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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

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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 months of age) and 14 young adult (2.5–6.5 months 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.

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 (OSHA, 2014; Bureau of Labor Statistics, 2012). Hand and wrist injuries are prevalent in occupations requiring upper extremity repetitive tasks, particularly with advancing age (Foss et al., 2011; Gerr et al., 2002). 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 (Bureau of Labor Statistics, 2011). 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 (Silverstein, 2008; Association of Occupational and Environmental Clinics, 2009; World Health Organization, 2007; WHO Scientific Group, 2003). 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 (NIOSH, 2011).

It is well known that chronic cyclical tissue overload, in general, affects bone morphometry (Green et al., 2011; Pearson and Lieberman, 2004; Warden et al., 2005; Sample et al., 2010; Robling et al., 2001; Burr et al., 2002). 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 (Ding et al., 2010; Solovieva et al., 2005; Vehmas et al., 2005).

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Bone scans of patients with upper extremity MSDs show increased blood flow and pooling (suggestive of inflammation) in affected forearm bones (Amorim et al., 2006; al-Nahhas et al., 1997). 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 (Erselcan et al., 2001; Savas et al., 2007). Although still under investigation, such bone changes may be due to increases in inflammatory cytokines occurring with prolonged performance of occupational tasks (Barbe et al., 2013a; Carp et al., 2007; Rechardt et al., 2013; Rechardt et al., 2011; Riondino et al., 2011; Jain et al., 2014), since similar cytokine increases in other chronic inflammatory conditions and in vitro stimulate osteoclastogenesis and activity and impair osteoblast differentiation (Guo et al., 2008; Kulkarni et al., 2012; Lacey et al., 2009; Lange et al., 2010; Thomas, 2010; Rani et al., 2010).

Aging is also linked to bone degradative changes and decreased bone mass (Khosla et al., 2011; Srinivasan et al., 2003; Turner, 2007; Turner et al., 1995). One possible cause may be the increased inflammatory cytokine levels and responses in tissues of aging mammals, compared to young adults (Ershler and Keller, 2000; Orjalo et al., 2009; Stowe et al., 2010; Xin et al., 2011a; Cevenini et al., 2010). 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 lead to either trabecular bone adaptation or pathological bone changes, dependent on repetition rate, force load, and duration of task (Jain et al., 2014; Rani et al., 2010; Barr et al., 2003; Barbe et al., 2013b). 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 (Barr et al., 2003; Barbe et al., 2013b). 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 are in the radius, concomitant with higher and more sustained increases in inflammatory cytokines (Barbe et al., 2013b; Driban et al., 2011; Rani et al., 2009). Although we have yet to examine the response of bones to prolonged performance of repetitive tasks in mature rats (14–18 months 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 (Xin et al., 2011a; Kietrys et al., 2012). The inflammatory cytokines were the same that affect bone cell homeostasis and induce net bone loss (interleukin-1beta and tumor necrosis factor alpha) (Lacey et al., 2009; Lange et al., 2010; Mihara et al., 1995).

Thus, the effects of performing repetitive tasks on bone architecture need 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 months 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 months 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 (Gerr et al., 2002; Srilatha et al., 2011; Ratzlaff et al., 2007; Cote, 2012); and 2) for comparison to bone data from our past studies on female rats using this model (Jain et al., 2014; Barr et al., 2003; Barbe et al., 2013b; Driban et al., 2011; Barbe et al., 2008). All rats were housed in a central animal facility in separate cages with a 12 h 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 Fig. 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 min/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 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 h/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 (Barbe et al., 2013b; Rani et al., 2009; Clark et al., 2004). 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 s 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