

# Ergonomic task reduction prevents bone osteopenia in a rat model of upper extremity overuse

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**Abstract:** We evaluated the effectiveness of ergonomic workload reduction of switching rats from a high repetition high force (HRHF) lever pulling task to a reduced force and reach rate task for preventing task-induced osteopenic changes in distal forelimb bones. Distal radius and ulna trabecular structure was examined in young adult rats performing one of three handle-pulling tasks for 12 wk: 1) HRHF, 2) low repetition low force (LRLF); or 3) HRHF for 4 wk and then LRLF thereafter (HRHF-to-LRLF). Results were compared to age-matched control rats. Distal forelimb bones of 12-wk HRHF rats showed increased trabecular resorption and decreased volume, as control rats. HRHF-to-LRLF rats had similar trabecular bone quality as control rats; and decreased bone resorption (decreased trabecular bone volume and serum CTX1), increased bone formation (increased mineral apposition, bone formation rate, and serum osteocalcin), and decreased osteoclasts and inflammatory cytokines, than HRHF rats. Thus, an ergonomic intervention of HRHF-to-LRLF prevented loss of trabecular bone volume occurring with prolonged performance of a repetitive upper extremity task. These findings support the idea of reduced workload as an effective approach to management of work-related musculoskeletal disorders, and begin to define reach rate and load level boundaries for such interventions.

**Key words:** Repetitive loading, Work-related musculoskeletal disorders, Repetitive strain injury, Osteopenia, Ergonomic intervention, Cytokine, Bone

## Introduction

Epidemiological studies have linked upper extremity overuse musculoskeletal injuries with occupational physical activities involving repetitive hand and arm movements, especially when those tasks included other risk factors for work-related musculoskeletal disorders (WMSDs), such as high force, long duration and female gender<sup>1–5</sup>.

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<sup>6–8</sup>. Bone scan studies of patients with upper extremity musculoskeletal disorders show increased blood flow and pooling (suggestive of inflammation) in affected forearm bones<sup>9, 10</sup>, which is important since presence of chronic inflammatory processes in bones is known to reduce bone quality by increased osteoclastic activity<sup>11, 12</sup>. In a large study examining job-related osteoarthritis, a significant association was found between hand osteoarthritis in females and high impact “jolting” of the hand<sup>13</sup>. Such changes

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can increase osteopenia and fracture risk, and therefore, preventive or ameliorative treatments are needed. The 2014 National Occupational Research Agenda emphasizes the need for research to develop effective preventions of work-related musculoskeletal disorder (WMSD) induced deterioration of tissues<sup>14</sup>.

The response of bone to the application of load, whether internal, external, static or dynamic, may be anabolic or catabolic depending on many factors, including load magnitude and duration<sup>15–17</sup>. Muscle force may place greater loads on bones than gravitational forces such as body weight<sup>18, 19</sup>, and bone quality can be enhanced by exercise and other forms of repeated muscle loading<sup>18, 19</sup>. This is consistent with studies showing a biomechanical link between muscles and bone<sup>20, 21</sup>, and that bones should adapt to increased loads imparted by muscles<sup>21, 22</sup>. There is also ample evidence that muscle strength can reduce the risk of fracture, and that muscle dysfunction can lead to significant bone degradation<sup>18, 20, 23</sup>. On the other hand, studies examining the effects of excessive dynamic loads induced by extremely intensive running on treadmills, performing prolonged repetitive jumping, or performing repetitive reaching and grasping tasks at high repetition high force levels (see further below), show that increasing the intensity of weight-bearing or muscle loading exercise/activities to excessive levels can be associated with declines in bone volume and quality<sup>16, 24, 25</sup>. These studies combined indicate that the response of bone to excessive applied loads, including that from muscle loading, can be maladaptive, while low loading is more likely to induce bone formation<sup>26</sup>.

Using a unique operant rat model of reaching and grasping in which forearm bones are loaded by muscle activity, we have recently shown that all measures of tissue adaptation versus damage (adaptation-related versus histopathology and inflammatory cytokine changes in muscles, tendons and cartilage, and morphometry and inflammatory and inflammatory changes in bone) demonstrate expected force x repetition interactions predicted by general adaptation and fatigue failure theories<sup>25, 27–30</sup>. Related to forelimb bone, we observed that prolonged performance of either a high repetition low force task or a low repetition high force task lead to trabecular bone adaptation, such as increased trabecular bone volume density<sup>25</sup>. In contrast, rats performing a high repetition high force (HRHF) upper extremity task for 12 wk had bone resorptive changes in distal metaphyseal trabeculae of the radius and ulna (with net decreases in trabecular bone volume and quality), articular cartilage degeneration, prolonged increases in

bone inflammatory cytokines, and increased serum biomarkers of bone and cartilage degradation<sup>25, 31–34</sup>. These changes were attenuated by secondary ibuprofen treatment provided in weeks 5–12 of a 12-wk HRHF task<sup>32, 35</sup> indicated that a HRHF-induced inflammatory response drove the bone resorptive and cartilage degeneration processes, inflammatory responses that were absent or resolved early in the lower demand tasks<sup>25, 31, 36</sup>. However, since long-term use of ibuprofen has negative side effects, including gastrointestinal bleeding, renal toxicity, increased risk of myocardial infarction and hypertension, we are interested in exploring forearm trabecular bone resorption versus formation in response to non-pharmacological conservative secondary interventions.

Thus, we sought here to determine for the first time, the effectiveness of an ergonomic workload reduction intervention of switching rats performing a prolonged high repetition high force upper extremity reaching and grasping task to a reduced force and reach rate task of low repetition and low force, in preventing high demand task-induced forearm bone loss. We hypothesized that net bone resorption would be prevented or at least reduced if 4-wk HRHF rats were switched to the LRLF task for 8 wk, compared to 12-wk HRHF rats, since the reduced reach rate and grasp force levels should reduce both biomechanical exposure and inflammatory bone responses produced by tissue overloading. This hypothesis was examined by analysis of trabecular bone structure in forelimb bones using micro-computerized tomography and histomorphometry, forelimb bone inflammatory cytokines, and serum biomarkers of bone turnover using ELISA.

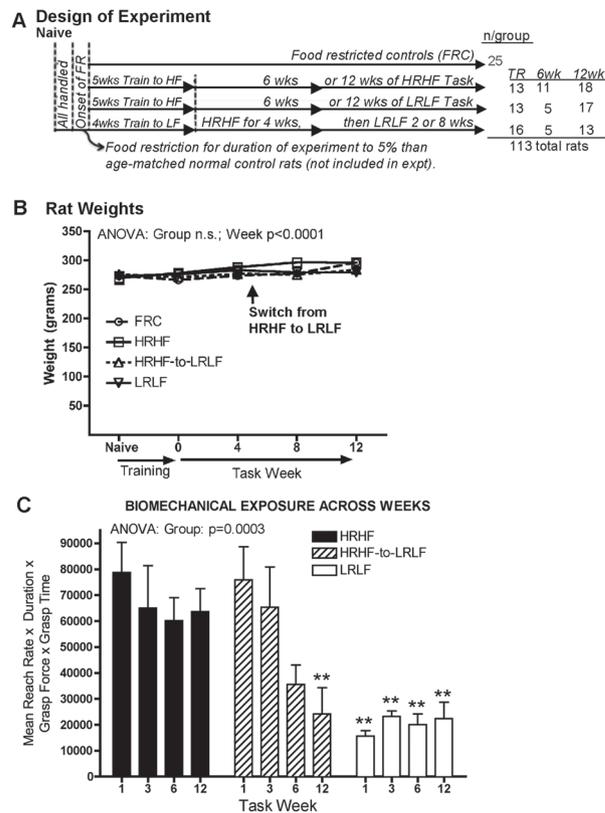
Female rats that were young adult to mature adult in age across the course of the experiment were used in this study for several reasons: (1) Human females have a higher incidence of work-related musculoskeletal disorders than males<sup>2, 37–39</sup>, although it is acknowledged in the literature that in empirical research with workplace populations, it is difficult to distinguish if observed male-female differences are due to biological or social factors<sup>40, 41</sup>; (2) and for comparison to data from our past studies on same aged female rats using this model, such as those examining HRHF task-induced reach performance and tissue degradative/degenerative changes<sup>25, 32–35, 42–46</sup>, and the effectiveness of ibuprofen intervention in reducing these changes<sup>32, 35, 46</sup>.

