The Final Progress Report, Completed Oct 29, 2012

Title Page

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List of Terms and Abbreviations

LRLF = low repetition low force
LRHF = low repetition high force
HRLF = high repetition low force
HRHF = high repetition high force
CTGF = Connective tissue growth factor
IL-1 = Interleukin 1
Macrophage inflammatory protein = MIP
MMP = Matrix metalloproteinase
RSI = repetitive strain injury
TNF-alpha = tumor necrosis factor alpha
TGFB1 = transforming growth factor beta 1
WDSD = work-related musculoskeletal disorder

Abstract (500 words or less)

Using a voluntary rat model of work overuse, we examined rats performing low to high demand work tasks of reaching and grasping for 12 to 24 weeks for tissue injury and inflammation, systemic inflammatory responses, tissue degradation and fibrosis, and associated sensorimotor behaviors, including sickness behaviors occurring as a consequence of performing repetitive work tasks with or without high force. Our research shows that work-related overuse injuries, particularly those resulting from long-term performance of repetitive and forceful tasks, lead to local and systemic inflammation, which drives tissue degradation and even sickness behaviors.

Our force-repetition interaction studies, in which rats perform different levels of tasks, indicate that even short-term performance of high repetition high force tasks leads to nerve damage, muscle and tendon injury, and eventually degeneration. High level tasks lead also to bone thinning, while moderate level tasks lead to bone growth.

We found that even moderate demand tasks lead to chronic low grade-chronic tissue and systemic inflammation that contribute to tissue degradation and fibrosis. The inflammation also lead to reduced motor performance and strength and increased discomfort. The sensory and motor declines were linked to increased inflammatory cytokines in the muscles, tendons, nerves and bones of the arm and hand used to perform this upper extremity work task. Thus, our laboratory has found a clear link between musculoskeletal overuse disorders and increased inflammatory cytokines in musculoskeletal tissues that contribute to weakness and pain.

We also observed sickness behaviors, specifically, decreased duration of social interaction and increased aggression in rats performing high demand tasks and in aged animals performing even moderate demand tasks. We assume the latter occurred since the systemic inflammatory response was greatly enhanced in aged animals compared to young adult animals performing the same work task. Several researchers have shown that increased inflammatory proteins in the blood, particularly pro-inflammatory cytokines can cause depression and other mood disorders, particularly in cancer patients treated with inflammatory cytokine drugs. And, several past studies have shown an increase in perceived job stress, depressed mood, increased absence from work due to sickness in patients with work-related musculoskeletal disorders. A link between perceived job stress and increased inflammatory proteins in blood has also been reported. However, we have demonstrated for the first time that there is a link between blood levels of pro-inflammatory cytokines, sickness behaviors, and work-related overuse injuries. We also found increased inflammatory cytokines in regions of the brain that form the blood brain barrier, and within the brain in regions that are responsible for sickness behaviors. In humans and animals, systemic infection causes many behavioral changes, including mood changes (such as anxiety and depression), fatigue, decreased motivation, reduced social interaction, and sleep disturbances. These symptoms are part of a coordinated brain response that changes the body's physiological and motivational state to preserve body integrity during illness.

The possibility that patients with overuse musculoskeletal disorders may develop tissue degradation, fibrosis, and sickness behaviors as a consequence of work-induced tissue and systemic inflammation should be considered when treating these patients.

Section 1 of the Final Progress Report (2-page limit)

Significant (Key) Findings.

Force-repetition interactions: We found that the ability of the rats to maintain target loads per week (force * repetitions) reduced over time in all but LRLF rats, with a positive interaction between force and interaction by week 12 between the groups. Signs of repetition and force-induced neuritis, injury and spinal cord sensitization were observed with training and task performance in the forepaw, median nerve and dorsal horns of cervical segments of the spinal cord. Repetition and force-induced forearm grip strength, flexor digitorum muscle and tendon inflammation, and injury were observed with both training and task performance to high force levels. Levels of MMP2 and TGFB1 increased, although not significantly in 12 week HRHF tendons. Hsp 70 levels were increased in the tendons of 12 week HRHF rats, compared to NC and each of the other groups. Levels of inflammatory cytokines in the serum increased, depending on the cytokine, with either training, task performance, or both. The distal radius showed signs of bone resorption with high force task performance for 12 week, while serum analysis of bone turnover markers indicates a general increase in bone degradation with the high force tasks but bone formation with the LRHF and HRLF tasks.

