulling force (30 force-grams) for a food reward. Serum estrogen levels and flexor digitorum muscle size were similar in each age group. Thus, mature rats had increased bone degradative changes than in young adult rats performing the same repetitive task for 12 weeks, with increased inflammatory cytokine responses and osteoclast activity as possible causes.

*Corresponding Author: Mary F. Barbe, PhD, Department of Anatomy and Cell Biology, Temple University School of Medicine, 3500 North Broad St., Philadelphia, PA 19140, 215-707-6422 phone, mary.barbe@temple.edu.

7. Conflict of Interests

None of the authors have any conflicts of interest issues to declare.

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Keywords

Repetitive strain injury; musculoskeletal; bone; inflammation; microCT; aging; radius

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1. Introduction

Work-related musculoskeletal disorders (WMSDs), also known as repetitive strain injuries and work-related overuse injuries, are the most reported types of occupational illnesses. According to the U.S. Bureau of Labor Statistics, WMSDs account for 34% of lost workday injuries and illnesses in the US, and cost on the order of \$100 billion annually [1, 2]. Hand and wrist injuries are prevalent in occupations requiring upper extremity repetitive tasks, particularly with advancing age [3, 4]. People above 35 years of age have increased numbers of days away from work (an indicator of illness severity), and people aged 45–54 have a higher incidence of work-related injuries [5]. Since age is a risk factor for these disorders, and since the average age of the American and international work force is rapidly increasing due to economic realities, more WMSD cases are predicted [6–9]. Yet, the underlying mechanisms of these disorders are incompletely understood. The 2010 National Manufacturing Agenda of the National Institute of Occupational Safety and Health cites the need for etiologic research in determining the contribution of biomechanical mechanisms towards the development of tissue injury and musculoskeletal disorders [10].

It is well known that chronic cyclical tissue overload, in general, affects bone morphometry [11–16]. A small number of studies have examined changes occurring in upper extremity bones as a consequence of prolonged performance of occupational tasks. Increased incidence of hand/wrist osteoarthritis and reduced bone mass has been identified in female dentists and teachers with heavy or one-sided hand workloads [17–19]. Bone scans of patients with upper extremity MSDs show increased blood flow and pooling (suggestive of inflammation) in affected forearm bones [20, 21]. Such changes can increase osteopenia and fracture risk. Premenopausal women with carpal tunnel syndrome, a type of upper extremity MSD, have decreased bone mineral density in the distal radius, ulna, and metacarpal bones, compared to control subjects [22, 23]. Although still under investigation, such bone changes may be due to increases in inflammatory cytokines occurring with prolonged performance of occupational tasks [24–29], since similar cytokine increases in other chronic inflammatory conditions and *in vitro* stimulate osteoclastogenesis and activity and impair osteoblast differentiation [30–35].

Aging is also linked to bone degradative changes and decreased bone mass [36–39]. One possible cause may be the increased inflammatory cytokine levels and responses in tissues of aging mammals, compared to young adults [40–44]. Although not yet examined to date, the combination of both aging and prolonged performance of an upper extremity repetitive task, may also enhance forelimb bone osteopenia and increase fracture risk.

We have developed an operant rat model of upper extremity WMSDs in which rats learn a reaching and grasping task for a food reward. We have shown that prolonged repetitive tasks leads to either trabecular bone adaptation or pathological bone changes, dependent on repetition rate, force load, and duration of task [29, 35, 45, 46]. Performance of a negligible

or low force repetitive reaching and grasping task for 6–12 weeks by young adult rats induces radial bone adaptation, concomitant with transient increases in bone inflammatory cytokines [45, 46]. In contrast, performance of a high repetition high force (HRHF) task for 12 weeks by young adult rats leads to significant losses in trabecular bone volume and cortical thinning in the radius, concomitant with higher and more sustained increases in inflammatory cytokines [46–48]. Although we have yet to examine the response of bones to prolonged performance of a repetitive task in mature rats (14–18 mo of age), similar aged rats have increased inflammatory cytokines in serum and tendons after a 12-week high repetition low force (HRLF) task, compared to young adult rats performing the same task [43, 49]. The inflammatory cytokines were the same that affect bone cell homeostasis and induce net bone loss (interleukin-1 β and tumor necrosis factor α) [32, 33, 50].

Thus, the effects of performing repetitive tasks on bone architecture needs further evaluation to assess if aging combined with prolonged performance of a moderate demand repetitive task enhances bone inflammation and loss, compared to young adult rats performing the same task. We sought to determine if mature adult rats (14–18 mo of age) performing an upper extremity high repetition low force (HRLF) task for 12 weeks have increased bone degradative changes, compared to young adult rats performing the same task (2.5–6.5 mo of age) in parallel with task- and age-related increases in bone inflammatory cytokines. We also examined for the first time, the effects of prolonged task performance on cortical microarchitecture at the mid-diaphyseal region of the radius. We hypothesized that aging combined with prolonged HRLF task performance would negatively affect both trabecular and cortical microarchitecture in the radius due to increased osteoclastic activity and reduced osteogenic adaptation, compared to young adult task rats (which will show increased trabecular bone volume and other forms of bone adaptation).

2. Materials and Methods

2.1 Animals and Experimental Design Overview

The Temple University Institutional Animal Care and Use Committee approved all experiments in compliance with NIH guidelines for the care and use of laboratory animals. Female rats were used in this study because: 1) Human females have a higher incidence of work-related musculoskeletal disorders than males [4, 51–53]; and 2) for comparison to bone data from our past studies on female rats using this model [29, 45–47, 54]. All rats were housed in a central animal facility in separate cages with a 12 hour light:dark cycle, and free access to water and environment enrichment toys. All rats were provided with equal rations of food reward pellets and Purina rat chow daily. All rats were inspected weekly and again post-mortem for presence of illness or tumors in order to reduce confounders for serum cytokine increases (if present, rats were excluded). To further reduce illness-related confounders, additional sentinel rats were examined for presence of viral or other infections as part of the regular veterinary care (none were detected).

As shown in Figure 1A, 55 young adult rats (2.5 months of age at the onset of experiments, and 6.5 months of age at completion) were randomly assigned to 3 groups: normal control rats (NC, n=20), food restricted control rats (FRC, n=21), and 12 weeks high repetition low force task rats (HRLF, n=14). Fifty-four retired-breeders (14 months of age at the onset of

experiments, and 18 months of age at completion) were randomly assigned to similar groups. However, 7 mature rats were excluded from the study due to renal failure, presence of tumors, or mortality. Thus, only 47 mature rats were included by the end of the study: NC, n=18; FRC, n=13; HRLF, n=16 rats (Fig. 1A). The total number of rats by study end was 102.

The experimental design was as follows (diagramed in Fig. 1A). First, all rats were handled for 10 minutes/day for 1 week. Then, all but normal control rats were food-restricted for 5–7 days to no more than 10–15% less than their naive weight to initiate interest in food reward pellets. After that week, all food-restricted rats (FRC and HRLF rats) were provided extra rat chow to gain weight quickly back to only 5% less than the age-matched NC rats, where they were maintained for the duration of the experiment. All rats were weighed weekly and food allotments were adjusted accordingly so that young adult rats were gained weight across the weeks of the experiment as a consequence of normal growth, and so that mature rats maintained weight. HRLF task rats first trained to learn the task in an approximately 4-week training period of 15 min/day for 5 days/week (described further below), before moving on to performing the HRLF task for 2 hours/day, 3 days/week for 12 weeks (described further below). The NC and FRC rats rested until euthanasia at age-matched time points as HRLF rats.

Data from FRC rats was compared to NC rat data. No significant differences were observed between these two groups for any outcome analyzed. Therefore, results of FRC and NC groups were combined into a single control (C) group for each age group: Young Adult C=41; Mature C=31.

2.2 Task Apparatuses

Custom-designed operant behavior chambers were used (Fig 2A–G), as previously described [46, 48, 55]. Briefly, rats were trained to reach through a portal located at shoulder height (Fig. 2A–C) to isometrically pull on a vertical 1.5 mm metal bar attached to a load cell (Futek Advanced Sensor Technology, Irvine, CA) positioned 2.5 cm outside of the chamber wall (Fig. 2D), for a food reward (Fig. 2E). Light and auditory indicators (Med Associates) lasting 5 seconds each, cued the animal to attempt a reach (Fig. 2F,G). The load cell output was interfaced with a signal conditioner (Analog Devices, Norwood, MA), which amplified and filtered the signal before it was sampled digitally at 100 Hz with Force Lever software (Med Associates, St. Albans, VT). The metal lever bar and its load cell were interfaced with custom written Force-Lever software that allowed a choice of a set force level that the rat had to pull and then hold for at least 50 milliseconds (ms) [56], before a food reward was provided (version 1.03.02, Med Associates, St. Albans, VT). The force transducer was encased in a custom made metal holder bolted to the floor of the chamber (Fig. 2G). The rats were trained to grasp the force lever bar and pull toward the chamber wall at a force effort of 15% of their mean maximum pulling force (30 force-grams = 0.23 Newtons) for at least 50 milliseconds [56]. If reach and force criteria for the task (defined below) were met within a 5 second cueing period, a 45 mg food pellet was dispensed via a tube into a trough located at floor height for the animal to lick up (Fig. 2E–G). A 1:1 mix of purified grain and banana-flavored food reward pellets (Bioserve, NJ, USA) was used throughout the study.