## Subjects and Methods

### Animals and overview

All experiments were approved by the Temple University Institutional Animal Care and Use Committee and were in compliance with NIH guidelines for humane care and use of laboratory animals. A total of 136 young adult, female, Sprague-Dawley rats were used. Rats were procured at 2 months of age, a point slightly older than the onset of puberty at 50 d of age<sup>47</sup>, and housed for 4 wk so that they were 3 months of age (90 d of age), and therefore young adult rats<sup>48, 49</sup>, at the onset of the experiments. They were 7 months of age at completion of the experiments (mature adults)<sup>47, 48</sup>. All were food-restricted to body weights of 5% less than age-matched normal controls (the latter were used for weight-matching purposes only). These rats were randomly divided into groups as shown in Fig. 1A: (1) 25 food-restricted control rats that did not undergo training or task performance (FRC rats); (2) 26 food-restricted rats that underwent an initial training period for 10 min/d, 5 d/wk, for 5 wk to learn a high force reaching task (TRHF) before euthanasia and division into two groups of 13 each in order to serve as statistical controls for the two HRHF task groups; (3) 16 food-restricted rats that trained to learn a low force reaching task (TRLF) before euthanasia, 4) 29 food-restricted rats that underwent the high force training before performing a high repetition high force task for 2 h/d, 3 d/wk, for 6 or 12 wk (HRHF rats, n=11 or 18, respectively); 5) 18 food-restricted rats that underwent the low force training before performing a low repetition low force task for 2 h/d, 3 d/wk, for 6 or 12 wk (LRLF rats, n=5 or 13, respectfully); and 6) 22 rats performed that underwent the high force training before performing the HRHF task for 4 wk, before switching to the LRLF task for 2 or 8 wk (HRHF-to-LRLF rats, n=5 or 17, respectfully).

Some of these data have been previously reported, and used since the FRC, LRLF and HRHF rats serve as negative and positive controls, respectively. This includes: some of the microCT, bone cell counts and serum biomarker results from one-third of the FRC rats (n=7), half of the TRHF rats (n=6), the eight 12-wk HRHF rats, and 3 of the 12-wk LRLF rats (the latter for which only radial bone data has been previously reported)<sup>25, 35</sup>. MicroCT data has been extended for these groups to include not only additional rats, but also to include degree of anisotropy and structural model index analysis in the microCT data for radial and ulna trabeculae, since they are indices of trabecular bone strength. Importantly, this is the first time we have reported dynamic bone histomorphometry in this model, IL-1alpha



**Fig. 1. Experimental design showing onset of food restriction, training period, duration of repetitive task performance, and number of animals utilized.**

FR: food restricted; training period: rats were trained to perform a high force or a low force task (Trained to high force (HF) or Low force (LF)). After training, rats performed a high repetition high force task (HRHF) or a low repetition low force (LRLF) task for 6 or 12 wk. HRHF-to-LRLF rats performed the HRHF task for 4 wk before switching to a LRLF task for 2 or 8 wk. Ends of arrows indicate points of euthanasia. (B) Rats were weighed weekly from the naïve time point to euthanasia. n.s.: not significant. The switch from HRHF to LRLF occurred at beginning of week 5, as indicated. (C) The biomechanical exposure per week was estimated by multiplying mean reach rate per minute, duration of task performance per week, mean voluntary grasp force in grams, and mean voluntary grasp time in seconds that rats held the force lever. &&:  $p < 0.01$ , compared to age-matched HRHF rats. Arrow indicates onset of intervention in HRHF-to-LRLF rats. ANOVA and posthoc findings are shown in individual panels; and mean + SEM shown here and hereafter.

in diaphyseal bone, estimated load per task week, ulnar trabeculae microCT data for 12-wk LRLF rats, serum biomarker data for 6-wk HRHF and 6-wk LRLF rats, and all LRLF and HRHF-to-LRLF rat data.

All rats were housed in an AAALAC-accredited animal facility in separate cages with a 12 h light:dark cycle, and free access to water and environment enrichment toys. In addition to 45 mg food pellet rewards (a 1:1 mix of

purified grain and banana flavored pellets; Bioserve, NJ, USA), all rats received Purina rat chow daily (i.e. same food reward and chow rations). Since rats were 3 months of age at onset, and 7.5 months of age at the point of tissue collection, all were allowed to gain weight as a consequence of normal growth (Fig. 1B).

#### *Behavioral apparatuses, training and task regimens*

The custom-designed behavioral apparatuses used were as previously described and depicted<sup>43, 44</sup>. Briefly, rats reached through a shoulder height portal and then pulled 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. The load cell output was interfaced with a signal conditioner (Analog Devices, Norwood, MA, USA), which amplified and filtered the signal before it was sampled digitally at 100 Hz with Force Lever software (Med Associates, St. Albans, VT, USA). Custom written Force-Lever software allowed us to choose a set force level before a food reward was provided (version 1.03.02, Med Associates). Every 15 or 30 s, a series of auditory indicators (Stimulus Clicker; Med Associates, St. Albans, VT, USA) lasting 5 s cued the animal to attempt a reach. The animal was trained to grasp the force lever bar and pull toward the chamber wall with a graded effort that was a percentage of the maximum grip strength of the included task rats and normal control rats (the latter not included in study) for at least 50 milliseconds<sup>43, 46</sup>. If reach and force criteria (defined below) were met within a 5 s cueing period, a 45 mg food pellet was dispensed into a trough located at floor height for the animal to lick up.

Prior to the initiation of the experiments, and as shown in Fig. 1A, all rats were handled for 10 min/d for 1 wk. All rats were initially food-restricted for 7 d to no more than 10–15% less than their naive weight to initiate interest in the food reward pellets (a 1:1 mix of grain-based and banana-flavored pellets). After that week, they were given extra rat chow to gain weight quickly back to only 5% less than age-matched normal control rats (not included in study). Rats were weighed weekly, maintained at 5% less than age-matched normal controls until euthanasia, and allowed to gain weight (Fig. 1B). Twenty-five rats were randomly chosen as food-restricted controls (FRC rats) that rested until euthanasia at time points matched to HRHF rats (Fig. 1A).

The remaining food-restricted rats were randomly chosen to be TRHF, TRLF, HRHF, LRLF or HRHF-to-LRLF rats. The TRHF, HRHF and HRHF-to-LRLF rats learned a high force reaching task, while the TRLF and LRLF rats

learned a low force reaching task across a 5-wk training period of 10 min/d, 5 d/wk, as described in detail previously<sup>25, 35</sup>. By the end of the 5-wk training period, the rats were able to perform either the LRLF task of two reaches/min at 15% of their maximum voluntary force (30 g) or a HRHF task of four reaches/min at 60% of their maximum voluntary force (110 g). The rats reached the HRHF level only during the last days of their 5th week of training. TRHF and TRLF rats were euthanized and tissues collected at this time point.

