**Skeletal site-specific effects of endurance running on structure and strength of tibia, lumbar vertebra, and mandible in male Sprague-Dawley rats**

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<td>13-Jan-2016</td>
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<td>Keyword:</td>
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https://mc06.manuscriptcentral.com/apnm-pubs
Skeletal site-specific effects of endurance running on structure and strength of tibia, lumbar vertebra, and mandible in male Sprague-Dawley rats

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Abstract

Bone microarchitecture, bone mineral density (BMD), and bone strength are positively affected by impact activities, such as running, however there are discrepancies in the magnitude of these changes. These inconsistencies are mainly a result of varying training protocols, analysis techniques, and whether skeletal sites measured are weight bearing or not. This study's purpose was to determine the effects of endurance running on sites that experience different weight bearing and load. Eight week old male Sprague-Dawley rats (n = 20) were randomized to either a progressive treadmill running protocol (25 m/min for 1 hour, incline of 10%) or a non-trained control group for 8-weeks. Trabecular structure of the tibia, lumbar vertebra (L3), and mandible, and cortical structure at the tibia midpoint were measured using µCT to quantify bone volume fraction, trabecular number, trabecular thickness, trabecular separation, and cortical thickness. BMD at the proximal tibia, lumbar vertebra (L1-3), and mandible was measured using DXA. Tibia midpoint strength was measured by three-point bending using a materials testing system. Endurance running resulted in superior bone structure at the proximal tibia (12% greater BV/TV, \( p = 0.03 \), 14% greater Tb.N, \( p = 0.01 \), and 19% lower Tb.Sp, \( p = 0.05 \)) but not other sites. Contrary to our hypothesis, mandible bone structure was altered following endurance training (8% lower BV/TV, \( p < 0.01 \), 15% lower Tb.Th, \( p < 0.01 \)), which may be explained by a lower food intake, resulting in less mechanical loading from chewing. These results highlight the site-specific effects of loading to the skeleton.

Keywords: bone morphology, bone mineral density, three-dimensional structure, trabecular bone, cortical bone, exercise, endurance training, microcomputed tomography
Introduction

Exercise as a form of mechanical loading improves or maintains overall bone mineral areal density, which is important for overall bone health (Calatayud et al. 2013). Exercise is defined as an activity requiring physical effort (i.e. running, swimming, or jumping) to maintain or improve health and fitness. Although there is controversy on the type, duration, and intensity of exercise, impact activities (i.e., running and jumping) apply mechanical loading to weight bearing bones and increase bone mineral mass, bone structure, and bone strength (Notomi et al. 2000; Rizzoli et al. 2010; Gomez-Cabello et al. 2012). Benefits to bone health have been observed in young growing animal models (Raab et al. 1990; Iwamoto et al. 1999; Joo et al. 2003; Wu et al. 2004; Wallace et al. 2007; Huang et al. 2008), aging animal models (Beyer et al. 1985; Raab et al. 1990; Yeh et al. 1993; Davicco et al. 1999), ovariectomized (Peng et al. 1997; Sakakura et al. 2001) and orchidectomized animal models (Horcajada et al. 1997), as well as during food restriction in conjunction with voluntary wheel running (Hattori et al. 2013). The ‘mechanostat’ theory has widely been used to explain these changes in bone mass and architecture in response to functional loading. It suggests that the bone and other signals that convey the mechanical usage of bone are involved in the feedback loop for bone to adapt its mechanical properties to match its functional needs (Frost 1987). Although the focus of the present paper was physiological exercise, we recognize the importance of work done investigating this ‘mechanostat’ theory and the modeling and remodeling of bone with *in vivo* animals studies using artificial loads applied to bones in rodent models (Rubin et al. 1985; Torrance et al. 1994; Mosley et al. 1997; De Souza et al. 2005).
Non-weight bearing exercise, such as swimming, can also have positive affects on bone, because there are loads to the bone being generated internally by the muscle that modulate bone. Our rationale for potential systemic effects of exercise is based on findings that non-weight bearing swimming exercise has positive effects on bone (Swissa-Siva et al. 1990; Synder et al. 1992; Huang et al. 2003) and that running exercise could prevent not only tibia bone loss but also mandible bone loss in an ovariectomized rat model (Sakakura et al. 2001). However, there remains limited evidence on the systemic effects of exercise on bone.

