Abstract

Increased Tendon Calcification and a Bone Mineralization Protein in Musculoskeletal Tissues with Repetitive Reaching Task

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Introduction

Work-related musculoskeletal disorders (WMSDs) arise from repeated performance of tasks at submaximal levels of physical exertion that eventually lead to tissue damage due, perhaps, to insufficient recovery of tissues between bouts of performance. Our laboratory has developed a rat model of repetitive and forceful reaching and grasping. Our results indicate that performance of a high rate, low force (HRLF) task regimen results in injury, inflammation and fibrosis in bone, muscles, tendons, nerves and associated loose connective tissues.[1–3] Osteoactivin (OA) is a recently identified factor that plays a role in bone mineralization and possibly in wound healing and inflammation. Enhanced expression of this protein would suggest that repetitive and/or forceful tasks lead to accelerated bone remodeling and tendon matrix changes, which would further our understanding of the etiology of MSDs. The purpose of this current study was to examine the production of OA in musculoskeletal tissues and tendon calcification following performance of these repetitive and/or forceful tasks for up to 12 weeks.

Methods

Young adult, female Sprague-Dawley rats were used. Experimental rats were trained in one of two repetitive tasks. These tasks consisted of reaching forward to pull a lever at a rate of 4 reaches/min at either 15% of maximum grip strength (HRLF) for 2 hours/day in 30 min sessions, 3 days/week for up to 12 weeks. Results were compared to control rats, which are considered week 0 in this study.

Following euthanasia using Nembutal (120 mg/kg body weight), rats were perfused intracardially with 4% paraformaldehyde. Musculoskeletal tissues were harvested. Soft tissues were dissected out and frozen sectioned en bloc into 15 micrometer longitudinal sections, while bones were paraffin embedded and cut into 5 micrometer sections, before mounting on coated slides. Tissue sections on slides were analyzed for the presence of calcium salts using von Kossa staining. Sections with tendons were stained in 3% silver nitrate solution for 1.5 hours under intense light, rinsed in distilled water, 5% sodium thiosulfate for 2 minutes, and counterstained with nuclear red.

For localization of OA, sections were blocked for endogenous peroxidase, washed, permeabilized with 0.05% Pepsin solution in 0.01N HCL, washed and blocked for nonspecific binding with 10% goat serum for 20 minutes. Primary antibody raised against the c-terminal sequence of osteoactivin was diluted 1:100 with PBS and incubated on slides overnight at room temperature. After washing, sections were incubated with appropriate secondary antibody conjugated to HRP, and visualized using DAB with or without cobalt (black versus brown, respectively). Percent area with OA immunoreactivity was quantified using the Osteo II Bioquant program using methods described previously.[3] Two-way ANOVA ($p \leq 0.05$) was used to examine differences across weeks and between regions, followed by Bonferonni post hoc analysis.

Results

Discrete sites of von Kossa staining were present in forelimb tendons at the level of the wrist in week 6 and 12 HRLF rats (Fig. 1B), but not in controls (Fig. 1A). Von Kossa staining was greatest in HRLF week 12 forelimb tendons in matrix regions surrounding tendon fibroblasts. Supraspinatus tendons were also examined, but no von Kossa staining was present at any time point in either exposure group.

OA immunoreactivity increased in osteoclasts, periosteal cells, osteoctyes and osteoblasts (Fig. 1C), as well as in chondrocytes, tendon fibroblasts and mast cells, in 6–12 week HRLF rat radius and ulna. The highest OA immunoreactivity was seen in radius periosteum at sites of muscle and tendon attachments in week 5 (Fig. 2A), and in forelimb tendons at the level of the wrist in week 8 (Fig. 2B).

Discussion

Increases in osteoactivin (OA) staining in the periosteum parallels previously reported inflammatory responses in this same tissue, suggesting that OA may play a role in inflammation-induced bone remodeling by a repetitive reaching

and grasping task. The tendon OA increases in week 8 indicate matrix remodeling changes that are delayed compared to bony tissues possibly due to the avascular nature of tendon. Tendon von Kossa staining may indicate the onset of pathological calcific tendonitis as a result of performing repetitive and forceful tasks. Thus, this study shows that repetitive tasks catalyze changes at the cellular level in a variety of tissues.

References

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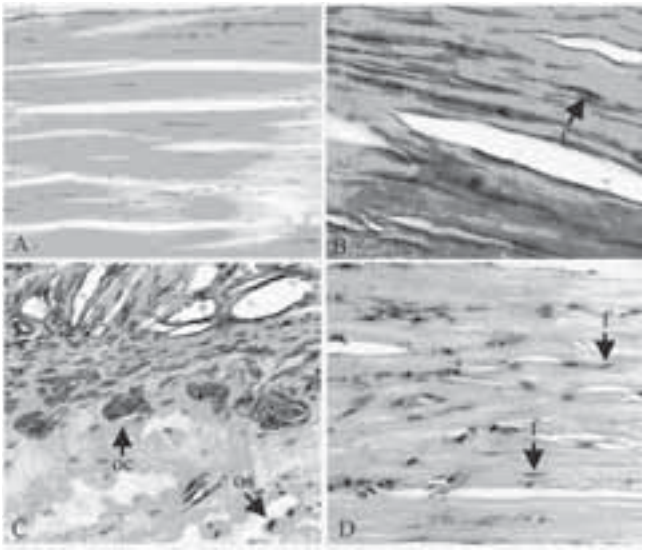

Figure 1. Flexor forelimb tendon vonKossa staining dramatically increased in HRHF 12 week rat tendon **(B)** compared to controls **(A). (C)** In HRLF week 12, OA increased in bone osteoclasts (oc) and osteocytes (os). **(D)** OA also increased in fibroblasts (f) in HRLF 12 week tendon.

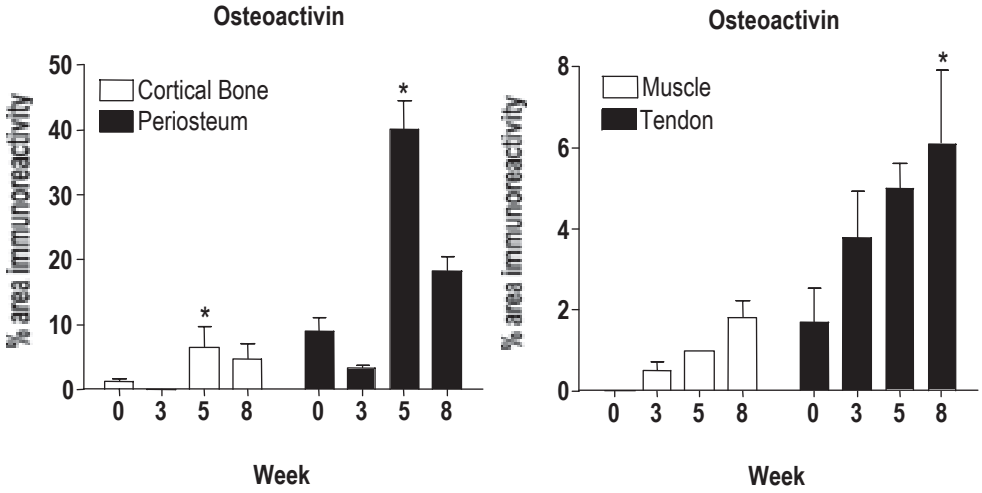

Figure 2. Graph shows that % area of osteoactivin immunoreactivity significantly increased in week 5 in cortical bone and periosteum, and in week 8 in tendon of HRLF rats. *$p < 0.001$.

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