2.3 Training Regimen

HRLF rats were first trained to learn the reaching and handle-pulling task during a 4-week period for 10 min/day, 5 days/wk, in which they ramped upwards towards the HRLF task level force. During this period, the rats moved through several stages of training, as previously described [29, 57]. Briefly, in week 1 of training, they were placed in a plastic box outfitted with Plexiglas portal and plastic trough located at shoulder height, and introduced to 45 mg food reward pellets. When rats learned to reach (without a specified reach rate) into the trough for the food pellets (typically 3 days), they were moved to operant chambers, where they learned to pull the force lever attached to a force transducer. In week 1, rats learned to grasp and pull on the force lever bar with a negligible force without any specified repetition rate, for a food reward. In week 2, rats were required to pull at 11 grams of force (5% of their maximum pulling force). By the beginning of week 4, they were required to pull at 15% of their maximum pulling force (30 grams of grip force), without any specified repetition rate. By the end of the 4-week training period, rats were able to perform the HRLF task of four reaches/min at 15% of their maximum pulling force. Trained rats reached this HRLF level only during the last 3–4 days of their 4th week of training at 10 min/day, 5 days/week.

2.4 Task Regimen

Trained rats went on to perform the high repetition low force (HRLF) task at a low force at a reach rate of 4 reaches/minute, for 2 hours/day, four 30 min sessions/day, 3 days/week, for a total of 12 weeks. The daily task was divided into four 30-minute sessions separated by 1.5 hours each in order to avoid satiation. The rats had to grasp the force handle and exert an isometric pull for at least 50 milliseconds with a graded force effort of $15\% \pm 5\%$ of their naïve maximum voluntary pulling force. Rats were allowed to use their preferred limb to reach (the “reach” limb) (Fig. 2C,D).

2.5 Determination of Voluntary Reach Performance Behaviors and Grip Strength

Force lever data were recorded continuously during task sessions for later calculation of dependent variables (reach rate, reach phase time, grasp phase time, duration of task performance, and voluntary grasp force on the lever bar) via an automated script (MatLab; Mathworks, Natick, MA), as previously described [55, 58, 59]. Briefly, reach rate was the mean number of reaches performed per minute. Reach phase time was defined as the mean time spent in seconds performing the reaching task on a given day. Grasp phase time was the mean time spent in seconds pulling on the force lever bar for all reaches on a given day. Duration of task performance was the mean number of minutes per day that a rat participated in the task (expressed as a percentage of the target of 120 minutes). Voluntary grasp force was mean grasp force on the lever bar, and was required to be a graded effort of 15% of their mean maximum grip strength. Data for each variable was calculated on the last day of week 12 for eight young adult HRLF rats and thirteen mature HRLF rats, and averaged across the four sessions. Force lever data could not be determined for control rats, as they did not perform the task.

Maximum reflexive grip strength was measured in all C and HRLF animals using a rat grip strength recording unit (Ugo-Basile-Grip-Strength-Meter, Stoelting, Wood Dale, IL), by an

examiner that was naïve to group assignment. This was assayed at baseline (the naïve time point), after food restriction (1 week later), after training (week 0, which was 4 weeks later), and every 3 weeks thereafter. The test was repeated 5 times/trial, and the maximum grip strength per trial for the preferred reach limb is reported.

2.6 Analysis of Serum Bone Turnover Markers and estrogen using ELISA

All rats were euthanized with an overdose of sodium pentobarbital (Nembutal; 120 mg/kg body weight) at 18 hours after completion of the final task session to avoid any “exercise” induced changes in blood or bone cytokine profiles, as previously described [43]. Blood was collected by cardiac puncture using a 23-gauge needle, stored on ice for 30 min, then centrifuged at 1,800 g for 20 min at 4°C, flash-frozen, and stored at –80°C until analyzed for serum osteocalcin (Elisa kit from Immunodiagnostic systems, Rat-MID Osteocalcin EIA # AC-12F1), a marker of bone formation from: young adult NC rats, n=6; young adult FRC rats, n=6, young adult HRLF rats, n=8; mature NC rats, n=7; mature FRC rats, n=7, mature HRLF rats, n=13. Estradiol levels (Elisa kit from Calbiotech SKU: ES180S-100) were also analyzed in young adult rats (n=31) and mature rats (n=10). Osteocalcin is a non-collagenous protein of the bone matrix that is synthesized by osteoblasts, making the measure of serum an indicator of bone formation. Serum estradiol levels were analyzed to determine if age-related hormonal changes were contributing to bone changes. The individuals carrying out these ELISA assays were naïve to group assignment and age.

2.7 Morphological analyses

Approximately one-half of the animals were utilized for microCT and histological analyses: young adult NC rats, n=10; young adult FRC rats, n=7; young adult HRLF rats, n=8; mature NC rats, n=9; mature FRC rats, n=6, and mature HRLF rats, n=8. These animals were euthanized by lethal overdose (Nembutal, 120 mg/kg body weight), perfused transcardially with 4% paraformaldehyde in 0.1M PO₄ buffer (pH 7.4), forelimb bones were collected, cleaned of soft tissues, and stored in phosphate buffered saline with sodium azide until microCT and histological analysis of the radius and ulna from the reach limbs. Young adult MicroCT analyses was performed on n=5–9/group and then for histomorphometry (described below). The remaining bones of this subcohort were processed for histomorphometry.

2.7.1. MicroCT Imaging and Analysis—MicroCT analysis of bones from n=5–9/group was performed according to recent guidelines [60] and as previously described [29, 57]. Skyscan volume rendering software (CTVox) and volume rendering software (CTVol) was used to render the 3D models and transaxial sections, shown in Figure 1B and C,D, respectively. The forelimb bones (radius and ulna) of each rat’s reach limb were scanned from their distal ends proximally towards the elbow, to a diaphyseal site that was 10 mm proximal from the growth plate, as shown in Figure 1B, using a SkyScan 1172, 12 megapixel, high-resolution cone-beam microCT scanner (Bruker, Kontich, Belgium), using the following settings: a pixel resolution size of 5.89 µm, x-ray source spot size of 300 nm, Al 0.5mm filter, voltage of 59 kV, current of 167 µA, rotation step of 0.40°, frame averaging of 5. Each scan was approximately 45 minutes per set of forelimb bones. During reconstruction of the images using cone-beam reconstruction software based on the

Feldkamp algorithm (Skyscan NRecon), a ring artifact correction of 10, and a beam hardening correction of 60% were applied to all samples. This process yielded more than 2700 tomographic sections, 5.89 μm in thickness, in the axial plane, for each set of forelimb bones.

Since we have previously shown that performance of this reaching and grasping upper extremity task induces greater changes in the radius than in the ulna, we focused our morphological assays on the radius [29]. Using the Skyscan CT Analyzer (CTAn) software, two regions of interest (ROI) of the radius were delineated using a region of interest tool, and then binarized separately. The metaphyseal trabecular bone ROI was delineated from 1.5 to 2.5 mm below the center of the distal growth plate (Fig 1B). The volume of interest (VOI) for the trabecular microarchitecture variables was a consistent circle shape within a few pixels of the endocortical margin (Fig 1C). The cortical diaphyseal ROI was delineated from 5 to 5.5 mm below the distal growth plate (Fig 1B). The VOI was defined by circling the outside of the cortical bone surface (Fig 1D). The saved data sets were segmented into binary images. Because of a low noise and the relative high resolution of the data sets, we used simple global thresholding methods. For trabecular bone, an upper threshold of 255 and a lower threshold of 95 were used to delineate each pixel as “bone” or non-bone. For the cortical analysis, the upper threshold remained 255 while the lower threshold was increased to 125. Despeckling was performed at a 2D setting of 50 pixels prior 2D and 3D analyses, and the shrink-wrap feature was set to cover holes of more than 50 pixels for the cortical analysis. The person carrying out the microCT analyses was naive to group assignment and age.

Trabecular bone morphometric traits were computed from binarized images using direct 3D techniques that do not rely on prior assumptions from the underlying structures. Trabecular bone volume per total volume (BV/TV), mean trabecular thickness (Tb.Th), mean trabecular number (Tb.N), mean trabecular separation (Tb.Sp), and degree of anisotropy (DA) indices were measured (in DA, 0 represents isotropic organization). Also, the structure model index (SMI) was measured to determine the prevalence of plate- or rod-like trabecular structures, where 0 represents “plates”, 2 represents “rods” and 3 “cylinders” [61].

Cortical bone morphometric traits were computed from binarized images using 2D techniques. Total cross-sectional area inside the periosteal envelope (Tt.Ar), cortical bone area (Ct.Ar), cortical area fraction (Ct.Ar/Tt.Ar), marrow area (Ma.Ar), periosteal perimeter (Ps.Pm), and average cortical thickness (Ct.Th) were analyzed. Additionally, data about closed pore volume (Po.V (cl)) was gathered.

For assay of bone mineral density (BMD), a set of 3 calcium hydroxyapatite phantoms of rat bones was scanned (partially in air; partially in water) using the same settings as for the bones. Hounsfield units for the phantoms, air and water were calculated and then used to compute volumetric BMD of the segmented metaphyseal trabeculae with surrounding marrow space for the radius of animal analyzed using microCT. BMD of the mid-diaphyseal cortical bone (segmented from marrow space) was also calculated.