After training, task rats went on to perform one of three tasks for 2 h/d, 3 d/wk for up to 12 wk. The daily task was divided into four 30-min sessions separated by 1.5 h each in order to avoid satiation. The first group of task rats, the HRHF rats, were cued to reach at a rate of 8 reaches/min and to grasp the force lever bar at a target force effort of  $60 \pm 5\%$  of the mean maximum pulling force<sup>25</sup>. The second group of task rats, the LRLF rats, were cued to reach at a rate of 2 reaches/min and to grasp the force lever bar at a target force effort of  $15 \pm 5\%$  of the mean maximum pulling force<sup>25</sup>. The third group of task rats, the HRHF-to-LRLF rats, performed the HRHF task for 4 wk before switching to the LRLF tasks in weeks 5–12 of this 12 wk task. All rats had to grasp the force lever bar and exert an isometric pull at their respective target level for at least 50 milliseconds to receive a food reward. Rats were allowed to use their preferred limb to reach (the “reach” limb), as described and depicted previously<sup>44</sup>. Tissues were from task rats were collected and assayed from the dominant reach limb.

#### *Estimation of biomechanical exposure per week*

Force lever data were recorded continuously during each task session for later calculation of reach performance data (reach rate, duration, voluntary grasp force, and voluntary grasp time) via an automated script (MatLab; Mathworks, Natick, MA, USA). Force lever data were obtained from: a) twenty HRHF animals at week 1, and sixteen at weeks 3 and 6, and 8 at week 12; b) eight HRHF-to-LRLF rats at weeks 1, 3, 6 and 12; and c) twelve LRLF animals at weeks 1, 3, 6 and 12. Week 1 was used as the baseline for reach performance variables since that was the first week that task rats actually performed the 2 h/d task regimens. As described previously<sup>46</sup>, reach rate was the average number of all reaches performed per minute; duration was the mean number of minutes that the rats actually performed the task on a given day; mean voluntary grasp force was the average force (in grams) applied to the force handle per reach; and mean voluntary grasp time was the time (in seconds)

that the rats held the force handle per reach. Data for each variable was calculated on the last day of weeks 1, 3, 6 and 12. The biomechanical exposure per week was then estimated by multiplying the reach rate per minute (RR), duration of task performance per day (Dur) and the number of days of task performance per week (3 d/wk) in order to estimate the duration of task performance per week, mean voluntary grasp force in grams (GF), and mean voluntary grasp time in seconds (GT).

#### *MicroCT analysis*

Approximately one-half of the animals were utilized for microCT analysis. These animals were euthanized by lethal overdose (Nembutal, 120 mg/kg body weight), perfused transcardially with 4% paraformaldehyde in 0.1 M PO<sub>4</sub> buffer (pH 7.4), forelimb bones were collected, cleaned of soft tissues, and stored in phosphate buffered saline with sodium azide until micro-CT analysis of the radius and ulna from the reach limbs of the following groups: FRC (n=12), TRHF (n=14), TRLF (n=8), 12 wk HRHF (n=8), 12 wk HRHF-to-LRLF (n=6), and 12 wk LRLF (n=4).

MicroCT analysis was performed according to recent guidelines<sup>50</sup> and as previously described<sup>25</sup>. Briefly, a Skyscan 1172 microCT scanner (Skyscan, Ltd., Antwerp, Belgium) was used to scan a 6 mm length of distal radial and ulna bones: source voltage of 59 kV, source current of 167  $\mu$ A, aluminum 0.5 mm filter, rotation steps of 0.40°, frame averaging of 4, ring artifact correction of 10, beam hardening correction of 60%, and an isotopic voxel size of 8 micrometers. Image slices were reconstructed using cone-beam reconstruction software (Skyscan NRecon). Using Skyscan CT Analyzer software, morphological traits of distal metaphyseal trabeculae were assessed starting 1 mm proximal to the growth plates, and extending proximally for 1 mm (112 slices). The volume of interest for trabecular microarchitectural variables was bounded to a few pixels within the endocortical margin. An upper threshold of 255 and a lower threshold of 75 were used to delineate each pixel as bone or non-bone, using simple global thresholding methods. 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, where 0 represents isotropic organization) were measured in 3 dimensions (3D). 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”<sup>51</sup>. Bone mineral density (BMD) was also assayed in metaphyseal trabeculae in

the 12-wk HRHF-to-LRLF rat bones, using previously described methods<sup>35</sup>. The BMD results were compared to previously published FRC and 12-wk HRHF data<sup>35</sup>.

#### *Histomorphometry of the distal radius metaphysis*

Cohorts of FRC, TRLF, TRHF and HRHF bones assayed for microCT were used for histomorphometry, as well as bones from additional LRLF rats, so that n=6/group were analyzed. Histomorphometry was performed according to the recommendations of the American Society for Bone and Mineral Research<sup>52</sup>. To measure dynamic bone formation parameters, calcein (i.p., 10 mg/kg body weight) had been previously injected at 9 and 2 d before euthanasia. Then, for plastic embedding, fixed forearm bones (n=4–6/gp) were preserved in 70% ethanol and embedded, undecalcified, into methyl methacrylate (MMA) resin. Bones were sectioned into 5  $\mu$ m longitudinal sections, placed onto charged slides (Fisher Scientific, Tissue Path Superfrost Slides), and dried at 60 °C overnight. Unstained plasticized longitudinal sections were used for the measurement of calcein fluorochrome label, using a Nikon microscope, digital camera, and image analysis system (Bioquant Osteo 2012, v12.1, Nashville, TN, USA). Dynamic parameters of trabecular bone formation were collected from unstained at 50  $\mu$ m below the growth plate and in from the cortical bone using a 20  $\times$  objective. Bone formation rate (BFR) was assessed by measuring single-labeled surface for single perimeter (sL.Pm), double-labeled surface (dL.Pm), and the interlabel distance in the dL.Pms. Mineral apposition rate (MAR, mm/d) and bone formation rate normalized to bone surface (BFR/BS) were calculated<sup>53</sup>. Adjacent sections were deplasticized and then stained with Masson’s Trichrome for counting osteoclasts, or were stained immunohistochemically using a mouse anti-rat anti-CD68 (a marker of osteoclast, macrophages and their progenitors<sup>54–57</sup>); MCA341R Serotec, Raleigh, NC, USA) at a dilution of 1:250 for overnight at 4 °C. This was followed by a secondary goat anti-mouse antibody tagged with horseradish peroxidase (Jackson ImmunoResearch, West Grove, PA, USA) at a dilution of 1:100 for 2 h at room temperature. The horseradish peroxidase was visualized using diaminobenzidine (DAB) with a metal enhancer substrate system (SigmaFast D0426, Sigma-Aldrich). Only CD68+ multinucleated stained cells counted as osteoclasts. Numbers of osteoclasts and osteoblasts were normalized to bone surface (N.Oc./BS and N.Ob/BS). Histomorphometry was performed on 3 sections/limb by a person naive to group assignment.

### Biochemical analyses

To study serum biomarkers of bone turnover, as well as bones for presence of inflammatory biomarkers, the remaining animals were euthanized with an overdose of sodium pentobarbital (Nembutal; 120 mg/kg body weight) at 18 h after completion of the final task session to avoid any “exercise” induced changes in blood profiles, as previously described<sup>58</sup>. Blood was collected by cardiac puncture using a 23-gauge needle from: FRC rats (n=17), TRHF (n=16), TRLF (n=6), 6-wk and 12-wk HRHF (n=6 and 11, respectfully), 6-wk and 12-wk HRHF-to-LRLF (n=6 and 11, respectfully), and 6-wk and 12-wk LRLF rats (n=6 and 9, respectfully). Blood was centrifuged at 1,800 g for 20 min at 4 °C, serum collected and flash-frozen, and then stored at -80 °C until analyzed using commercially available kits for CTX-1 (Immunodiagnostic systems, RatLaps EIA, # AC-06F1) and osteocalcin (Immunodiagnostic systems, Rat-MID Osteocalcin EIA # AC-12F1), in duplicate, and presented as pg of protein per ml of serum.