In rodents, there are inconsistencies in the magnitude of responses associated with treadmill running. These inconsistencies are a result of variations in training protocols used (i.e., duration, intensity, mode), analysis techniques, and bone sites measured (weight bearing vs. non-weight bearing sites within a bone). Prior to microcomputed tomography ($\mu$CT), the primary bone outcome measures used to evaluate effects of training were bone mineral content (BMC) and bone mineral density (BMD) using dual energy X-ray absorptiometry (DXA) that do not provide information about bone structure and strength.

Running has been documented to improve weight bearing bones using a variety of techniques (i.e., DXA, mechanical strength testing), but there is limited research using $\mu$CT that measures structural changes in bone, and compares multiple bone sites to elucidate the systemic effects of exercise on bone. Our hypothesis was that endurance running would have a positive effect on areal BMD, structure, and strength due to direct loading and potential systemic effects. We further hypothesized that endurance running would have the greatest affect on weight bearing bones, the tibia, as well less weight
bearing, the lumbar vertebra, and systemic effects on non-weight bearing bones, the mandible. We determined the effects of an 8-week endurance treadmill protocol in young male rats on bone sites varying in weight bearing with prolonged running: tibia (weight bearing), lumbar vertebra (somewhat weight bearing), and mandible (non-weight bearing).

**Material & Methods**

**Animals**

Male Sprague-Dawley rats (age 51-53 days) were purchased from Charles River Laboratories (Quebec, Canada). All experimental protocols and procedures conformed to the Canadian Council on Animal Care guidelines (Olfert E.D. 1993) and were approved by Brock University Animal Care Committee. The present study was apart of a larger study that examined the effects of endurance running on muscle lipid metabolism (Turnbull et al. 2015). Rats were housed in pairs from the same condition (endurance-trained or control) in the Comparative Bioscience Facility in a reverse 12:12 light-dark cycle. Rats had *ad libitum* access to water and food, a standard rodent chow (Tekland Global 14% protein, Harlan Tekland Global, Mississauga, ON) throughout the study.

**Training Protocol**

Following a 5 day acclimatization period to allow for recovery from transport, rats were randomly assigned to either the control condition (n = 10) or trained condition (n = 10), however one rat was unable to complete the training protocol resulting in a n = 9. Rats were housed in pairs with a cage mate from the same condition (either control or trained). Trained rats began the treadmill training protocol at a speed of 18 m/min for 30 minutes and progressed to a speed of 25 m/min for 1 hour by week 7, all at an incline of 10% as previously described (Duan et al. 1994; Dyck et al. 2000). Weekly food intake
was calculated with the following equation: food intake = (food provided-food remaining)/2, since rats were housed in pairs. Body weight was also measured weekly.

Anesthesia

Rats were anaesthetized with isoflurane (Benson Medical Industries Inc., Markham, ON) with an induction rate of 5% inhalant and monitored between 3-5% during the surgical procedures for the extraction of tissues for the muscle lipid metabolism study prior to euthanasia (Turnbull et al. 2015). Following the procedure the rats were euthanized under anesthesia and tissues including the tibia, whole vertebral column and mandible were excised, cleaned of tissue, wrapped in gauze soaked in phosphate buffered saline solution (Sigma-Aldrich, St. Louis, MO) and stored at -80°C until further analysis.