2.7.2 Histomorphometry—Bones that had been assayed for microCT, as well as from additional rats as indicated above in section 2.7, were used for histomorphometry according to the recommendations of the American Society for Bone and Mineral Research [62, 63]. Half of the bones from each group underwent plastic embedding, and half underwent paraffin embedding. The individuals carrying out all histomorphometric analyses and cell counts were blinded to treatment.

To measure dynamic bone formation parameters, calcein (i.p., 10 mg/kg body weight) had been previously injected at 9 and 2 days before euthanasia. Then, fixed forearm bones underwent plastic embedding, in which they were first preserved in 70% ethanol, before being embedded, undecalcified, into methyl methacrylate resin. These bones were sectioned into 5 μm longitudinal sections, placed onto charged slides (Fisher Scientific, Tissue Path Superfrost Slides), and dried at 60°C overnight. Unstained plastic-embedded sections of the radius were used for dynamic histomorphometric analysis (i.e., measurement of calcein labeling) and static histomorphometric analysis to assess changes in bone structure and remodeling. Trabeculae were assayed in the distal metaphysis beginning 150 μm below the chondro-osseous junction of the secondary spongiosa and 50 μm in from the surrounding cortical bone using a 20x objective and image analysis software (BIOQUANT Osteo II, Bioquant Image Analysis Corp., Nashville, TN), and using methods described by Parfitt et al [63]. Bone formation rate (BFR) was assessed in unstained calcein-labeled sections by measuring single-labeled surface for single perimeter (sL.Pm), double-labeled surface (dL.Pm), and the interlabel distance in the dL.Pms. From this, mineral apposition rate (MAR, mM/day) and bone formation rate normalized to bone surface (BFR/BS) were calculated [64].

Unstained plastic embedded sections were also analyzed using both bright field and polarized light microscopy to visualize if woven bone formation had occurred. The area of a field at 300 x magnification containing woven bone volume was chosen using Bioquant manual thresholded selection methods, as was the area of bone volume. Woven bone volume/total bone volume (Wo.V./BV. %) was reported.

Adjacent sections were deplasticized and stained with Masson's trichrome to detect osteoblasts, von Kossa with toluidine blue counterstain for the staining of mineralized bone, or were processed for tartrate-resistant acid phosphatase (TRAP) staining to detect osteoclasts, as per manufacturer's instructions (Sigma-Aldrich Acid Phosphatase kit 387A). Osteoid volume per bone volume (OV/BV), osteoid width (O.Wi), osteoid surface per bone surface (OS/BS), and osteoblast numbers per bone surface (Ob.N/BS) on trabecular surfaces were determined in Masson's trichrome stained sections. Numbers of osteoclasts (multinucleated TRAP+ cells) per bone surface (N.Oc/BS) were counted in TRAP stained sections on trabecular surfaces in the secondary spongiosa, using a Nikon E800 microscope interfaced with an image analysis program (Bioquant Osteo 2012 v12.1). Since TRAP is also known to stain macrophages and other mononuclear cells of the monocyte-macrophage lineage [65–68], only large cells with 3 or more nuclei were counted, as previously described. Osteoclast resorption spaces (number per bone surface area; defined as spaces in cortical bone in which osteoclasts were present) and arterial spaces were counted in mid-diaphyseal cortical bone.

Flexor digitorum muscles were collected from the same rats and limbs, postfixed by immersion overnight in 4% paraformaldehyde in phosphate buffer (pH 7.4). A cross-sectional piece of the entire flexor digitorum muscle, of 1.5 mm in thickness, was removed with a scalpel from the mid-region of the muscle at its widest point. This cross-sectional piece was equilibrated in sucrose for 3 days, and then cryosectioned into cross-sectional slices of 14 μm thick each. These sections were mounted on slides, dried and stained with H&E. An image analysis program (Bioquant) was used to measure the cross-sectional area (CSA) of the entire muscle belly in mm^2 using a 2X objective. Three sections were counted per histomorphometric analysis parameter.

2.8 Analysis of Bone Inflammatory Cytokines using ELISA

After collection of blood, the radial and ulnar bones were collected and flash-frozen from: young adult NC rats, n=8; young adult FRC rats, n=8, young adult HRLF rats, n=6; mature NC rats, n=9; mature FRC rats, n=7; mature HRLF rats, n=8. Bones were powdered, homogenized and assessed for interleukin (IL)-1 β , tumor necrosis factor -alpha (TNF- α), IL-6 and IL-10 using commercially available ELISA kits (BioSourceTM, Invitrogen Life Sciences, CA), as described previously [54]. Each sample was run in duplicate in a blinded manner. ELISA assay data (pg cytokine protein) were normalized to μg total protein, which was determined using a bicinchoninic acid (Pierce BCA Protein assay #23225. Thermo Scientific, MA). The individual carrying out these ELISA assays was blinded to treatment.

2.9 Statistical Analyses

An unpaired, two-tailed, t-test was used to compare the reach performance variables in young adult versus mature HRLF rats. For the microCT, histomorphometric and ELISA data, mixed-model two-way and one-way ANOVAs were performed to determine differences between and among groups. Repeated measures ANOVAs were used to compare body weight and grip strength data across weeks. For each ANOVA, the Bonferroni post-hoc method for multiple comparisons was used. Adjusted p-values are reported, and after adjustment, a p-value of <0.05 was considered statistically significant. Two-tailed Pearson's correlation tests were used to compare to trabecular BV/TV with serum estrogen levels, and with the animal weights at euthanasia. Data are expressed as mean $\pm$ standard error of the mean (SEM). Two-way ANOVA results and significant posthoc findings are listed with the individual graphs.

3. Results

3.1. Voluntary task parameters were similar in each age group of HRLF rats

Each age group of HRLF rats was able to perform the task at the expected reach and force parameters (Table 1). For example, each age group was able to pull at the required submaximal 15% of mean voluntary pulling force (30 grams of voluntary grip force).

3.2 MicroCT analysis shows trabecular bone adaptation in the radius to the HRLF task in young adult rats, yet degradative changes in mature HRLF rats

There were no significant differences in any microCT or bone cell count variables observed between NC and FRC of young adult rats, or between NC and FRC of mature rats (data not

shown), likely due to the food restriction of only 5% less than the age-matched NC rats (data shown later in results). Therefore, NC and FRC data were combined by age, into one control (C) group per age group, hereafter.

Figure 3A–D shows representative 3D images of the distal trabecular metaphyseal region of the radius, and shows an increase in trabecular bone volume in young adult HRLF rats, compared to the other groups. We quantified these differences using microCT (Table 2), and observed loading-induced bone adaptation in young adult HRLF rats as increased BV/TV, Tb.Th, Tb.N and trabecular BMD, compared to young adult C rats. The degree of anisotropy (DA) also improved (decreased) in trabeculae of young adult HRLF rats, compared to young adult C rats. The trabecular shape changed from rod-like to plate-like with HRLF task performance, indicated as decreased SMI, a change known to increase bone strength [69]. An increase in bone volume in young adult HRLF rats was also seen in von Kossa stained sections, compared to the other groups (Fig. 3E–H). Note, 3D and histological images also show an increase in trabecular bone proximally towards the mid-diaphyseal region of the radius in young adult HRLF rats, than in young adult C rats (Fig. 3B versus A).

In contrast, mature rats performing the HRLF task showed several indices of bone loss in the distal radial trabeculae, including decreased BV/TV and Tb.Th, compared to mature C rats and to young adult HRLF rats (Fig 3A–H; Table 2). Mature HRLF rats also had decreased trabecular numbers (Tb.N) and increased trabecular separation (Tb.Sp), compared to young adult HRLF rats (Table 2). The microCT 3D and histological images show loss of trabecular bone proximally towards the mid-diaphyseal radius in mature HRLF rats (Fig. 3D,H), findings not seen in young adult HRLF or C rats. Mature HRLF rats had increased DA, compared to mature C and young adult HRLF rats, indicative of more unevenly distributed trabeculae (Table 2). On a positive note, mature HRLF rats showed one index of bone adaptation to the task, that being a lower SMI than mature C rats, although the magnitude of this change was less than seen in young adult HRLF rats and did not reach significance. Trabecular BMD was also increased in mature HRLF rats, compared to mature C rats, although this increase did not reach significance (Table 2).

3.3 MicroCT analysis shows cortical bone growth in the radius of young adult HRLF rats, but thinning and increased porosity in mature HRLF rats

Mid-diaphyseal bone growth was observed in the radius of young adult HRLF rats (Table 2 lower half). This was observed as decreased Ma.Ar (indicative of endosteal bone formation), increased Ps.Pm (indicative of periosteal growth), and increased cortical bone thickness (Ct.Th), compared to young adult C rats. There was also an increase in cortical bone BMD in young adult HRLF rats, compared to young adult C rats.