Radius and ulna bones were collected for biochemical assays from rats from the following groups: FRC rats (n=13), TRHF (n=12), TRLF (n=8), 12-wk HRHF (n=11), 12-wk HRHF-to-LRLF (n=12), and 12-wk LRLF (n=8) rats. Distal and diaphyseal regions were analyzed separately for interleukin (IL)-1alpha using a commercially available ELISA kit (BioSource™, Invitrogen Life Sciences, CA, USA), using described methods<sup>31</sup>. 12-wk HRHF-to-LRLF rat bones were also examined for levels of IL-1beta and TNF-alpha, and results compared to previously published FRC and 12-wk HRHF data<sup>35</sup>. Data for ELISA assay data (pg cytokine protein) were normalized to µg total protein, determined using a bicinchoninic acid (BCA) protein assay kit.

### Statistical analyses

A two-way ANOVA was used to compare rat weights across time using the factors of time (naïve through week 12) and group (FRC, HRHF, HRHF-to-LRLF and LRLF). A two-way ANOVA was used to compare biomechanical exposure across time using the factors of time (task weeks 1, 3, 6 and 12) and group (HRHF, HRHF-to-LRLF and LRLF). Two-way ANOVAs were also used for the radial and ulnar microCT trabecular attributes, osteoblast and osteoclast numbers, and for the bone levels of IL-1alpha data, using the factors of group (HRHF, HRHF-to-LRLF and LRLF) and week in task (FR, trained, and 12 wk). To provide data for these two-way ANOVAs, FRC rats were randomly divided into three even subgroups, as were TRHF rats, with n=4–9/subgroup depending on the vari-

able. In this manner, there was enough FR data and trained only data for week in task factor for each group. Two-way ANOVAs were also used for the serum CTX-1 and osteocalcin data, using the factors of group (HRHF, HRHF-to-LRLF and LRLF) and week in task (FR, trained, 6 wk, and 12 wk). To provide data for these serum biomarker two-way ANOVAs, FRC rats were randomly divided into three even subgroups, as were TRHF rats, with n=4–9/subgroup depending on the subgroup. One-way ANOVAs were used for the BMD, MAR and BFR/BS data. For each two-way and one-way ANOVA, the Bonferroni post-hoc method for multiple comparisons was used, with comparisons to each other. Adjusted *p*-values are reported, and after adjustment, a *p* value of <0.05 was considered statistically different. All data are expressed as mean ± standard error (SEM). The *p* values for the ANOVAs are reported in individual graphs, as are post hoc findings.

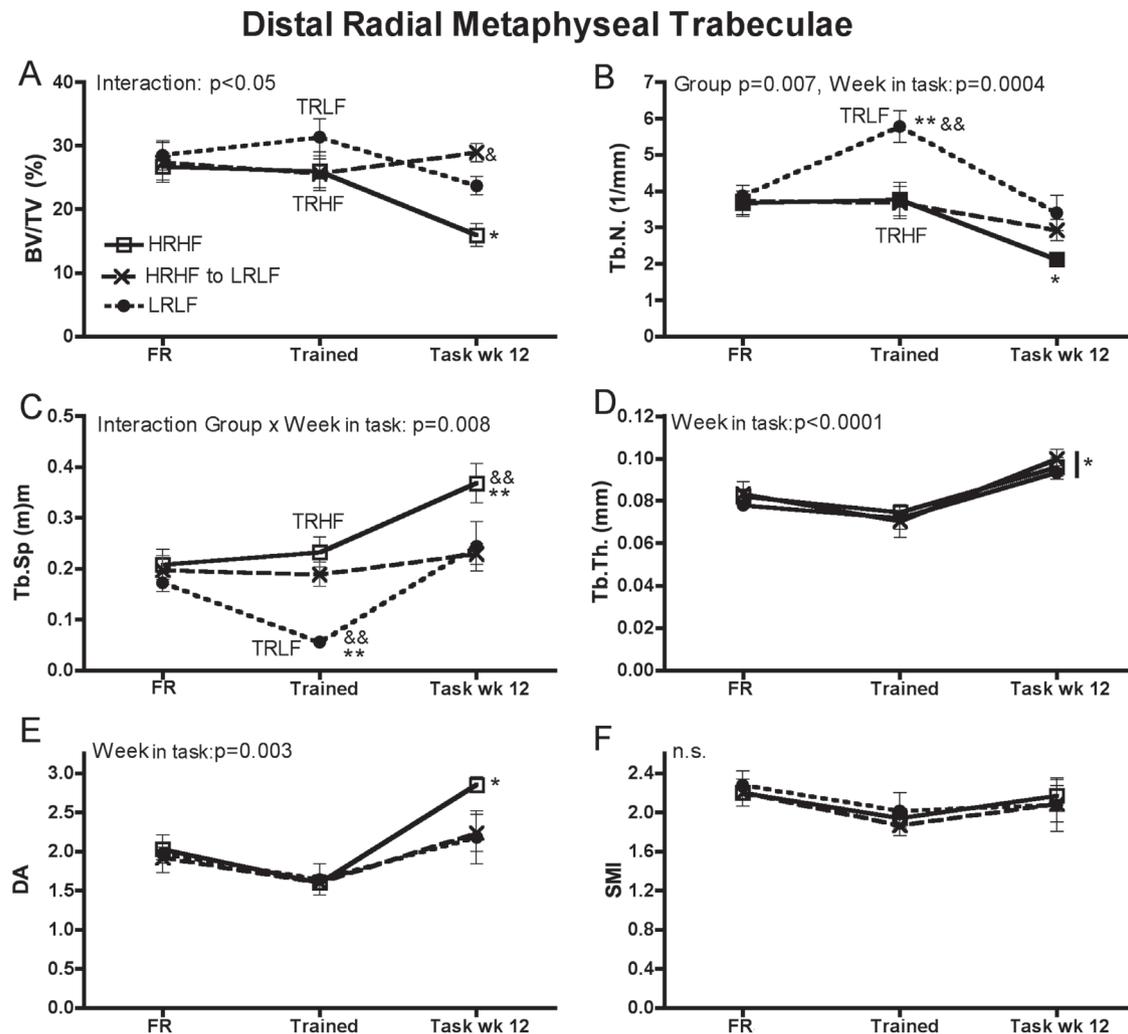
## Results

### *No changes in rat weights between groups*

A two-way ANOVA indicated that there were no significant differences in weight between the task rat groups, compared to FRC rats (not significant). All rats gained weight in a consistent manner across the weeks of the experiment (Fig. 1B). Thus, any changes observed in bone structure between groups were not due to changes in body weight.

### *Biomechanical exposure estimates indicate HRHF rats performed at highest exposure, LRLF rats at lowest exposure, and HRHF to LRLF rats successfully reduced their exposure*

As described in detail in the methods, the amount of biomechanical exposure per week, per group, was determined by multiplying several performance parameters: reach rate/min × duration/day × number of days per week × voluntary grasp force in grams, and voluntary grasp time in seconds. There was a significant difference in biomechanical exposure levels between the groups (Fig. 1C). HRHF rats had at a statistically consistent exposure amount across task weeks, as did LRLF rats. LRLF rats had at a significantly lower exposure level than the HRHF rats each week (Fig. 1C). HRHF-to-LRLF rats worked at the HRHF exposure level in weeks 1 and 3, less in week 6 (at a level that was not statistically different from either the HRHF or the LRLF groups), and at the LRLF exposure level in week 12, indicating that the reduced exposure intervention was achieved.



**Fig. 2.** MicroCT analysis of trabeculae of the distal radial metaphysis.

Results for trabecular bone volume (BV/TV), trabecular number (Tb.N), trabecular separation (Tb.Sp), trabecular thickness (Tb.Th), degree of anisotropy (DA) in which 0=isotropic 1=anisotropic, and structural model index (SMI) in which 1=plates, 2=rods, 3=cylinders, are shown. \* and \*\*:  $p < 0.05$  and  $p < 0.01$ , compared to age-matched FRC rats; & and &&:  $p < 0.05$  and  $p < 0.01$ , compared to HRHF rats.