Bone Microarchitecture Using Microcomputed Tomography

The right tibia, lumbar vertebra (L3), and right mandible were scanned ex vivo using µCT (Bruker SkyScan 1176, Kontich, Belgium). The bones were wrapped in parafilm, secured in foam holders, and scanned at a 9 µm resolution. For the tibia the X-ray source was set at a voltage of 65kV with a current of 385µA, the LV was set at a voltage of 77mV with a current of 313µA, and the mandible was set at a voltage of 86kV with a current of 278µA. For all scans an integration time of 1140ms, rotation step of 0.2°, and 1mm aluminum filter were used. Acquired images were reconstructed using NRecon (software version 1.6.9.10, Bruker, Kontich, Belgium) and image reorientation was performed and transaxial images were saved using Dataviewer software (software version 1.5.1.3, Bruker, Kontich, Belgium). For each bone site the same parameters were applied to all the samples to ensure consistency (tibia: smoothing= 1, ring artifact= 3, beam
hardening= 30%; lumbar vertebra: smoothing= 1, ring artifact= 5, beam hardening= 30%; and mandible: smoothing= 0, ring artifact= 3, beam hardening= 40%;). Regions of interest were selected and analyzed using CTAn software (software version 1.14.4.1+, Bruker, Kontich, Belgium). To examine the structural properties of trabecular bone at the proximal tibia metaphysis, a region of interest consisting of 200 slices (1.8 mm) with a separation of 30 slices (0.27 mm) from the distal end of the growth plate was selected by manually contouring a few pixels within the endocortical shell. To examine cortical bone at the tibia midpoint, a region of interest of 200 slices (1.8 mm), 100 slices above and below the midpoint, was selected by manually contouring a few pixels away from the endocortical boundary. A region of interest spanning the height of L3 vertebral body varying between samples, excluding 30 slices (0.27 mm) from the proximal and distal growth plates, was selected by manually contouring a few pixels within the endocortical shell. The mandible region of interest was drawn at the interalveolar bone beginning where the four roots of the first molar appear and ending where all the roots become no longer visible, with the region of interest varying between samples, as previously described (Johnston et al. 2015). Global thresholding was used to segment bone from the background. The upper threshold value was 255 and the lower threshold for each sample was automatically assigned using the Otsu threshold method (Otsu 1979). Prior to scanning an alignment test was performed, as well as uncorrected and corrected flat fields for each scanning day. The flat fields were performed to test the X-ray source and signal transmission to the detector. During the scan period the unadjusted flat fields were consistent indicating that there were no deviations in the X-ray source or signal transmission to the detector.
Following guidelines suggested by (Bouxsein et al. 2010) the following \( \mu \)CT parameters were reported. The trabecular outcome measures for the proximal tibia, lumbar vertebra (L3), and mandible included the bone volume fraction (i.e., bone volume divided by total volume (BV/TV)), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp). Cortical outcome measures for the tibia midpoint included cortical bone area (Tt.Ar), total cross-sectional area (Tt.Ar) and cortical area fraction calculated by cortical bone area over total area (Ct.Ar/ Tt.Ar), and cortical thickness (Ct.Th).

The coefficient of variation (100 x standard deviation/mean) was calculated for experimenter error by selecting the region of interest five times from randomly selected bones at the proximal tibia, lumbar vertebra, and mandible. The coefficient of variation for the bone outcome measures, BV/TV, Tb.Th, Tb.N, and Tb.Sp, at each bone site was below 1%.

Bone Mineral Density Using DXA

Areal BMD of the right tibia, specifically the proximal one third, the lumbar vertebrae (L1-L3), and the right mandible bones were analyzed \textit{ex vivo} using DXA (Orthometrix, Naples, Florida) with a scan speed of 10 mm/s and a resolution of 0.2 x 0.2. The \textit{ex vivo} scans were analyzed using specialized software (Host Software version: 3.9.4; Scanner Software version: 1.2.0). Coefficient of variation (100 x standard deviation/mean) was calculated for areal BMD from randomly selected bones, scanned five times, for each skeletal site: 0.9% for the proximal tibia, 0.9% for the lumbar vertebrae and 0.6% for the mandible. Quality assurance and quality control phantoms were scanned prior to each scanning session.
Bone Strength Testing Using a Materials Testing System