In contrast, mature HRLF rats had increased Ma.Ar and cortical thinning, compared to mature C rats (despite starting with thicker cortical bones than young adult C rats; Table 2). Mature task rats also showed an increased volume of closed pores (Po.V (cl)), indicative of increased cortical bone porosity, compared to mature C rats and young adult HRLF rats (Table 2). Cortical bone BMD was lower in mature HRLF rats, than in young adult HRLF rats, although not significantly lower than mature C rats. Representative 3D images show an increase in cortical bone porosity in the mature HRLF rats and cortical thinning, compared

to the other groups (Fig. 3I–L). The increased cortical bone porosity observed using microCT appeared to be due to increases in osteoclast resorptive spaces and vascular profiles in cortical radial bones of mature HRLF rats (Fig. 3P), compared to mature C rats and young adult HRLF rats (Fig 3N,O); Table 2). A qualitative increase in osteoclast resorptive spaces was also observed in distal radial cortical of mature HRLF rats than in the other groups (Fig 3H inset, arrows). Lastly, the mature HRLF rats showed increased woven bone in the mid-diaphyseal cortical bone (Fig. 3P), compared to the other groups (Fig. 3M–O; Table 2).

3.4. Static and dynamic bone histomorphometry shows increased radial trabecular osteoblast activity in young adult HRLF rats, compared to mature HRLF rats

Several indices of osteoblast activity and proliferation were observed in distal radial metaphyseal trabeculae of young adult HRLF rats, including increased percent osteoid volume per bone volume (OV/BV), osteoid width (O.Wi), osteoblast surface per bone surface (Ob.S/BS), and numbers of osteoblasts per bone surface (N.Ob/BS), compared to young adult C and mature HRLF rats (Fig. 4A–D). Similar adaptive bone changes were not observed in the mature HRLF rats (Fig 4A–D). Both mineral apposition rate (MAR) and bone formation rate per bone surface (BFR/BS) were increased in distal radial metaphyseal trabeculae of young adult HRLF rats, compared to young adult C rats and mature HRLF rats (Fig 4E and F). Representative images show increased distance between calcein double labeling in trabeculae of young adult HRLF rats, particularly compared to young adult C rats (Fig. 4H versus G). The mature C and HRLF rats showed only random areas of calcein labeling and few clear double-labeled bands (Fig. 4I and J); the latter findings indicative of very little bone growth in the radius of mature animals, with or without repetitive loading. Serum osteocalcin levels showed similar increases in young adult HRLF rats, compared to young adult C rats, and lower levels in both mature C and mature HRLF rats, compared to their young adult counterparts (Fig. 4K).

3.5. Osteoclast activity and numbers increased in mature HRLF rats

Quantification of TRAP-stained osteoclasts in the distal radial metaphyseal trabeculae showed increases in both osteoclast surface and number per bone surface (Oc.S/BS and N.Oc/BS) in mature HRLF rats, compared to mature C rats and young adult HRLF rats (Fig 5A and B).

3.6. Greater task-induced inflammatory cytokine responses in mature rats

We next analyzed inflammatory cytokine levels in forelimb bones (radius and ulna), since we have reported increased pro-inflammatory cytokines in serum and tendons of mature HRLF rats, compared to young adult counterparts [49, 70], and since inflammatory cytokine levels are known to stimulate osteoclastogenesis [30–34]. Levels of each pro-inflammatory cytokine examined (IL-1beta, TNF-alpha and IL-6) were higher in mature HRLF rat bones (Fig. 6A–C), and IL-1beta and TNF-alpha were increased in mature C rats, compared to young adult C rats (Fig. 6A,B). Interestingly, IL-10 levels (a key anti-inflammatory cytokine) was lower in mature HRLF rats (Fig. 6D), compared to mature C and young adult HRLF rats. Both TNF-alpha and IL-10 were increased in young adult HRLF rats, compared to young adult C rats (Fig. 6B,D).

3.7 Animals weights were not significantly affected by food restriction

To determine if differences in body weight could be a contributing factor to the observed bone changes, body weights were compared across the weeks and between groups at matched time points. Body weights of young adult rats showed that young adult rats in each group weighed more at week 12, than at naïve, indicating that they each gained weight similarly across the course of the experiment (Fig. 7A). Mature HRLF rats did not show any significant changes in weight, compared to mature FRC and NC rats (Fig. 7B). However, mature rats of each group were heavier than the young adult rats at each time point (Fig. 7C), although this increase in weight did not translate to increased bone in the mature rats, compared to young adult rats, as shown in the microCT results.

3.8 Maximum reflexive grip strength declines are evident in mature task rats

Young adult HRLF rats showed increased maximum reflexive grip strength in weeks 3 and 6 of task performance, compared to their naïve grip strength (Fig. 7D). By week 12, maximum reflexive grip strength of young adult HRLF rats had returned to NC and FRC levels (Fig. 7D). In contrast, mature HRLF rats showed a progressive decrease in maximum reflexive grip strength (Fig. 7E), compared to naïve. When comparing the two HRLF cohorts, young adult HRLF rats showed lower maximum reflexive grip strength than mature rats at naïve, but greater maximum reflexive grip strength than mature rats by week 6 (Fig. 7F).

3.9. No significant changes in the forelimb flexor muscles were observed with task or age

Differences in muscle size between the age groups were also examined as a possible contributing factor to the observed bone changes. Young adult HRLF rats had a similar mean cross sectional area as young adult C rats (56.08 ± 2.04 versus 50.84 ± 3.17 , $p = 0.11$), as did mature HRLF rats, compared to mature C rats (47.90 ± 8.06 versus 40.09 ± 4.38 , $p = 0.21$). There were also no significant differences between age groups (Two way ANOVA: Age $p = 0.08$, Task $p = 0.10$, Interaction $p = 0.97$).

3.10. Estrogen levels were similar in young adult and mature rats, and do not correlate with trabecular bone volume

To determine if bone changes observed were caused by sex hormone changes, we tested serum estradiol levels and found that mature rats had similar estrogen levels as the young adult rats (Supplemental Fig. 1A), indicating that the mature rats were pre-menopausal. No significant correlation was observed between serum estrogen levels and BV/TV in either group separately, or when both age groups were combined (Supplemental Fig. 1B), or between serum estrogen levels and Ct.Th and Ma.Ar changes (data not shown, $p > 0.05$). There was also no correlation between trabecular BV/TV and the rats' weights in either group separately, or when both age groups were combined (Supplemental Fig. 1C).

4. Discussion

4.1. Bone adaptation occurs in young adult HRLF rats by week 12

We found that 12 weeks of performance of a HRLF task by young adult rats induced bone remodeling and net bone formation in the distal radial metaphyseal trabeculae, as well as positive adaptive changes in the mid-diaphyseal cortical bone. This adaptation was evident in the distal radius as increased trabecular bone volume ratio, thickness and numbers, and bone mineral density. Trabecular bone adaptation in the young adult HRLF rats was also supported by a decrease in DA, a change associated with improved trabecular bone organization, and a decrease in the SMI, indicative of a transition from rod-like to plate-like trabecular shape, a change associated with higher mechanical strength [61, 71–73]. In the mid-diaphyseal cortical bone of young adult HRLF rats, marrow area was decreased (indicative of growth at the endosteal surface), periosteal perimeter was increased, osteoclastic resorption (indicative of periosteal growth), cortical thickness was increased, and the cortical BMD was increased. Each are positive adaptive changes that should reduce the risk of bone fracture [39, 74]. The increased osteoblast numbers and activity and increased MAR and BFR in the young adult HRLF rats, compared to unloaded control rats, indicate an increase in bone formation in response to performance of this moderate level task for 12 weeks. These data extend prior findings in this model, showing increased trabecular bone volume ratio in the distal radius of young adult rats that had performed the HRLF task for 12 weeks, compared to rats performing easier or more challenging tasks [46]. Combined, they show that the level of loading in this particular reaching and grasping task (4 reaches/day at 30 force-grams), consistently applied 3 days/week, 2 hours/day, for 12 weeks, was sufficient to induce osteogenesis and net bone growth in the radius of young adult rats.

4.2. Trabecular bone loss, cortical thinning and increased porosity in mature HRLF rats

In contrast, performance of the same HRLF task for 12 weeks by mature rats induced decreases in trabecular bone volume/tissue volume, thickness and numbers, compared to mature C rats and compared to young adult HRLF rats. This was in spite of taking care to complete the studies using the same experimental methodology (see Table 1) and equipment. Animals were purchased from the same vendor, housed in the same room, and fed the same diet; the only obvious difference in this regard was their age and weights. The mature rats were heavier than the young adult rats, although this increase in weight did not translate to increased bone in the mature rats. We observed a decrease in SMI in both mature and young adult HRLF rats, a change typically associated with improved trabecular bone structure [61, 71–73]. The mature HRLF rats showed a mix of both rods and plates (less of a decrease in SMI), while the young adult HRLF rats transitioned primarily to the stronger plate-like structure (a greater decrease in SMI). These SMI findings on their own suggest that longer performance of the HRLF task should have positive effects on trabecular bone structure in mature rats. However, the mature HRLF also had significant increases in the degree of anisotropy (DA) in the distal radial trabeculae. Increased DA is indicative of disorganization in trabecular distribution and a weaker bone that is less resistant to mechanical loading due to increased stiffness [71]. The mature HRLF rats failed to demonstrate osteogenesis (no change in osteocalcin or osteoblast numbers or activity) after

task performance. This low osteogenic response in the mature HRLF rats is consistent with prior studies showing that more vigorous loading or longer loading periods may be necessary to induce an osteogenic response in aging mammals [38, 39, 74, 75]. Reports about how aging affects the osteogenic potential of mesenchymal cells are conflicting, with some studies stating that aging does not affect osteoblast recruitment, differentiation and activity [76], while others state that there is an age-related decline in the osteogenic potential of bone marrow cells [77] that can be improved by physical activity in rats [78]. We did not observe such improvement in the mature rats with performance of this 12-week high repetition low force task. The increased osteoclast and inflammatory cytokine response with this 30 force-grams of loading regimen suggests that more vigorous loading may be counterintuitive; thus, longer loading periods at even a lower loading force may be needed to induce bone formation in the mature rats.