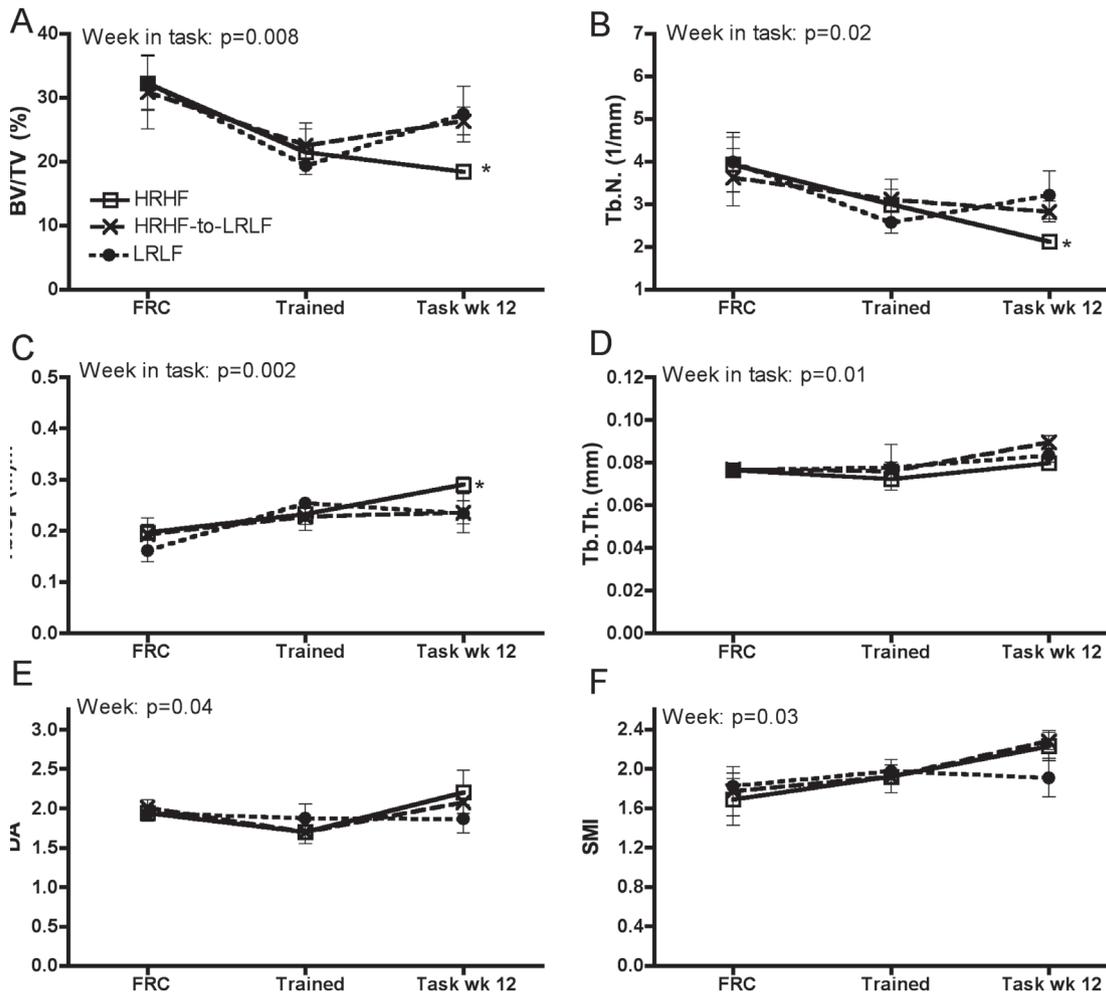
#### *Training to low force enhances indices of bone formation*

Training to the low force level (TRLF) lead to bone formation changes in trabeculae of the distal radial metaphysis, such as increased bone volume (BV/TV), trabecular number (Tb.N) and reduced trabecular separation (Tb.Sp), compared to FRC rats (Fig. 2A–C). Training to the low force level induced no detectable changes in distal ulna trabeculae (Fig. 3). However, training to the high force level (TRHF) induced no statistically detectable changes in the radial or ulnar metaphyseal trabeculae, compared to FRC rats (Figs. 2 and 3). These results indicate a positive effect of training to low force, but not to this level of high force, with regard to radial trabecular bone quality.

#### *Ergonomic task reduction prevents HRHF-induced trabecular bone resorption and allowed net bone formation*

Twelve weeks of HRHF loading induced resorptive changes in the radial and ulnar metaphyseal trabeculae, compared to FRC rats (Figs. 2, 3 and 4A), as previously described<sup>25, 35</sup>, despite inclusion of additional rats from those past studies. These changes included decreased trabecular bone volume density (BV/TV), decreased trabecular number (Tb.N) and increased trabecular separation (Tb.Sp) in both the radius and ulna, compared to FRC rats (Figs. 2A–C; 3A–C). We report here for the first time that the degree of anisotropy (DA) was increased in radial trabeculae of 12-wk HRHF rats, compared to age-matched FRC rats (Fig. 2E), indicative of disorganization

### Distal Ulnar Metaphyseal Trabeculae



**Fig. 3. MicroCT analysis of trabeculae of the distal ulnar metaphyseal regions metaphysis.**

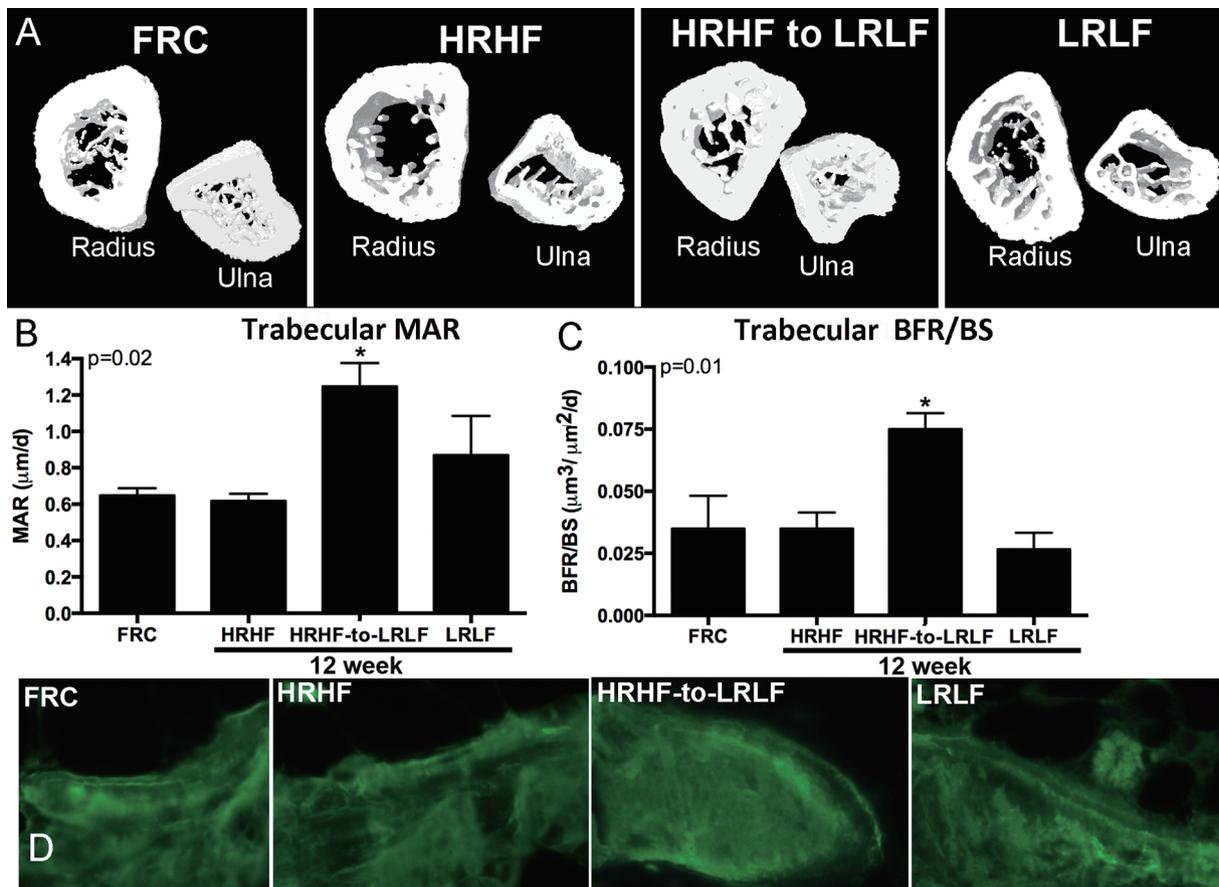
Results for trabecular bone volume (BV/TV), trabecular number (Tb.N), trabecular separation (Tb.Sp), trabecular thickness (Tb.Th), degree of anisotropy (DA), and structural model index (SMI) are shown. \* and \*\*:  $p < 0.05$  and  $p < 0.01$ , compared to age-matched FRC rats; & and &&:  $p < 0.05$  and  $p < 0.01$ , compared to HRHF rats.