Tibia bones were physically fractured by a three point bending test using a materials testing system (Instron 3300 Single Column Universal Testing System, Norwood, MA) fitted with a customized jig. Prior to strength testing, bones were soaked in 1x phosphate buffer saline solution (Sigma-Aldrich, St. Louis, MO) for 3 hours. The length of the tibia bones was measured using an electronic caliper (Electron Microscopy Sciences, Hatfield, PA) to determine the midpoint. The lateral surface of the tibia was placed on two 1 mm wide base supports with a jig span width of 15 mm so that the midpoint was positioned directly under the crosshead. The crosshead was then lowered at a speed of 2 mm/min applying force to the tibia midpoint until fracture occurred to determine the peak load, the maximum force a bone can withstand until fracture. All data from the strength testing was recorded using the Bluehill program (software version 2.26.815, Instron, Norwood, MA).

Statistical Analysis

One-tailed unpaired t-tests were conducted for each outcome measure at each bone site. Body weights and food weights were analyzed as a repeated two-way analysis of variance (ANOVA) for training status x time (week). Correlation analysis was also performed for food intake and mandible bone outcomes to determine if these variables covaried and the extent of the relationship. Statistical significance for all tests was accepted at p < 0.05.

Results

Body Weight and Food Intake
This study was a part of a previously published larger study that examined alterations in muscle lipid metabolism (Turnbull et al. 2015). Food intake and body weight measures from that study are repeated here because they are important to help explain the observed bone outcomes. The endurance training protocol resulted in lower body weight ($p < 0.001$, Table 1) and lower food intakes throughout the study starting at the first week ($p < 0.001$, Table 2) compared to the control group.

**Ex vivo Tibia Outcomes**

At the proximal tibia the endurance training protocol resulted in a 12% greater BV/TV ($p = 0.03$, Fig.1A) and a 14% greater Tb.N ($p = 0.01$, Fig.1B) compared to the controls. In addition, the trained group had a 19% lower Tb.Sp ($p = 0.05$, Fig.1D) compared to the controls. However, there was no significant difference in Tb.Th ($p = 0.10$, Fig.1C) between the endurance-trained and control groups. At the tibia midpoint there were no significant differences for any of the cortical bone outcomes, Ct.Ar ($p = 0.15$), Tt.Ar ($p = 0.13$), Ct.Ar/ Tt.Ar ($p = 0.27$), and Ct.Th ($p = 0.27$) between groups. BMD at the proximal tibia ($p = 0.22$, Table 3) did not differ between groups. Peak load at tibia midpoint was also similar between groups ($p = 0.21$).

**Ex vivo Lumbar Vertebra Outcomes**

For lumbar vertebrae (L3) there were no significant differences between groups for any of the microarchitectural outcomes: BV/TV ($p = 0.13$, Fig.2A), Tb.Th ($p = 0.09$, Fig.2B), Tb.N ($p = 0.36$, Fig.2C), or Tb.Sp ($p = 0.10$, Fig.2D). In addition, no significant differences in the L1-L3 BMD ($p = 0.48$, Table 3) were observed between groups.

**Ex vivo Mandible**
At the mandible, trained rats had 8% lower BV/TV ($p < 0.01$, Fig.3A) and 15% lower Tb.Th ($p < 0.001$, Fig.3B). However, the endurance-trained had 5% more Tb.N ($p = .02$, Fig.3C) compared to control rats, but this did not improve the overall change in percentage bone volume in endurance-trained rats. There was no significant difference in Tb.Sp ($p = 0.19$, Fig.3D) between groups. In addition, no significant differences in the mandible BMD ($p = 0.09$, Table 3) were observed between groups. Significant positive correlations were found between food intake and mandible BV/TV ($R^2 = 0.35$, $p = 0.02$) and Tb.Th ($R^2 = 0.51$, $p < 0.01$), but not for Tb.N ($R^2 = 0.17$, $p = 0.11$) and Tb.Sp ($R^2 = 0.06$, $p = 0.37$).