Muscle and tendon inflammation precede degradation and fibrosis (tendinitis preceeds tendinosis): We observed persistent declines in forearm grip strength with long-term overuse activity at a moderate task level. These declines were associated initially with a low-grade inflammatory response in forearm flexor digitorum muscles and tendons (evidenced by a small but significant increase in IL-1beta and TNF-α in the muscles and tendons immediately after training). As the task continued, serum levels of TNF-α rose significantly above baseline levels in week 12, an increase that was matched temporally by significant increases in TNF-α in flexor digitorum tendons. By week 18 of task performance, the serum inflammatory response had resolved, only to resurged\ in serum, tendons and muscle in week 24, albeit a key anti-inflammatory protein, such as IL-10 also increased in tissues in week 24. At the later time points of 18 and 24 weeks, serum biomarkers of degradation (MMP2), fibrosis (TGFB1), and repair also increased. The increased serum MMP2 and TGFB1 was coincident with increased MMP2 and TGFB1 in tendons. These findings show that even moderate demand tasks can induce tissue degradative changes if the work is performed for long periods of time. Also, the temporal association of grip strength declines with both the low-grade tissue inflammatory cytokine response and the degenerative changes support a contribution from each process to functional declines occurring with overuse. Lastly, serum biomarkers MMP2 and TGFB1 were pinpointed as markers of underlying tissue degenerative and fibrotic changes. Serum levels of CTGF levels were not tested in this study, but may also serve as a biomarker of tissue degradative processes induced by long-term performance of repetitive tasks.

A link between systemic inflammation and sickness behaviors with performance of high demand work tasks and aging: We have shown in a rat model of work-related overuse that prolonged performance of repetitive tasks increases serum inflammatory cytokines that correlate with sensorimotor declines. Since virally induced increases in serum inflammatory cytokines also correlate with increased brain cytokines and sickness behaviors, e.g. decreased social interaction and increased aggression, we examined these in our rat model. Young adult and aged female Sprague-Dawley rats were trained for 4-5 wks for 10 min/day, 5 days/wk at either a low force (TRLF; 15% max force) or a high force (TRHF; 60% max force) task level. The rats then performed a high repetition low force task (HRLF) of 8 reaches/min, 15% max force, for 2 hrs/day, 3 days/wk, for 9-24 wks. Duration of social interaction and incidence of aggression with novel juvenile rats were assessed every 3 wks. Results were compared to age-matched food-restricted controls (FRC). Following euthanasia, serum and brains were collected and analyzed for inflammatory cytokines. Behaviorally, at end of initial training period, both aged TRHF and TRLF rats had decreased social interaction with juvenile rats than at naïve, and FRC. Both aged and young TRHF rats had increased displays of aggression towards the juveniles than at naïve, behavior not observed in aged or young TRLF, NC or FRC rats. These behavioral changes in aged

TRHF rats did not resolve until week 9 of the HRLF task (termed TRHF-HRLF), but resolved by week 3 in the young adult TRHF-HRLF and in aged TRLF-HRLF rats. These behaviors were not observed in young adult TRLF-HRLF rats, even those performing the HRLF task for 24 weeks. There was an increase of serum interleukin 6 (IL-6) in aged and young TRHF ($p=0.01$ and $p=0.05$, respectfully) and in aged TRHF-HRLF rats that performed the task for 3 weeks; this increase resolved in these aged rats thereafter. No increase in serum cytokines was detected in young adult TRLF or TRLF-HRLF rats. Increased IL-6 and IL-6 receptor was also detected immunohistochemically in brains of aged rats with these sickness behaviors, specifically in the anterior cingulate cortex and paraventricular nuclei ($p<0.05$ each). No increase of IL6 or IL-6 receptor was observed in aged TRLF-HRLF rats or any young adult rats. Thus, training to high force, even for a short time period, induced increased serum IL6 and sickness behaviors. Aging also contributed to increased sickness behaviors and brain IL6 and IL-6 receptor

Translation of Findings.