The mature HRLF rats also showed several negative morphometric changes in their mid-diaphyseal cortical bone, including increased marrow area, thinning, porosity, osteoclastic resorptive spaces, arterial spaces, and woven bone. An increase in marrow area can offer increased torsion strength and flexibility if cortical thickness and bone diameter are maintained, since bending strength of bone is exponentially related to its diameter [79]. However, in the mature HRLF rats, the cortical thinning was due to a lack of a compensatory increase in the periosteal perimeter as the endosteal perimeter increased. Such cortical thinning is typically linked to reduced cortical bone quality [36, 80]. Increased porosity is considered to not affect bone strength if pores are concentrated to the endocortical region [81]. However, the increased pores were evenly distributed throughout the cortical bone of the mature rats (Fig 3L) and were the result of evenly distributed increases in osteoclastic resorptive spaces and vascular profiles. This matches a prior report of increased osteoclastic resorption spaces contributing to increased microCT-detected porosity [82]. Increased intra-cortical porosity has been linked to increased fragility, microdamage and fracture risk, and is considered a key indicator of osteopenia [83–86]. These findings combined show increased metaphyseal trabecular and diaphyseal cortical bone degradative changes, with only a few indices of adaptation, in mature rats performing this moderate demand upper extremity task.

4.3. Links between aging, task-induced tissue inflammation and bone remodeling

Inflammatory changes were present in forelimb bones involved in performing the HRLF task in both mature and young adult rats. The small, yet significant, increases in TNF α and the increased osteoblastic response in young adult HRLF rats are similar to findings from other studies showing that low doses of TNF- α can stimulate osteoblast proliferation and osteogenic differentiation [87–89]. However, much greater increases in inflammatory cytokines were seen in forelimb bones of mature HRLF rats. These results are similar to our prior reports of increased inflammatory cytokine in tendons and serum of mature HRLF rats, compared to young adult HRLF rats [43, 49]. Increased inflammatory cytokine level variations in bones are known to affect both bone formation and resorption [32, 34, 35, 87, 90]. TNF- α and IL1- β are produced by osteoclasts and interfere with osteoblast differentiation and proliferation [32, 33] in a dose-dependent manner by promoting loss of bone morphogenetic proteins [30, 45, 91]. We have previously shown in

this model that anti-inflammatory doses of ibuprofen, provided as a secondary intervention, reduced TNF-alpha and IL-1beta levels, and prevented loss of bone volume in young adult rats performing a high repetition high force task for 12 weeks [29]. An ergonomic task reduction intervention in which young adult rats were moved from a high repetitive high force task in week 4, to a low repetition low force task for another 8 weeks, also reduced inflammatory cytokine levels and lead to enhanced bone formation [92]. These two studies support an underlying bone inflammatory cytokine mechanism in this model that was further enhanced by aging in rats involved in performing this repetitive loading task for 12 weeks.

The lower IL-10 response in the mature HRLF rats is consistent with other studies showing a dysregulation of the IL-10 response as a consequence of aging [44, 93]. The decline in IL-10 response in the mature rats may also be contributing to the increased pro-inflammatory cytokine response.

4.4. Other possible contributing factors to observed bone changes in mature rats

Several potential contributing factors can be ruled out. There were no significant differences in reach performance parameters assayed or estrogen levels in the young adult HRLF versus mature HRLF rats, ruling out these factors as contributors to the observed group differences. Body weight did not appear to be a contributing factor to the degradative bone changes observed in the mature rats in this study since they weighed more than the young adult rats. There was also no significant correlation between the rats' weights and bone volume ratios of either age group (Supplemental Fig 1C). Measurements of the cross-sectional area of the flexor muscle at its widest point showed no statistically significant increases between or among the 4 groups, although there was a marginal increase in muscle girth in mature HRLF rats, compared to mature C rats. This marginal increase was apparently not enough to trigger an osteogenic bone response and also failed to rescue maximum reflexive grip strength declines. Regarding the maximum reflexive grip strength declines, we have previously shown that increased inflammatory cytokines in serum, nerves and tendons of mature HRLF rats correlated with declines in maximum reflexive grip strength [49, 70, 94]. The decrease in maximum reflexive grip strength in the mature rats appears to be a myalgia-generated or even central sensitization pain response, as reported previously [54, 58, 94–96], that did not affect the mature HRLF rats' abilities to perform this submaximal grasping task in which the HRLF rats had to reach at 15% of their maximum grip strength (see Table 1).

4.5 Limitations

There are some limitations in this study. For consistency in loading, the 30 gram grip force used as the pulling force target (15% of maximum pulling force was used in both age groups, even though the mature rats weighed more and had a slightly higher grip strength at naïve than the young adult rats. While this may seem like a limitation, increasing the pulling force target would likely have increased the bone degradative changes further in the mature HRLF rats. Second, for the cytokine analyses, we collected and homogenized together both ulna and radial bones. Therefore, the inflammatory cytokine data is representative of changes in both of these bones, rather than in just the radius. Next, the serum levels of osteocalcin are representative of bone formation occurring in all bones involved in performing the task, not just the radius. Therefore, another limitation of this study is that we

did not examine other bony sites of the upper extremity for morphological changes. Lastly, this study was performed in rats and until a similar longitudinal study is performed in young adult and mature human subjects with WMSDs, caution is warranted.

5. Conclusions

The radial bones of young adult female rats show positive adaptation and bone growth to this low force repetitive task. In contrast, in the mature rats performing the same reaching and grasping task, fewer osteogenic changes, and metaphyseal trabecular and cortical bone degradative changes were observed. The observed group differences in bone formation versus bone loss responses were not due to differences in strain rate (repetition rate or grasping force levels), estrogen levels, or flexor muscle cross-section area. The reduced radial bone quality in the mature rats indicates that prolonged performance of even moderate demand upper extremity, hand intensive task may increase the risk of fracture or osteopenia, matching results from human subjects showing reduced bone mass in females with heavy or one-sided hand workloads [17–19], and decreased bone mineral density in distal forearm bones of subjects with carpal tunnel syndrome [22, 23]. Our findings of increased bone inflammatory cytokines and osteoclast activity in the mature HRLF rat bones, combined with recent findings of reduced cytokines and preservation of bone volume after anti-inflammatory treatments [29, 92], supports an underlying inflammatory mechanism that should be considered in future intervention studies on animal and human subjects with WMSDs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Literature Cited

1. OSHA. Prevention of work-related musculoskeletal disorders. 2014. [cited 2014 06/22/2014]; Available from: https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=UNIFIED_AGENDA&p_id=4481
2. Bureau of Labor Statistics. NONFATAL OCCUPATIONAL INJURIES AND ILLNESSES REQUIRING DAYS AWAY FROM WORK. U.S. Department of Labor; 2012. <http://www.bls.gov/news.release/osh2.nr0.htm>. 2012 01/05/2011; Available from: <http://www.bls.gov/news.release/osh2.nr0.htm>
3. Foss L, et al. The impact of workplace risk factors on long-term musculoskeletal sickness absence: a registry-based 5-year follow-up from the Oslo health study. *J Occup Environ Med*. 2011; 53(12): 1478–82. [PubMed: 22076041]
4. Gerr F, et al. A prospective study of computer users: I. Study design and incidence of musculoskeletal symptoms and disorders. *Am J Ind Med*. 2002; 41(4):221–35. [PubMed: 11920966]