in trabeculae distribution<sup>59</sup>). The structural model index (SMI) was unchanged in radial trabeculae (Fig. 2F), yet slightly increased in distal ulna trabeculae of 12-wk HRHF rats (ANOVA  $p = 0.03$ ; Fig 3F), although there were no significant posthoc findings. The latter shows a slight change from the more favorable plate-like trabeculae to the rod-shaped that could contribute to decreased bone strength<sup>60</sup>).

In contrast, there were no signs of trabecular bone resorption in radial or ulnar trabeculae of HRHF-to-LRLF and LRLF rats, compared to FRC rats (Figs 2, 3 and 4A), and we observed an increase in trabecular bone volume density (BV/TV) in HRHF-to-LRLF rats, compared to HRHF rats (Fig. 2A). We also observed increased BV/TV and decreased Tb.Sp in radial trabeculae of HRHF-

to-LRLF, compared to HRHF rats (Fig. 2A, C). The structural model index (SMI) was unchanged in radial trabeculae of HRHF-to-LRLF rats (Fig. 2F), yet slightly increased in distal ulna trabeculae of 12-wk HRHF and HRHF-to-LRLF rats (ANOVA  $p = 0.03$ ; Fig 3F). HRHF-to-LRLF rats had similar bone mineral density (BMD) levels in radial trabeculae as FRC rats ( $p > 0.05$ ; HRHF-to-LRLF:  $1.28 \pm 0.01$ , versus FRC:  $1.08 \pm 0.05$ ), a level that was significantly lower than in HRHF rats ( $0.92 \pm 0.02$ ;  $p < 0.01$ ). Combined, these results indicate that bone resorptive changes observed in trabeculae of radial and ulnar metaphyses of 12-wk HRHF rats were prevented by switching HRHF rats in week 4 to a LRLF task for 8 wk.

Another index of trabecular adaptation was seen in



**Fig. 4. Representative images of distal radius and ulna, and dynamic bone histomorphometry.**

(A) Representative 3D models of transaxial microCT slices through the metaphyseal region of the left ulnar and radial bones of FRC, and 12-week HRHF, HRHF-to-LRLF or LRLF rats. These transaxial reconstructions are located from 1.0 to 2.0 mm proximal to the respective growth plates, and are viewed from the bottom looking towards the growth plate. (B) Trabecular mineral apposition rate in the radius (MAR). (C) Trabecular bone formation rate (BRF), normalized to bone surface (BS), in the distal radial metaphyseal trabeculae. (D) Representative microscope images showing calcein double labeling in radial trabeculae of each group. \*:  $p < 0.05$ , compared to age-matched FRC rats.

each of the 12-wk task rats (HRHF, LRLF and HRHF-to-LRLF), in that each showed increased trabecular thickness in distal radial metaphyses, compared to FRC rats (Fig. 2D).

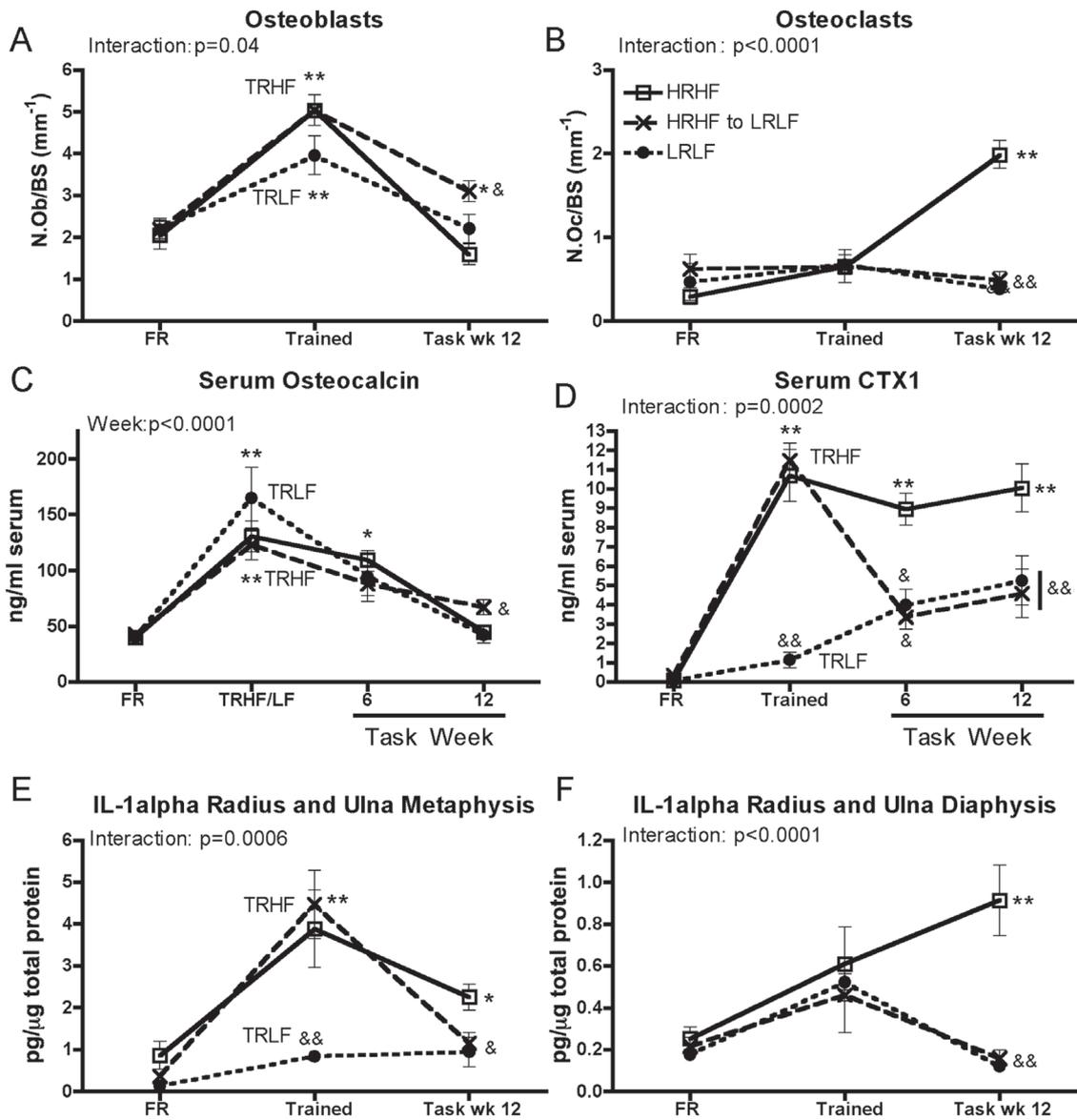
#### *HRHF-to-LRLF task promotes an osteogenic response and prevents an inflammatory and osteoclastic response*

After sectioning the bones and using bone histomorphometric methods, we observed that the mineral apposition rate (MAR) in distal radial metaphyseal trabeculae was increased in 12-wk HRHF-to-LRLF rats, compared to FRC rats, as was bone formation rate/bone surface (BFR/BS) (Fig. 4B, C). Such increases in bone formation were not observed in the other groups. Representative photographs of trabeculae show increased distance between calcein double-labeling in 12-wk HRHF-to-LRLF rats, compared to the other groups (Fig. 4D).