Training to high force, even for a short time period, induced increased serum IL6 and sickness behaviors. Aging also contributed to increased sickness behaviors and brain IL6. Our studies document that a robust cytokine and chemokine response pattern is induced by prolonged performance of a repetitive task and leads an increased tissue and serum cytokine inflammatory response (Barbe et al 2003; Elliott et al, 2008, 2009, 2010; Rani et al, 2010, Xin et al, 2011), and then to increased sickness behaviors (Xin et al, submitted).

We followed up our initial studies showing systemic inflammation (Barbe et al, 2003), with studies on patients with upper extremity repetition strain injuries (Carp et al, 2007). We, and now other groups, Our group have reported that patients with RSIs of the upper extremity have increases in several serum pro-inflammatory markers and mediators, including C-reactive protein (CRP), IL-1, IL-1RII, IL-1ra, IL-6, IL-18, and TNF (Carp et al., 2007; Gotoh et al., 2001; Rechardt et al., 2011). In the Carp study, increased inflammatory markers correlated with the severity of upper-extremity signs and symptoms in patients short-term RSIs, including increased pain and an increase in pain frequency at multiple anatomical sites (Carp et al., 2007). Levels of CRP and TNF were predictive of RSI severity. Gotoh found that differential regulation of two forms of IL-1 receptor antagonist (IL-1ra) mRNAs also appear to play important roles in shoulder pain in rotator cuff diseases (Gotoh et al., 2001). Rechardt's group showed that levels of circulating soluble IL-1RII and IL-18 were associated with incipient upper extremity soft tissue disorders, suggesting an important role for IL-1 family members in these disorders (Rechardt et al., 2011). The possibility for patients with chronic inflammatory conditions to succumb to the sickness or depressive effects of local and systemic pro-inflammatory cytokines has implications in the management of RMI.

Outcomes/ Impact.

It is clear from the force-repetition interaction results that performance of a high repetition high force tasks for prolonged periods leads to chronic inflammatory and concomitant catabolic effects on many tissues types, even those at a distance from the localized injury site in involved tissues. Treatments needs to take into account the differential healing characteristics of each affected tissue.

A more complete understanding of the relationship between prolonged performance of repetitive tasks and the induction of the sickness response would help direct effective workplace and clinical management strategies. Such findings would also reduce the negative stigmatization often imposed by health care providers upon patients who present with such vague and apparently psychological complaints.

Although the findings of animal models of WMSDs cannot be directly generalized to human workers, the findings of such studies provide strong evidence of a complex pathophysiological process that involves multiple systems and tissues and leads to motor decline. Because the pathophysiology of repetitive motion injury is complex, the most effective therapeutic approach needs to be multifaceted. However, it is quite clear that earlier or preventive interventions are needed to avoid persistent motor dysfunction.

A summary figure of findings and papers in which they were published are shown below on page 6.

Section 2 of the Final Progress Report
Scientific Report.

A. SPECFIC AIMS AS FUNDED for 2006-2011 (the aims were changed from those stated in the original application because of large budgetary reductions (60%) made by NIOSH before the award.

The new aims as now stated on CRISP:
Specific Aim 1) To determine the extent to which long-term exposure to 2 task regimens, low repetition-low force (LRLF), and high repetition-low force (HRLF) causes tissue changes indicative of inflammation.
Specific Aim 2) To determine the extent to which long-term exposure to 2 task regimens (LRLF and HRLF) causes motor behavior changes indicative of inflammation.

Original aims before budget cuts:
Specific Aim 1) To examine if long-term exposure to lower demand tasks, e.g. low repetition-low force (LRLF) or even high repetition-low force (HRLF) regimens, causes compensatory, adaptive remodeling in musculoskeletal tissues.
Specific Aim 2) To examine if long-term exposure to the moderate demand task regimens, e.g. low repetition-high force (LRLF) and the HRLF regimens, causes persistent tissue injury and inflammation in musculoskeletal tissues.
Specific Aim 3) To examine if long-term exposure to the higher demand tasks, e.g. high repetition-high force (HRHF or even the LRHF regimens causes pathological tissue reorganization and degeneration in musculoskeletal tissues.
Specific Aim 4) To examine the effects of long-term exposure to the four task regimens on motor performance.