5. Bureau of Labor Statistics. Nonfatal occupational injuries and illnesses requiring days away from work, 2011. Nov 8. 2012 <http://www.bls.gov/news.release/osh2.nr0.htm> News Release USDL-12-2204
6. Silverstein M. Meeting the challenges of an aging workforce. *Am J Ind Med.* 2008; 51(4):269–80. [PubMed: 18271000]
7. Association of Occupational and Environmental Clinics. Electronic Library of Construction Occupational Safety and Health. CPWR - The Center for Construction Research and Training; 2009. Healthy Aging for a Sustainable Workforce. <http://www.elcosh.org/document/1684/957/d000987/2b-.html>
8. World Health Organization. Workers' health: global plan of action. 2007.
9. WHO Scientific Group. The burden of musculoskeletal conditions at the start of the new millenium. 2003.
10. NIOSH. National Manufacturing Agenda - June 2010. 2011. [cited 2011 October 23]; Available from: <http://www.cdc.gov/niosh/nora/comment/agendas/manuf/>
11. Green JO, et al. Age-related changes in human trabecular bone: Relationship between microstructural stress and strain and damage morphology. *J Biomech.* 2011; 44(12):2279–85. [PubMed: 21724189]
12. Pearson OM, Lieberman DE. The aging of Wolff's "law": Ontogeny and responses to mechanical loading in cortical bone. *American Journal of Physical Anthropology.* 2004; 125(S39):63–99. [PubMed: 15605390]
13. Warden SJ, et al. Bone adaptation to a mechanical loading program significantly increases skeletal fatigue resistance. *J Bone Miner Res.* 2005; 20(5):809–16. [PubMed: 15824854]
14. Sample SJ, et al. Systemic effects of ulna loading in male rats during functional adaptation. *J Bone Miner Res.* 2010; 25(9):2016–28. [PubMed: 20499374]
15. Robling AG, Burr DB, Turner CH. Skeletal loading in animals. *J Musculoskelet Neuronal Interact.* 2001; 1(3):249–62. [PubMed: 15758499]
16. Burr DB, Robling AG, Turner CH. Effects of biomechanical stress on bones in animals. *Bone.* 2002; 30(5):781–6. [PubMed: 11996920]
17. Ding H, et al. Hand osteoarthritis and pinch grip strength among middle-aged female dentists and teachers. *Scand J Rheumatol.* 2010; 39(1):84–7. [PubMed: 20132076]
18. Solovieva S, et al. Hand use and patterns of joint involvement in osteoarthritis. A comparison of female dentists and teachers. *Rheumatology (Oxford).* 2005; 44(4):521–8. [PubMed: 15728421]
19. Vehmas T, et al. Hand workload and the metacarpal cortical index. a study of middle-aged teachers and dentists. *Osteoporos Int.* 2005; 16(6):672–80. [PubMed: 15502963]
20. Amorim BJ, et al. Low sensitivity of three-phase bone scintigraphy for the diagnosis of repetitive strain injury. *Sao Paulo medical journal = Revista paulista de medicina.* 2006; 124(3):145–9. [PubMed: 17119691]
21. al-Nahhas AM, et al. 99Tcm-MDP blood-pool phase in the assessment of repetitive strain injury. *Nuclear medicine communications.* 1997; 18(10):927–31. [PubMed: 9392793]
22. Erselcan T, et al. Carpal tunnel syndrome leads to significant bone loss in metacarpal bones. *J Bone Miner Metab.* 2001; 19(5):317–20. [PubMed: 11498735]
23. Savas S, et al. Metacarpal Bone Density in Carpal Tunnel Syndrome Patients Without Thenar Atrophy. *(Rheumatism.* 2007; 22:15–19.
24. Barbe MF, et al. The interaction of force and repetition on musculoskeletal and neural tissue responses and sensorimotor behavior in a rat model of work-related musculoskeletal disorders. *BMC Musculoskelet Disord.* 2013; 14:303. [PubMed: 24156755]
25. Carp SJ, et al. Inflammatory biomarkers increase with severity of upper-extremity overuse disorders. *Clinical science.* 2007; 112(5):305–14. [PubMed: 17064252]
26. Rechardt M, et al. Associations of metabolic factors and adipokines with pain in incipient upper extremity soft tissue disorders: a cross-sectional study. *BMJ Open.* 2013; 3(8):e003036.
27. Rechardt M, et al. Soluble IL-1RII and IL-18 are associated with incipient upper extremity soft tissue disorders. *Cytokine.* 2011; 54(2):149–53. [PubMed: 21371906]

28. Rioldino S, et al. Functional impairment in video terminal operators is related to low-grade inflammation. *Int Arch Occup Environ Health*. 2011; 84(7):745–51. [PubMed: 21153734]
29. Jain NX, et al. Bone loss from high repetitive high force loading is prevented by ibuprofen treatment. *J Musculoskelet Neuronal Interact*. 2014; 14(1):78–94. [PubMed: 24583543]
30. Guo R, et al. Ubiquitin ligase Smurf1 mediates tumor necrosis factor-induced systemic bone loss by promoting proteasomal degradation of bone morphogenetic signaling proteins. *J Biol Chem*. 2008; 283(34):23084–92. [PubMed: 18567580]
31. Kulkarni RN, et al. Mechanical loading prevents the stimulating effect of IL-1beta on osteocyte-modulated osteoclastogenesis. *Biochem Biophys Res Commun*. 2012; 420(1):11–6. [PubMed: 22390927]
32. Lacey DC, et al. Proinflammatory cytokines inhibit osteogenic differentiation from stem cells: implications for bone repair during inflammation. *Osteoarthritis Cartilage*. 2009; 17(6):735–42. [PubMed: 19136283]
33. Lange J, et al. Action of IL-1beta during fracture healing. *J Orthop Res*. 2010; 28(6):778–84. [PubMed: 20041490]
34. Thomas T. New actors in bone remodelling: a role for the immune system. *Bull Acad Natl Med*. 2010; 194(8):1493–503. discussion 1503–4. [PubMed: 22046712]
35. Rani S, et al. Role of TNF alpha and PLF in bone remodeling in a rat model of repetitive reaching and grasping. *J Cell Physiol*. 2010; 225(1):152–67. [PubMed: 20458732]
36. Khosla S, et al. Forum on aging and skeletal health: summary of the proceedings of an ASBMR workshop. *J Bone Miner Res*. 2011; 26(11):2565–78. [PubMed: 21915901]
37. Srinivasan S, et al. Enabling bone formation in the aged skeleton via rest-inserted mechanical loading. *Bone*. 2003; 33(6):946–55. [PubMed: 14678854]
38. Turner CH. Aging and fragility of bone. *J Musculoskelet Neuronal Interact*. 2007; 7(4):342–3. [PubMed: 18094504]
39. Turner CH, Takano Y, Owan I. Aging changes mechanical loading thresholds for bone formation in rats. *J Bone Miner Res*. 1995; 10(10):1544–9. [PubMed: 8686511]
40. Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. *Annu Rev Med*. 2000; 51:245–70. [PubMed: 10774463]
41. Orjalo AV, et al. Cell surface-bound IL-1alpha is an upstream regulator of the senescence-associated IL-6/IL-8 cytokine network. *Proc Natl Acad Sci U S A*. 2009; 106(40):17031–6. [PubMed: 19805069]
42. Stowe RP, et al. Plasma cytokine levels in a population-based study: relation to age and ethnicity. *J Gerontol A Biol Sci Med Sci*. 2010; 65(4):429–33. [PubMed: 20018825]
43. Xin DL, et al. Aging enhances serum cytokine response but not task-induced grip strength declines in a rat model of work-related musculoskeletal disorders. *BMC musculoskeletal disorders*. 2011; 12:63. [PubMed: 21447183]
44. Cevenini E, et al. Age-related inflammation: the contribution of different organs, tissues and systems. How to face it for therapeutic approaches. *Curr Pharm Des*. 2010; 16(6):609–18. [PubMed: 20388071]
45. Barr AE, et al. Repetitive, negligible force reaching in rats induces pathological overloading of upper extremity bones. *J Bone Miner Res*. 2003; 18(11):2023–32. [PubMed: 14606516]
46. Barbe MF, et al. The interaction of force and repetition on musculoskeletal and neural tissue responses and sensorimotor behavior in a rat model of work-related musculoskeletal disorders. *BMC Musculoskelet Disord*. 2013; 14(1):303. [PubMed: 24156755]
47. Driban JB, et al. Joint inflammation and early degeneration induced by high-force reaching are attenuated by ibuprofen in an animal model of work-related musculoskeletal disorder. *J Biomed Biotechnol*. 2011; 2011:691412. [PubMed: 21403884]
48. Rani S, et al. Periostin-like-factor and Periostin in an animal model of work-related musculoskeletal disorder. *Bone*. 2009; 44(3):502–12. [PubMed: 19095091]
49. Kietrys DM, et al. Aging contributes to inflammation in upper extremity tendons and declines in forelimb agility in a rat model of upper extremity overuse. *PLoS One*. 2012; 7(10):e46954. [PubMed: 23056540]