Osteoblast numbers were increased in distal radial tra-

beculae after training in TRHF and TRLF rats, compared to FRC rats, and in 12-wk HRHF-to-LRLF rats, compared to age-matched FRC and 12-wk HRHF rats (Fig. 5A). Osteoclast numbers were highest in 12-wk HRHF rats, compared to FRC rats (Fig. 5B), as previously described<sup>25, 54</sup>, and at baseline levels in TRHF, TRLF, HRHF-to-LRLF and LRLF rats (Fig. 5B).

Serum osteocalcin, a biomarker of bone formation, was increased in TRHF, TRLF, and 6-wk HRHF rats, compared to FRC rats, and in 12-wk HRHF-to-LRLF rats, compared to 12-wk HRHF rats (Fig. 5C). Serum CTX1, a biomarker of bone resorption, was increased in TRHF, and 6- and 12-wk HRHF rats, compared to FRC rats (Fig. 5D). Serum CTX1 was significantly lower in TRLF rats, compared to TRHF rats, and in HRHF-to-LRLF and LRLF rats, compared to HRHF rats at both 6 and 12 wk, which was 2 and 8 wk after onset of the ergonomic intervention (Fig. 5D).



**Fig. 5. Osteoblast and osteoclast histomorphometric counts, serum levels of biomarkers of bone turnover, and radial bone levels of IL-1alpha.** (A&B) Cellular density of osteoblasts (N.Ob.) and osteoclasts (N.Oc.), normalized to bone surface (BS), in distal radial metaphyseal trabeculae. (C&D) Serum levels of osteocalcin and CTX1, assayed using ELISA. (E&F) IL-1alpha in distal (metaphyseal) and diaphyseal radius and ulna, respectively, assayed using ELISA. \* and \*\*:  $p<0.05$  and  $p<0.01$ , compared to age-matched FRC rats; & and &&:  $p<0.05$  and  $p<0.01$ , compared to age-matched HRHF rats.

Since IL-1alpha is a potent stimulator of bone resorption<sup>61, 62</sup>, its levels were assayed in distal versus diaphyseal regions of the radial and ulnar bones. Bone IL-1alpha was increased in the distal radius and ulna of TRHF and 12-wk HRHF rats, compared to the other groups (Fig. 5E), and in the diaphyseal region of 12-wk HRHF rats, compared the other groups (Fig. 5F). HRHF-to-LRLF rats also had similar levels of IL-1beta in radial trabeculae as FRC rats ( $p>0.05$ ; HRHF-to-LRLF:  $1.23 \pm 0.24$ , versus FRC:

$1.09 \pm 0.13$ ), a level that was significantly lower than in HRHF rats ( $2.43 \pm 0.33$ ;  $p<0.01$ ). Similar results were observed for TNFalpha ( $p>0.05$ ; HRHF-to-LRLF:  $0.26 \pm 0.01$ , versus FRC:  $1.16 \pm 0.19$ ), a level that was significantly lower than in HRHF rats ( $7.19 \pm 1.04$ ;  $p<0.01$ ). Thus, ergonomic task reduction reduced bone inflammatory cytokines in HRHF-to-LRLF rats to baseline levels.

These results combined show that an ergonomic workload reduction intervention enhanced bone formation

processes and reduced osteoclastic and inflammatory responses in the HRHF-to-LRLF rats, despite continued task performance, all likely due to the reduction in high force loading on the bones.

## Discussion

Our biomechanical exposure calculation in which continuous measures of reach performance variables were multiplied (reach rate, duration, grasp force, and grasp time) indicates that the reduced exposure intervention was achieved by the HRHF-to-LRLF rats by week 12 (biomechanical exposure of  $24,120 \pm 10,160$ ), compared to the HRHF rats in week 12 (biomechanical exposure of  $63,620 \pm 8,822$ ,  $p < 0.01$ ) and the LRLF rats in week 12 ( $22,319 \pm 6,347$ ,  $p$  value not significant.).

Our hypothesis that net bone resorption would be reduced if 4-wk HRHF rats were switched to the LRLF task for 8 wk, was supported in that no indicators of bone resorption were observed in trabeculae of 12-wk HRHF-to-LRLF rats and that trabecular bone microarchitecture was similar to that in FRC rats. A pro-inflammatory cytokine response that began during training in TRHF rats, and present in the HRHF group, was not present in 12-wk HRHF-to-LRLF rats. Instead, osteogenic responses were observed in 12-wk HRHF-to-LRLF rats (such as increased trabecular mineral apposition rate and bone formation rate/bone surface). However, although there was increased trabecular bone volume in HRHF-to-LRLF rats, compared to HRHF rats, this increase was not greater than that observed in FRC rats, suggesting that adjustments to this ergonomic intervention are needed in future experiments in order to enhance bone formation rather than just prevent trabecular bone loss.

The initial period of low force training had a positive adaptive effect on distal radial trabeculae of TRLF rats. Increased osteoblasts and serum osteocalcin in TRHF rats were tempered by small increases in osteoclast numbers<sup>35</sup>, and increased serum CTX-1 and bone IL-1 $\alpha$ , the latter a potent stimulator of bone resorption<sup>61, 62</sup>. The combination likely contributed to the lack of a net increase in trabecular bone volume in TRHF rats. The trained only rats were young adult rats of 3 months of age at the onset of the experiments<sup>48, 49</sup>, and 4.5 months of age at the end of training and time of their tissue collection, a period of rat growth and moderate weight gains (Fig. 1A)<sup>47–49, 63</sup>. Mechanical deformation serves as an epigenetic guiding stimulus for skeletal morphology<sup>64</sup>, particularly during growth, a point of peak bone mass accrual<sup>65</sup>. The

contribution of cyclical low loading to bone growth and cyclical high loading to bone resorptive changes was highlighted by the bone quality differences observed in TRLF and TRHF rats, respectively.

Many indicators of net bone resorption and weaker trabecular bone microarchitecture were present in 12-wk HRHF rats, matching our past results showing that this level of high repetition high force task is past the fatigue failure point for the tissues<sup>25, 27–30</sup>, compared to lower demand reaching and pulling tasks which show no response or bone adaptation depending on task demands<sup>25</sup>. Such changes are attenuated by secondary ibuprofen treatment at anti-inflammatory levels, when provided in weeks 5–12 of this 12-wk HRHF task<sup>32, 35</sup>. This latter finding indicates that a HRHF-induced inflammatory response helps drive the bone resorptive and cartilage degeneration processes. We extended our prior work here to include additional animals as well as trabecular DA and SMI examination using microCT, and found a significant increase in DA in radial trabeculae of 12-wk HRHF rats and a small increase in SMI in ulnar trabeculae of HRHF as well as HRHF-to-LRLF rats. The increased DA is indicative of disorganization in trabeculae distribution and a weaker bone that is less resistant to mechanical loading due to increased stiffness<sup>59</sup>. The increase in SMI in 12-wk HRHF and HRHF-to-LRLF rats is a remodeling change that increases bone surface available for resorption and a change linked to osteoporosis<sup>51</sup>. Longer performance of the HRHF task may lead to higher increases in SMI, although further studies are needed to be certain. The 12-wk HRHF rats lacked dynamic osteogenic responses, and showed only one sign of bone adaptation (increased trabecular thickness). The presence of several catabolic changes and one anabolic response in the HRHF rats is indicative of the complex response of bone to higher load conditions in which the adaptive response may fall short of injury-inducing demands being placed on the tissues as a result of the high force high repetition loading<sup>66</sup>, particularly when localized load concentrations develop as a result of increasing external task demands<sup>16</sup>. To be clear, it is thought that the higher the demands of a task, the heavier the bone damage becomes<sup>25, 66, 67</sup>.