**Discussion**

The present study is the first to examine the effects of a treadmill running protocol on both weight bearing (i.e., tibia and lumbar vertebra) and non-weight bearing (i.e., mandible) bones using µCT and DXA to provide a comprehensive set of bone outcomes: BMD, bone structure, and bone strength. The 8-week endurance training protocol had no effect on tibia, lumbar vertebrae, or mandible BMD and improved bone structure at the proximal tibia, had no significant effect at the lumbar vertebra (L3), and altered bone structure at the mandible, possibly due to a lower food intake in the endurance-trained rats. This study highlights the site-specific effects of loading with an 8-week progressive treadmill training, with direct loading required to have an affect on bone structure.

The greater trabecular percentage bone volume at the proximal tibia was a result of greater trabecular number and lower trabecular separation. Results for the proximal tibia are consistent with findings from another study that examined the effects of endurance running using a similar training protocol and found similar changes in the
trabecular microarchitecture using µCT (Joo et al. 2003). They found a greater trabecular bone volume that was attributed to the creation of new trabecular, and lower trabecular separation, but also found greater trabecular thickness. Although the cortical geometry of the tibia in the present study did not differ between groups, these investigators found greater cortical width and bone area with endurance training in the femoral diaphysis (Joo et al. 2003). It is important to note the differences in protocols, as the animals in the previous study began training at a younger age (4 weeks) and trained for a longer duration (10 weeks), which may explain the differences seen in the results with the present study. In addition only the femur was analyzed, although long bones of the lower limbs are expected to respond similarly, the proximal tibia is more responsive to training than the femur, at least in terms of areal BMD (Hagihara et al. 2005). Since the tibia is closer to the ground than the femur it experiences more of the ground reaction forces and is considered to be the more loaded bone. In other rodent models, the ground squirrel and chipmunk, it was reported that the tibia incurs significantly more stress than the femur (Biewener 1983). In addition, the proximal tibia a trabecular rich region is responsive to changes in load and is commonly studied (Iwamoto et al. 2000; Sakakura et al. 2001; Huang et al. 2003; Iwamoto et al. 2004; Hagihara et al. 2005; Hattori et al. 2013).

The lack of association between body mass and the bone outcomes suggests that the endurance training protocol was the cause of the improved bone outcomes seen at the tibia. The loading on the bones from the endurance running protocol had a greater affect on the tibia than the increased body weight of the controls.

The present study demonstrated that chronic endurance exercise does not affect BMD at the proximal tibia or biomechanical strength at the tibia midpoint. Some studies
have found a significant difference in the mechanical strength of the tibia midpoint (Huang et al. 2003), femur midpoint (Raab et al. 1990; Joo et al. 2003; Huang et al. 2003; Huang et al. 2008), and humerus midpoint (Raab et al. 1990) with exercise training, while other studies have found no difference at the tibia midpoint (Iwamoto et al. 2004), femur midpoint (Horcajada et al. 1997; Davicco et al. 1999; Hattori et al. 2013), and even reduced strength of the tibia midpoint (Li et al. 1991). Consistent with our study, Hattori et al. (2013) found no difference in BMD at the proximal tibia measured using DXA.

The present study demonstrated that lumbar vertebrae, a skeletal site considered to be partially weight bearing in rodents, showed no differences between the endurance-trained and control rats for any of the trabecular bone microarchitecture measures and ex vivo BMD. Findings from other rat studies have found similar results. One study showed that endurance exercise for 8 and 12 weeks in 4 week old female Sprague Dawley rats did not affect lumbar vertebra trabecular bone volume, as measured by dynamic bone histomorphometry (Iwamoto et al. 1999). Similar to the results in the present study, lumbar BMD appears not to change with endurance exercise training in other studies (Iwamoto et al. 2004). However, greater BMD at the lumbar vertebrae in female Sprague Dawley rats 14 months of age was observed with a lower intensity (17 m/min) but longer (16 weeks) endurance training protocol using DXA (Yeh et al. 1993). In this case, an aged female animal model was used, which is different from the young male animal model used in the present study. This suggests that age and sex is an important variable with respect to the positive effects of endurance training. As well, the duration of the training period appears to be extremely important. Studies using female animal models with longer training durations (11 and 12 weeks) report changes for some bone measures,
such as BMD, tibia bone formation rates as measured by dynamic bone histomorphometry, and serum bone markers, that were not observed with shorter training protocols (7 and 8 weeks) (Iwamoto et al. 1999; Iwamoto et al. 2000; Iwamoto et al. 2004). Therefore, we can speculate that with a longer training protocol than our 8 weeks, significant changes may have been detected at the lumbar vertebrae whereas tibia had improved microarchitecture as it is a weight bearing site, and thus more responsive to treadmill exercise training.