50. Mihara M, et al. Interleukin-6 (IL-6) induces the proliferation of synovial fibroblastic cells in the presence of soluble IL-6 receptor. *Br J Rheumatol*. 1995; 34(4):321–5. [PubMed: 7788145]
51. Srilatha, et al. Prevalence of Work-Related Wrist and Hand Musculoskeletal Disorders (WMSD) among Computer Users. *Journal of Clinical and Diagnostic Research*. 2011; 5(3):605–607.
52. Ratzlaff CR, Gillies JH, Koehoorn MW. Work-related repetitive strain injury and leisure-time physical activity. *Arthritis Rheum*. 2007; 57(3):495–500. [PubMed: 17394178]
53. Cote JN. A critical review on physical factors and functional characteristics that may explain a sex/gender difference in work-related neck/shoulder disorders. *Ergonomics*. 2012; 55(2):173–82. [PubMed: 21846285]
54. Barbe MF, et al. Serum and tissue cytokines and chemokines increase with repetitive upper extremity tasks. *J Orthop Res*. 2008; 26(10):1320–6. [PubMed: 18464247]
55. Clark BD, et al. Performance of a high-repetition, high-force task induces carpal tunnel syndrome in rats. *J Orthop Sports Phys Ther*. 2004; 34(5):244–53. [PubMed: 15189016]
56. Kietrys DM, Barr AE, Barbe MF. Exposure to repetitive tasks induces motor changes related to skill acquisition and inflammation in rats. *Journal of motor behavior*. 2011; 43(6):465–76. [PubMed: 22087754]
57. Barbe MF, et al. The interaction of force and repetition on musculoskeletal and neural tissue responses and sensorimotor behavior in a rat model of work-related musculoskeletal disorders. *BMC musculoskeletal disorders*. 2013; 14(1):303. [PubMed: 24156755]
58. Elliott MB, et al. Peripheral neuritis and increased spinal cord neurochemicals are induced in a model of repetitive motion injury with low force and repetition exposure. *Brain Res*. 2008; 1218:103–13. [PubMed: 18511022]
59. Kietrys DM, Barr AE, Barbe MF. Exposure to repetitive tasks induces motor changes related to skill acquisition and inflammation in rats. *J Mot Behav*. 2011; 43(6):465–76. [PubMed: 22087754]
60. Bouxsein ML, et al. Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2010; 25(7):1468–86.
61. Hildebrand T, Ruegsegger P. Quantification of Bone Microarchitecture with the Structure Model Index. *Comput Methods Biomech Biomed Engin*. 1997; 1(1):15–23. [PubMed: 11264794]
62. Dempster DW, et al. Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. *J Bone Miner Res*. 2013; 28(1):2–17. [PubMed: 23197339]
63. Parfitt AM, et al. Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. *J Bone Miner Res*. 1987; 2(6):595–610. [PubMed: 3455637]
64. Brennan T, et al. Abrogation of Cbl-PI3K interaction increases bone formation and osteoblast proliferation. *Calcif Tissue Int*. 2011; 89(5):396–410. [PubMed: 21952831]
65. Chen P, et al. Increases in BMD correlate with improvements in bone microarchitecture with teriparatide treatment in postmenopausal women with osteoporosis. *J Bone Miner Res*. 2007; 22(8):1173–80. [PubMed: 17451369]
66. Dempster DW. The contribution of trabecular architecture to cancellous bone quality. *J Bone Miner Res*. 2000; 15(1):20–3. [PubMed: 10646110]
67. Mueller D, et al. The 3D-based scaling index algorithm: a new structure measure to analyze trabecular bone architecture in high-resolution MR images in vivo. *Osteoporos Int*. 2006; 17(10):1483–93. [PubMed: 16847587]
68. Iolascon G, et al. Bone quality and bone strength: benefits of the bone-forming approach. *Clin Cases Miner Bone Metab*. 2014; 11(1):20–4. [PubMed: 25002875]
69. Liu XS, et al. Individual trabeculae segmentation (ITS)-based morphological analysis of high-resolution peripheral quantitative computed tomography images detects abnormal trabecular plate and rod microarchitecture in premenopausal women with idiopathic osteoporosis. *J Bone Miner Res*. 2010; 25(7):1496–505. [PubMed: 20200967]
70. Xin DL, et al. Aging enhances serum cytokine response but not task-induced grip strength declines in a rat model of work-related musculoskeletal disorders. *BMC Musculoskelet Disord*. 2011; 12:63. [PubMed: 21447183]

71. Ding M, Odgaard A, Hvid I. Changes in the three-dimensional microstructure of human tibial cancellous bone in early osteoarthritis. *J Bone Joint Surg Br.* 2003; 85(6):906–12. [PubMed: 12931817]
72. Akhter MP, et al. Transmenopausal changes in the trabecular bone structure. *Bone.* 2007; 41(1): 111–6. [PubMed: 17499038]
73. McLaughlin F, et al. Glucocorticoid-induced osteopenia in the mouse as assessed by histomorphometry, microcomputed tomography, and biochemical markers. *Bone.* 2002; 30(6): 924–30. [PubMed: 12052464]
74. Srinivasan S, Gross TS, Bain SD. Bone mechanotransduction may require augmentation in order to strengthen the senescent skeleton. *Ageing Res Rev.* 2012; 11(3):353–60. [PubMed: 22240208]
75. Leppanen OV, et al. Pathogenesis of age-related osteoporosis: impaired mechano-responsiveness of bone is not the culprit. *PLoS One.* 2008; 3(7):e2540. [PubMed: 18648530]
76. Leskela HV, et al. Osteoblast recruitment from stem cells does not decrease by age at late adulthood. *Biochem Biophys Res Commun.* 2003; 311(4):1008–13. [PubMed: 14623282]
77. Mueller SM, Glowacki J. Age-related decline in the osteogenic potential of human bone marrow cells cultured in three-dimensional collagen sponges. *J Cell Biochem.* 2001; 82(4):583–90. [PubMed: 11500936]
78. Hell RC, et al. Physical activity improves age-related decline in the osteogenic potential of rats' bone marrow-derived mesenchymal stem cells. *Acta Physiol (Oxf).* 2012; 205(2):292–301. [PubMed: 22168399]
79. Saine ME, et al. Hypothalamic suppression during adolescence varies by bone envelope. *Med Sci Sports Exerc.* 2011; 43(4):608–16. [PubMed: 20798664]
80. Keaveny TM, et al. Age-dependence of femoral strength in white women and men. *J Bone Miner Res.* 2010; 25(5):994–1001. [PubMed: 19874201]
81. Burr DB, et al. Intermittently administered human parathyroid hormone(1–34) treatment increases intracortical bone turnover and porosity without reducing bone strength in the humerus of ovariectomized cynomolgus monkeys. *J Bone Miner Res.* 2001; 16(1):157–65. [PubMed: 11149480]
82. Wu B, et al. Self-repair of rat cortical bone microdamage after fatigue loading in vivo. *Int J Endocrinol.* 2013; 2013:321074. [PubMed: 23662102]
83. Borah B, et al. Risedronate reduces intracortical porosity in women with osteoporosis. *J Bone Miner Res.* 2010; 25(1):41–7. [PubMed: 19580469]
84. Burr DB, et al. Bone microdamage and skeletal fragility in osteoporotic and stress fractures. *J Bone Miner Res.* 1997; 12(1):6–15. [PubMed: 9240720]
85. Dhainaut A, et al. Cortical hand bone porosity and its association with distal radius fracture in middle aged and elderly women. *PLoS One.* 2013; 8(7):e68405. [PubMed: 23844197]
86. Nishiyama KK, et al. Postmenopausal women with osteopenia have higher cortical porosity and thinner cortices at the distal radius and tibia than women with normal aBMD: an in vivo HR-pQCT study. *J Bone Miner Res.* 2010; 25(4):882–90. [PubMed: 19839766]
87. Mumme M, et al. Interleukin-1beta modulates endochondral ossification by human adult bone marrow stromal cells. *Eur Cell Mater.* 2012; 24:224–36. [PubMed: 23007908]
88. Cho TJ, Gerstenfeld LC, Einhorn TA. Differential temporal expression of members of the transforming growth factor beta superfamily during murine fracture healing. *J Bone Miner Res.* 2002; 17(3):513–20. [PubMed: 11874242]
89. Cho HH, et al. NF-kappaB activation stimulates osteogenic differentiation of mesenchymal stem cells derived from human adipose tissue by increasing TAZ expression. *J Cell Physiol.* 2010; 223(1):168–77. [PubMed: 20049872]
90. Gravallese EM. Bone destruction in arthritis. *Ann Rheum Dis.* 2002; 61(Suppl 2):ii84–6. [PubMed: 12379632]
91. Kaneki H, et al. Tumor necrosis factor promotes Runx2 degradation through up-regulation of Smurf1 and Smurf2 in osteoblasts. *J Biol Chem.* 2006; 281(7):4326–33. [PubMed: 16373342]
92. Barbe MF, et al. Ergonomic task reduction prevents bone osteopenia in a rat model of upper extremity overuse. *Ind Health.* 2015; 53(3):206–21. [PubMed: 25739896]

93. Salvioli S, et al. Inflamm-aging, cytokines and aging: state of the art, new hypotheses on the role of mitochondria and new perspectives from systems biology. *Curr Pharm Des.* 2006; 12(24):3161–71. [PubMed: 16918441]
94. Elliott MB, et al. Performance of a repetitive task by aged rats leads to median neuropathy and spinal cord inflammation with associated sensorimotor declines. *Neuroscience.* 2010; 170(3):929–41. [PubMed: 20673790]
95. Elliott MB, Barr AE, Barbe MF. Spinal substance P and neurokinin-1 increase with high repetition reaching. *Neurosci Lett.* 2009; 454(1):33–7. [PubMed: 19429049]
96. Elliott MB, et al. High force reaching task induces widespread inflammation, increased spinal cord neurochemicals and neuropathic pain. *Neuroscience.* 2009; 158(2):922–31. [PubMed: 19032977]

HIGHLIGHTS

- Prolonged performance of a high repetition low force reaching and grasping task by young adult rats induced adaptive bone remodeling in the distal metaphysis of the radius.
- Prolonged performance of a high repetition low force task by mature rats induced detrimental effects on radial bone morphology, in both distal metaphyseal trabeculae and mid-diaphyseal cortical bone
- Prolonged performance of a high repetition task, even at low force loads, lead to increased in bone pro-inflammatory cytokine levels and osteoclasts in mature rat forelimb bones, at levels higher than observed in young adult rats performing the same task.
- Prolonged performance of a high repetition low force reaching and grasping task by mature rats induces radial bone osteopenic changes that may increase fracture risk.