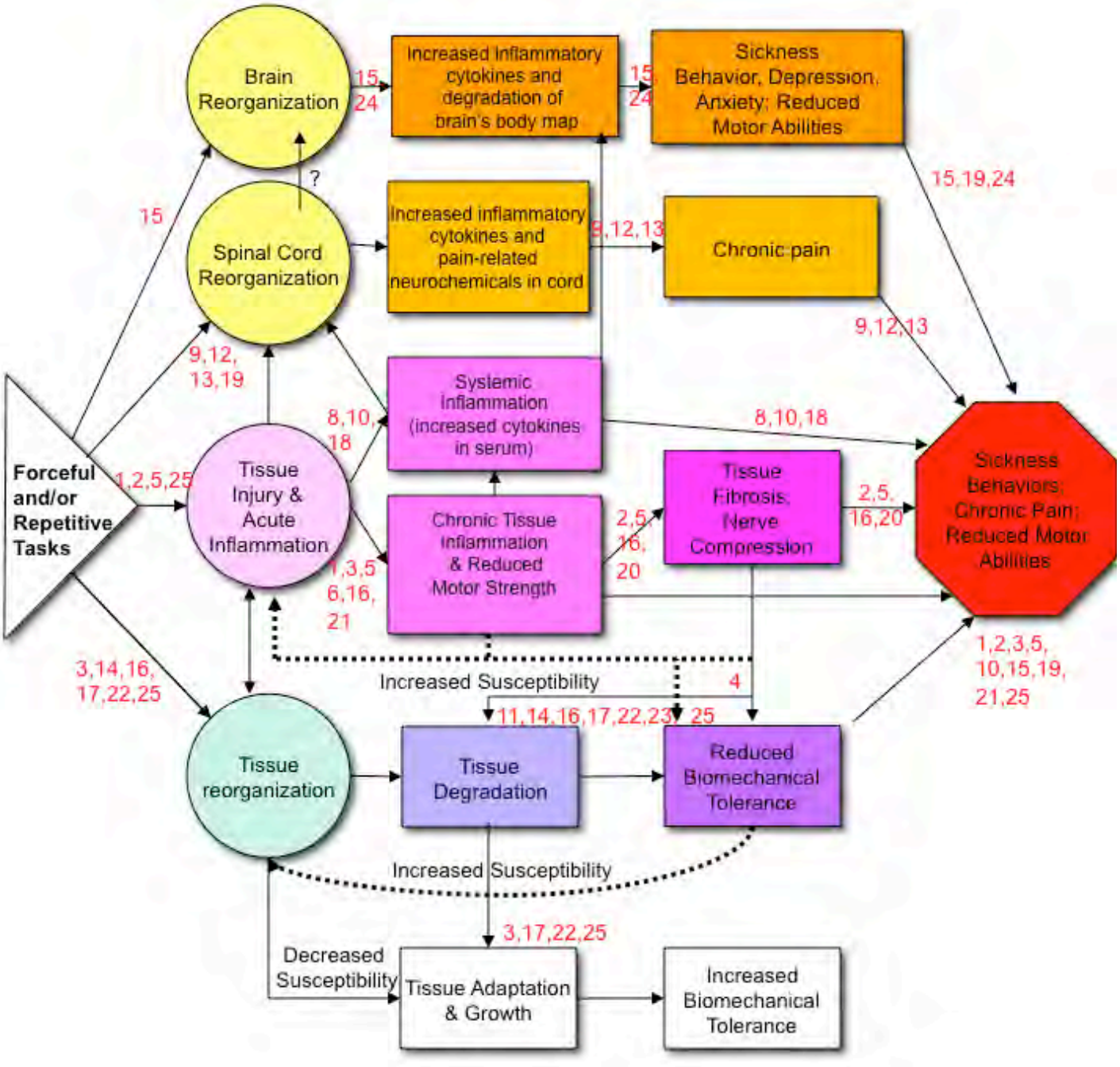
B. STUDIES AND RESULTS:

1. Funding issue and my ability to address some of those issues, and evidence of success (i.e. publications):

As noted above, the aims were changed from those stated in the original application to a smaller subset, due to a budgetary reduction of 60% of the amount requested at the time of the award. Although only able to put myself on the grant for 5% effort, I essentially worked as a full time postdoctoral fellow in my lab the last many years, devoting at least 50% of my effort to the grant project (much to the chagrin of my supervisors and administrators). With careful use of funds, including funds given back to investigators of 10% of the indirect monies generated from this and my prior NIOSH grant award (from a cost center which is now empty – I went for broke), Temple University departmental funds, utilization of PhD students from industry that did not cost salary monies, and utilization of banked tissues from past NIOSH funded projects – we were able to achieve more that the newly written aims. We were able to address many of the aims from the originally proposed grant.

As a consequence of this funding, I have been able to produce since 2006, 15 peer-reviewed manuscripts in PubMed indexed journals + 2 more that are submitted, 6 conference proceedings, 6 internal reports, 10 book chapters, 56 invited presentations and published abstracts of posters and platform talks, two educational materials, and 6 related human studies examining similar changes in subjects with overuse injuries. I am grateful for the opportunity that NIOSH gave me with this grant award. I am quite excited about the end results. The findings of the peer reviewed publications of both funding periods have allowed me to postulate the model below of how repetitive and forceful tasks lead to sensoritmotor declines and sickness behaviors. Numbers in red indicate my publications from this 10 year grant in support of this model.

Modified from Barbe and Barr, 2006 (ref 7)



References indicated in red: (1) Barbe et al, 2003; (2) Clark BD et al, 2003 (3) Barr et al, 2003. (4) Barr and Barbe, 2004. (5) Clark et al, 2004. (6) Al-Shatti et al, 2005. (7) Barbe et al, 2007. (8) Carp et al, 2007. (9) Elliott et al, 2008. (10) Barbe et al, 2008. (11) Rani et al, 2009a. (12) Elliott et al, 2009a. (13) Elliott et al, 2009b. (14) Rani et al, 2009b. (15) Coq et al, 2009. (16) Fedorczyk et al, 2010. (17) Rani et al, 2010. (18) Xin et al, 2011. (19) Kietrys et al, 2011. (20) Abdelmagid et al, 2012. (21) Kietrys et al, 2012. (22) Jain et al, submitted 2012. (23) Gao et al, submitted 2012. (24) Xin et al, submitted 2012. (25) Barbe et al, in prep.

2. My first goal was to complete studies with each of the task paradigms to 12 weeks: 1) low repetition with low force (LRLF), 2) high repetition with low force (HRLF), 3) low repetition with high force (LRHF), and 4) high repetition with high force (HRHF). We had been examined the effects of task performance for up to 8 weeks only, which was not enough, apparently to see adaptive versus inflammatory versus catabolic effects on tissues. All musculoskeletal tissues of the forelimb were examined, as was the median nerve and the spinal cord in this study. Many parts of this work was published separately based on the individual task paradigms: LRLF *(Elliott et al, 2009; Kietrys et al, 2011), HRLF (Coq et al, 2009; Elliott et al, 2010; Barbe et al, 2008; Coq et al, 2010 book chapter, Xin et al, 2011; Abdelmagid et al, 2012), LRHF (Elliott et al, 2009, Neuroscience; Kietrys et al, 2011; Keitrys et al, 2012), and the effects of the HRHF task (Fedoczyk et al, 2010; Rani et al, 2009a,b, 2010; Kietrys et al, 2011; Abdelmagid et al, 2012).* For a complete listing of publications, please see below, Primary Outcomes.

I have now also completed an additional manuscript that is in its final stages of submission. This manuscript includes data from past studies as well as new original work, and excitingly, compares the effects of all four task paradigms in a true force x repetition interaction design. I was unfortunately able to afford only to 12 weeks of task performance for each group, but was fortunate to be able to include neural (peripheral and spinal cord), musculotendinous, serum and bone changes. This work will be described below, and addresses the following aims of this grant: Examination if long-term exposure (to 12 weeks in this project) to lower demand tasks or even higher demand tasks causes compensatory, adaptive remodeling in musculoskeletal tissues (aim 1), tissue injury and inflammation in musculoskeletal tissues (aim 2), pathological tissue reorganization and degeneration in musculoskeletal tissues (aim 3), or declines in sensorimotor function (aim 4).