Pathological trabecular bone remodeling and loss of bone volume density was avoided in the HRHF-to-LRLF rats that were switched from the HRHF task in week 4 to a LRLF task for 8 wk, a time period when the rats are more mature and are 154–210 d of age (5–7 months of age). The preservation of trabecular bone quality and volume was equal to age-matched FRC and LRLF task rats, and equal to that observed in a prior study using ibuprofen as a

secondary intervention in HRHF task rats<sup>35</sup>). The reduced IL-1 alpha, a cytokine known to stimulate osteoclastogenesis and activity<sup>11, 12</sup>, likely contributed to the baseline osteoclast numbers and absence of indices of bone resorption in the 12-wk HRHF-to-LRLF rats. Their increased osteogenic responses, yet no net increase in bone volume, could be because the 12-wk task rats were 7 months of age at the time of bone assessment, a point of maturity when musculoskeletal maturity is reached and bone remodeling is slowing<sup>18, 47-49, 63</sup>). On the other hand, we have previously shown that same aged (7 month old) adult female rats performing a high repetition low force task or a low repetition high force task had significant gains in bone volume density, compared to controls<sup>25</sup>), a point also shown in the calcein findings in this current study. Prior studies from other labs show that bone responds to loading along a continuum ranging from anabolism to catabolism, depending on the magnitude, frequency and duration of loading<sup>15, 17, 20, 68</sup>), and that bone cells accommodate to customary loading, making them less responsive and less likely to show signs of adaptation with routine loading signals<sup>69</sup>). Therefore, the more likely reason for similar but not increased bone volume density in the HRHF-to-LRLF rats in week 12 is that more dynamic loading may be needed to induce an increase in trabecular bone volume in these adult rats, in which the rate of strain is varied during short daily periods, leads to increased bone volume<sup>70-72</sup>).

We have previously reported that LRLF rats showed no morphological changes in the distal radius<sup>25</sup>). We show here for the first time that they also show no changes in bone quality in the distal ulna and no dynamic osteogenic responses. Thus, prolonged loading at low repetition rates with low force loads was neither osteogenic nor osteodegenerative. Bone cells are known to accommodate to customary loading, making them less responsive and less likely to show signs of adaptation with routine loading signals<sup>70</sup>). The 12-wk LRLF results match this idea discussed above that bones accommodate early and can stop responding after initial loading periods, if force loads remain the same or decrease over time<sup>73, 74</sup>).

Human females have a higher incidence of work-related musculoskeletal disorders than males<sup>2, 37-39</sup>), although it is acknowledged in the literature that it is difficult to distinguish if observed male-female differences in workplace populations are due to biological or social factors<sup>39-41, 75</sup>). There is some evidence of gender differences in the prevalence of neck/shoulder disorders, which is generally higher in women<sup>38, 76</sup>). Higher muscle fatigability is generally reported in men<sup>77, 78</sup>), while women have decreased

strength<sup>39</sup>), each perhaps due to findings of increased type 1 fibers in muscles of females<sup>79</sup>). Also, women are less able to rearrange shoulder muscle activity as males and report higher perceived pain<sup>80</sup>). Gender differences have been observed in activation patterns of primary and secondary muscles during an isometric task performed at 50% maximum force<sup>81</sup>) with women showing more activation of accessory muscles and less activation of primary muscle groups than men. Concerning bone, Fausto-Sterling has pointed out that a trait like bone density is influenced by sex-linked biological factors, such as sex hormones, and by gendered social dynamics, including dress, occupation, and physical activity differences that contribute to bone density because they can create gendered variations in factors, such as sun exposure and vitamin D synthesis, or weight-bearing activities<sup>63</sup>). One human study shows that there is structural variability in the distal radius of young healthy males versus females, although BMD was not different between the two sexes<sup>82</sup>). In 12-wk old Wistar rats, sexual dimorphism has been observed in trabecular bone structural parameters in response to exercise (treadmill training to 60% of maximum velocity, performed 5 times/week for 12 wk<sup>83</sup>). In that study, BV/TV was greater in female than male rats, irrespective of training, and exercise training increased this parameter in both sexes; Tb.N was higher in female rats, while Tb.Sp was greater in male rats, and exercise training did not affect Tb.N or Tb.Sp in either sex. Tb.Th increased with exercise training in male rats, while MAR was higher in female rats, and exercise training increased both MAR and BFR/BS in males but not in female rats<sup>83</sup>). Thus, female rats do not appear to have an increased brittleness to exercise (which would manifest as a significant decrease in BV/TV), although they did have differential adaptive responses. We saw different outcomes in our study examining female rats performing a work-related task, with Tb.Th increasing in all task groups by week 12, and MAR and BFR/BS increasing in the HRHF-to-LRLF rats by week 12, for example. Future experimental studies should consider the sexual dimorphism of bone in response to work-related tasks in their study design and data interpretation. Such studies would elucidate if male-female differences in human workplace populations are from sex-linked biological factors versus gendered social dynamics.

The observed differences in the radius and ulna in this current study are due to anatomical differences, since the radius has a direct articular with the carpal bones, whereas force applied to the ulna are transmitted across the interosseus membrane to the radius. For example, Schonau *et al.*

has shown that as much as 76% of distal radial bone variations can be explained by grip strength alone<sup>84</sup> (which would be indicated in our estimated loading levels as grasp force levels), while Myburgh showed that 67% of ulna bone quality is indirectly affected by biceps strength<sup>85</sup>.

There are several limitations in this study. The increases in serum osteocalcin and CTX-1 could be occurring at any bone site involved in task performance, such as the metacarpal bones, diaphysis of forelimb bones, or humerus. Therefore, one limitation of this study is that we did not examine other bony sites of the upper extremity for bone changes. We used an operant rat model of overuse. The effectiveness of this ergonomic type intervention should be repeated in human subjects. We used studied young adult female rats to the point of musculoskeletal maturity (7 months of age). The effectiveness of this ergonomic type intervention should be repeated in more mature rats and in male rats. Lastly, we used some previously published data from the 12-wk HRHF and LRLF rats for comparison purposes<sup>25, 35</sup>. However, we believe that this data was needed to show the effectiveness of this ergonomic task intervention in preventing HRHF-task induced trabecular bone loss.

In conclusion, this study shows task modification to reaching and pulling at lower rates and load levels prevented overload-induced trabecular bone resorptive changes in association with reduced bone inflammatory cytokines and osteoclasts, and enhanced indices of bone formation. The amount of trabecular bone preservation was equal to that observed in a prior study using ibuprofen as a secondary intervention<sup>35</sup>. These findings support the idea of reduced workload as an effective approach to management of WMSDs and begin to define the reach rate and load level boundaries for establishing activity ranges for such interventions. Future studies should be directed at using job modification such as reduced repetition rates and force to enhance bone volume in workers showing signs of osteopenia. However, we suggest that types of strain should be more varied either across the day or across the week, to induce greater gains in bone volume as bones accommodate early and can stop responding if force loads remain the same or decrease over time<sup>73, 74</sup>.

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