We chose the mandible bone as an example of a non-weight bearing bone during treadmill exercise but did not anticipate the endurance-trained rats would eat less than their sedentary counterparts (Table 2). The systemic effects of exercise were not observed in the present study, as there was an overall lower percentage bone volume that appeared to be due to decreased trabecular thickness. Paradoxically, there was greater trabecular number that did not affect the overall percentage bone volume and may have been an indication of a potential systemic effect of the endurance training. The endurance-trained rats ate significantly less than the controls and showed no symptoms or signs of stress. Therefore, they had less loading at the mandible than the controls due to differences in chewing and potentially overshadowed any systemic effects of exercise. It remains unknown whether trabecular number or thickness is altered first with direct loading and further research is required.

A limitation of the present study is the use of the mandible as a representative non-weight bearing bone during exercise, since the difference in food intake between the endurance trained and control groups was unexpected and likely a confounding factor. In hindsight different bones of the skull that are not involved in chewing would have been
chosen, however in our lab the mandible has previously been used and characterized. We did not anticipate the significant difference in food intake since many exercise intervention studies do not measure food intake and if we had, we would have pair-fed the sedentary group to the intervention group to investigate the systemic effects of exercise on bone health. Strengths of this study are the combination of the multiple skeletal sites chosen, which allowed for a comprehensive analysis of the skeleton and using treadmill running, which is a physiologically relevant perturbation. As well, the combination of analysis techniques, particularly the µCT, detected the changes associated with endurance training that would have otherwise gone unnoticed had only DXA and mechanical strength testing been used.

Although some research suggests that endurance running may not improve bone health since lumbar vertebra areal BMD is lower, subjects evaluated are training above recreational activity levels, approximately 90 km/week (Bilanin et al. 1989; Hind et al. 2006). The present training protocol would be comparable to recreational training in humans, which has been found to have positive affects on areal BMD (Callreus et al. 2012). The results from the present study highlight the structural changes in bone with a running protocol.

In conclusion, endurance running improves tibia microarchitecture at the proximal but not the midpoint region, likely because trabecular bone is more responsive than cortical bone to loading. Lumbar vertebrae remains unchanged due to less direct loading with running. We speculate that the altered structure of the mandible can be attributed to the lower food intake observed during the endurance training protocol (Hichijo et al. 2015), although there was greater trabecular number in the mandible that is not fully explained.
Endurance exercise in growing male rats has skeletal-site specific effects, suggesting the effect of bone loading during treadmill running is localized.

Acknowledgements

This study was supported by a Discovery Grant from the Natural Sciences and Engineering Research (NSERC) and Ontario Graduate Scholarship from the Ontario provincial government. Funding for the μCT was provided through the Canadian Foundation for Innovation. Dr. Ward holds a Canada Research Chair in Bone and Muscle Development.