Figure 1. Experimental design and regions of radial bone analyzed. (A) Young adult (2.5 months at onset) and mature (15 months at onset) Sprague-Dawley rats were randomly assigned to normal control (NC), food restriction control (FRC), and 12 week high repetition low force (HRLF) task groups. Number of rats per group is shown. All rats were handled for one week before onset of food restriction (FR) to $\pm 5\%$ less than weights of age-matched NC rats. Both FRC rats and HRLF rats were food restricted until euthanasia to encourage interest in food reward pellets. After a training period of 4 weeks in which HRLF rats learned to perform the reaching and lever grasping task, this group performed the task for 12 weeks. (B) Distal metaphyseal trabeculae and diaphyseal cortical bone regions in the radius (R) that were analyzed using micro-computerized tomography (MicroCT) in subcohorts of rats from each group. Locations of regions of interest (ROI) are as indicated. The ulna (U) was not analyzed, as we have shown it to be less affected than the radius. (C,D) Transaxial slice regions of metaphyseal trabecular bone and mid-diaphyseal cortical bone used in volumes of interest for microCT are highlighted in red. Radius (R) and ulna (U) are indicated.

Figure 2.

Operant behavioral task apparatus. (A) Rat waiting for auditory and light cues with snout in portal. (B,C) Rat reaches into portal, and extends arm to the force lever bar with right forepaw. (D) Rat grasps and isometrically pulls a force lever bar attached to a force transducer (FT), until the force threshold (30 grams grip-force; 15% of their mean maximum pulling force) is reached and held for 50 milliseconds. (E) Rat retrieves a food pellet reward by mouth from the food trough. (F) Photo of inside of chamber showing light cue, portal and trough for food reward. (G) Photo of outside of chamber showing auditory clicker, food pellet dispenser and force lever bar attached to force transducer, which is in a metal stabilizing holder that is bolted down to the chamber floor.

Figure 3.

Representative microCT and microscope images of distal radius. (A–D) Representative 3D images generated using CTVox, a volume rendering software, of trabecular bone area analyzed in the radius for each group. These 3D reconstructions are located from the distal growth plate towards the mid-diaphysis. (E–H) Representative von Kossa stained sections of a distal radius from each group. Scale bar = 100 micrometers. The inset in panel H shows a number of osteoclast resorptive spaces in the distal radius of a mature HRLF rat. (I–L) Representative transaxial 3D microCT images, generated using a surface rendering software program, of the mid-diaphyseal cortical bone of the radius from each group. These transaxial reconstructions are located from 5 to 5.5 mm proximal to the distal growth plates. (M–P) Representative polarized light images from the mid-diaphyseal cortical bone of the radius from each group, taken with a 20x objective and differential interference contrast optics. The inset in panel P shows an osteoclast resorptive space in the distal radius of a mature HRLF rat; this section is stained with TRAP and a TRAP-stained osteoclast is visible in this space.

Figure 4.

Static osteoblast and dynamic histomorphometric measurements in distal metaphyseal trabecular region of radii of young adult and mature control (C) and 12-week high repetition low force (HRLF) rats. (A) Osteoid volume normalized to bone volume (OV/BV). (B) Osteoid width (O.Wi). (C) Osteoblast surface normalized to bone surface (Ob.S/BS). (D) Cellular density of osteoblasts (N.OB) normalized to bone surface (BS). (E) Trabecular mineral apposition rate in the radius (MAR). (F) Trabecular bone formation rate (BRF), normalized to bone surface (BS). (G–H) Representative microscope images showing calcein double labeling in radial trabeculae of young adult rats. (I–J) Representative microscope images showing calcein labeling in radial trabeculae of mature rats, and shows only spotty calcein labeling in each. (K) Serum levels of osteocalcin, assayed using ELISA. Two-way ANOVA results are shown in individual panels, and Mean $\pm$ SEM is shown. *: $p < 0.05$ and **: $p < 0.01$; n.s. not significant.

Fig 5.

Histomorphometric measurements of osteoclasts in distal metaphyseal trabecular region of the radius of young adult and mature control (C) and 12-week high repetition low force (HRLF) rats. (A) Osteoclast surface, normalized to bone surface (Oc.S/BS). (B) Number of osteoclasts, normalized to bone surface (N.Oc/BS). Two-way ANOVA results are shown in individual panels, and Mean $\pm$ SEM is shown. *: $p < 0.05$; n.s. = not significant.

Figure 6. Inflammatory cytokines in forelimb bones of young adult and mature control (C) and 12-week high repetition low force (HRLF) rats. (A) IL-1beta, (B) TNF-alpha, (C) IL-6, and (D) IL-10. Two-way ANOVA results are shown in individual panels, and Mean $\pm$ SEM is shown. **: p<0.01.

Figure 7.

Body weights and maximum grip strength of young adult and mature rats across weeks of experiment. Data shown for normal control (NC), food restriction control (FRC), and 12-week high repetition low force (HRLF) task rats from each age group. (A–C) Body weights of young adult control and HRLF rats, mature control and HRLF rats, and young adult versus mature HRLF rats. (D) Grip strength of young adult rats from naïve to task week 12. (E–F) Grip strength of young adult control and HRLF rats, mature control and HRLF rats, and young adult versus mature HRLF rats. Two-way ANOVA results are shown in individual panels, and Mean $\pm$ SEM is shown. *: $p < 0.05$, and **: $p < 0.01$, compared to naïve time point; #: $p < 0.05$ and ##: $p < 0.01$, compared to age-matched NC and FRC rats; &&: $p < 0.01$, compared to matching group of mature HRLF rats.

Table 1

Task Performance Outcomes in Week 12

Attribute	Young adult 12-wk HRLF	Mature 12-wk HRLF	Statistical Findings
Reaches/min	4.83 ± 2.037	4.71 ± 1.1	n.s.
Reach Phase Time (sec)	1.69 ± 0.16	1.76 ± 0.13	n.s.
Grasp Phase Time (sec)	1.20 ± 0.20	0.92 ± 0.13	n.s.
Duration (% of 120 min)	72.78 ± 8.10	77.47 ± 8.53	n.s.
Mean Voluntary Pulling Force (%)	15.74 ± 4.40	16.49 ± 2.44	n.s.

Mean Voluntary Pulling Force of 15% = a mean of 30 grams of grip force; values shown are mean ± sem.

Table 2

MicroCT results for Radial Bone (6 micrometer voxel resolution)

Young Adult		Mature Adult		Statistical Findings	
Attribute	C	12-wk HRLF	C		12-wk HRLF
Distal Radial Metaphyseal Trabeculae					
BV/TV (%)	33.86±2.46	61.08±13.92 ^a	34.88±9.22	22.28±1.76 ^{a,b}	Interaction p=0.04
Tb.Th (mm)	0.06±0.004	0.14±0.04 ^a	0.10±0.03	0.06±0.003 ^{a,b}	Interaction p=0.01
Tb.N (1/mm)	4.14±0.35	5.84±0.94 ^a	4.57±0.62	4.06±0.18 ^b	Interaction p<0.05
Tb.Sp (mm)	0.12±0.008	0.12±0.03	0.18±0.01 ^b	0.20±0.006 ^b	Age: p=0.0001
DA	0.50±0.04	0.38±0.03 ^a	0.46±0.05	0.57±0.02 ^a	Interaction p=0.008
SMI	2.10±0.14	0.16±0.58 ^a	2.11±0.34	1.00±0.21	Task Gp p=0.001
BMD Tb (g/cm ³)	7.20±2.16	23.00±3.74 ^a	14.49±1.97	22.07±2.33	Task Gp p=0.0008
Mid-Diaphyseal Radial Cortical Bone					
Tt.Ar (mm ²)	1.51±0.022	1.45±0.10	1.62±0.08	1.73±0.06 ^b	Age p=0.01
Ct.Ar (mm ²)	1.38±0.05	1.36±0.20	1.53±0.06	1.60±0.046	Age p=0.03
Ct.Ar/Tt.Ar (%)	91.13±3.84	80.27±10.44	95.06±2.26	92.06±0.94	n.s.
M.Ar (mm ²)	0.09±0.02	0.05±0.004 ^a	0.04±0.015	0.14±0.02 ^{a,b}	Interaction p=0.004
Ps.Pm (mm)	4.79±0.03	5.57±0.38	4.99±0.15	5.16±0.09	Task p=0.03
Ct.Th (mm)	0.48±0.04	0.50±0.07 ^a	0.57±0.03 ^b	0.53±0.01 ^a	Interaction p=0.004
Po.V (cl) (mm ³)	0.0004±0.00008	0.006±0.003	0.04±0.002	0.01±0.005 ^{a,b}	Task p=0.004; Age p=0.03
BMD Ct (g/cm ³)	97.37±2.93	110.69±3.70 ^a	95.54±2.44	92.36±6.01 ^b	Interaction p=0.04
Wo.BV/BV (%)	12.25±3.57	4.20±2.13	14.80±4.81	37.43±5.64 ^{a,b}	Interaction p=0.006
Resorptive spaces (1/mm ²)	0.86±0.86	0.21±0.02	0.11±0.03	0.39±0.07 ^{a,b}	Task p=0.002
Vascular profiles (1/mm ²)	4.09±0.43	4.97±0.36	6.51±1.06	10.13±1.14 ^{a,b}	Task p=0.02

^a p<0.05, compared to age-matched control group;^b p<0.05, compared to matched young adult counterpart group (control or task); values shown are mean ± sem.