The authors declare there are no conflicts of interest.
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Table 1. Body weight

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<td>333 ± 3</td>
<td>314 ± 3*</td>
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<td>2</td>
<td>402 ± 3</td>
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<tr>
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<td>463 ± 5</td>
<td>399 ± 5*</td>
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<td>590 ± 7</td>
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<td>7</td>
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Body weight for the controls compared to the trained rats from week 0 to week 7.
Weights were recorded once every week on the same day for all animals. Data is reported as the mean±SE, *, signifies significant differences between the control and trained for each individual week ($p<0.05$). As explained in the text, the results were also reported in Turnbull et al. (2015).
Table 2. Food intake

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</tbody>
</table>

Food intake of control compared to trained rats from end of week 1 until end of week 6. Food intakes were recorded per cage (2 rats/cage) at the end of every week. The difference between the weight of the food at the beginning of the week and at the end of the week was used to calculate the food intake per cage and divided by 2 as an estimate of food intake per rat. Data is reported as the mean ± SE, *, signifies significant differences between the control and trained for each individual week (p<0.05). Across the 6-week the trained rats had significantly lower food intakes compared to the control group (p < .0001). As explained in text, these results were also reported in Turnbull et al. (2015).
Table 3. *Ex vivo* bone mineral density (BMD, g/cm$^2$)

<table>
<thead>
<tr>
<th>Bone Site</th>
<th>Control (g/cm$^2$)</th>
<th>Endurance (g/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibia Proximal 1/3</td>
<td>0.196 ± 0.002</td>
<td>0.199 ± 0.008</td>
</tr>
<tr>
<td>Lumbar Vertebra (L1-L3)</td>
<td>0.230 ± 0.004</td>
<td>0.230 ± 0.004</td>
</tr>
<tr>
<td>Mandible</td>
<td>0.148 ± 0.003</td>
<td>0.142 ± 0.002</td>
</tr>
</tbody>
</table>

*Ex vivo* bone mineral density (BMD, g/cm$^2$) measured using dual energy X-ray (DXA) at three different bone sites: the proximal 1/3 of the tibia, lumbar vertebra (L1-L3), and the mandible bone. Data is reported as the mean ± SE. Bones were scanned at a speed of 10 mm/s and at a resolution of 0.2 x 0.2. No significant differences were observed between the control and endurance-trained rats at any of the bone sites measured.
Fig 1. Microcomputed tomography (µCT) outcomes for the proximal tibia metaphysis comparing the endurance trained (n = 9) to the controls (n = 9). Data is reported as the mean±SE. (A) The endurance training protocol resulted in an improved percentage bone volume, calculated as bone volume over total volume (BV/TV) (p = 0.03). (B) No differences between the endurance trained and controls was observed for trabecular thickness (Tb.Th) (p = 0.10). (C) The endurance trained group had a greater trabecular number (Tb.N) (p = 0.01). (D) The endurance trained has a lower amount of trabecular separation (Tb.Sp) compared to the controls (p = 0.05).

Fig 2. Microcomputed tomography (µCT) outcomes for the lumbar vertebra (L3) comparing the endurance trained (n = 9) to the controls (n = 10). Data is reported as the mean±SE. (A) No differences between the endurance trained and controls were observed for percentage bone volume, calculated as bone volume over total volume (BV/TV) (p = 0.13). (B) No differences between the endurance trained and controls was observed for trabecular thickness (Tb.Th) (p = 0.36). (C) No differences between the endurance trained and controls were observed for trabecular number (Tb.N) (p = 0.09), however there was a trend towards an increase in Tb.N in the endurance trained. (D) No differences between the endurance trained and controls were observed for Trabecular separation (Tb.Sp) (p = 0.10), however there was a trend towards an increase in Tb.Sp in the endurance trained.

Fig 3. Microcomputed tomography (µCT) outcomes for the mandible alveolar bone comparing the endurance trained (n = 8) to the controls (n = 8). Data is reported as the mean±SE. (A) The endurance trained had lower percentage bone volume, calculated as
bone volume over total volume (BV/TV), than the controls ($p < 0.01$). (B) The endurance trained had lower trabecular thickness (Tb.Th) than the controls ($p < 0.001$). (C) The endurance trained had more trabecular number (Tb.N) that the controls ($p = 0.02$). (D) No differences between the endurance trained and controls were observed for trabecular separation (Tb.Sp) ($p = 0.19$), however there was a trend towards an increase in Tb.Sp in the endurance trained.
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233x214mm (300 x 300 DPI)
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