Barbe MF, Gallagher S, Massicotte V, Tytell M, Barr-Gillespie AE. The Interaction for Force and Repetition on Sensorimotor function and biochemical musculoskeletal and neural responses in a voluntary rat model of work. In preparation for submission to Ergonomics, Nov 2012.

Rationale of study:

It is evident that MSDs often develop as the result of the physical demands placed upon the musculoskeletal system in the workplace (Bernard, 1997; NRC-IOM 2000). Several risk factors associated with occupationally related MSDs have been identified – the most common being forceful exertions, repetitive motion, non-neutral body postures, and/or exposure to segmental or whole-body vibration (Punnett and Wegman, 2004). Of particular interest to the current article is the relationship of the critical musculoskeletal risk factors of force and repetition. A recent systematic review of MSD epidemiology literature investigated whether these risk factors exhibit an interaction between these two risk factors (Gallagher and Heberger, 2012). Results of this investigation found that 10 of 12 studies addressing an interaction between force and repetition demonstrated evidence of an interaction between these important MSD risk factors. The pattern of interaction between force and repetition and MSD risk was consistent across a number of joint disorders, with low force tasks showing a small to modest increase in risk with increased repetition, while high force tasks exhibited a rapid escalation of risk with increased repetition. The pattern of was similar to what is observed with failure of musculoskeletal tissues in biomaterials testing. A theoretical basis was provided for musculoskeletal tissues to incur damage as the result of fatigue failure (Gallagher and Heberger 2012). We sought to determine if this was the same for all tissue types using our rat model, in which nerve, muscle, tendon and bone can be examined.

Aims:

Our goads were to examine the physiological responses observed in response to exposure to varying degrees of force and repetition in a unique, voluntary rat model of repetitive reaching and handle pulling. We extended previous studies to examine exposure-dependent changes in several sensorimotor behaviors and

biochemical responses occurring with performance of a handle-pulling task for 12 weeks at 4 different repetition and force levels: 1) low repetition with low force (LRLF), 2) high repetition with low force (HRLF), 3) low repetition with high force (LRHF), and 4) high repetition with high force (HRHF). Some of this data has been published previously, such as some of the 0- and 12-week LRHF data (Elliott et al, 2009). We have indicated in the methods specifically when data has been published previously. However, to our knowledge, this is the first time that hsp 70 and MMP2 has been examined in a voluntary animal model of upper extremity repetitive loading. Also, this is the first time, to our knowledge, that all of the behavioral and tissue analytes have been compared across all four loading paradigms in a controlled animal study. Based on our conceptual model, we hypothesized that HRHF loading will be the point of tissue fatigue for each tissue type examined (neural, muscle, tendon and bone) and that this will be reflected in sensorimotor functional assays.

Methods:

Animals

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 231 young adult (14 weeks of age at onset of study) female Sprague-Dawley rats were used: Control rats that were either normal controls (NC, n=35) or food restricted controls (FRC, n=6); Task rats that performed one of 4 different tasks for 6 or 12 weeks: low repetition low force task (LRLF, n=11), high repetition low force task (HRLF, n=27), low repetition high force task (LRHF, n=33), and high repetition high force task (HRHF, n=37); and trained only rats that trained to the LRLF level (LRLF wk 0, n=17), trained to the HRLF level (HRLF wk 0, n=41), trained to the LRHF level (LRHF wk 0, n=6), and trained to the HRHF level (HRHF wk 0, n=27), before euthanasia. Also 5 animals that had performed the HRHF task for 4 weeks before moving to the LRLF task for 8 weeks (termed HRHF-to-LRLF) were analyzed for bone mass, since bones from LRHF has already been sectioned and could no longer be used for that analysis. The task, sensorimotor functional assay, and many of the biochemical methods used are as described previously (Elliott et al, 2009; Kietrys et al, 2011; Coq et al, 2009; Elliott et al, 2010; Barbe et al, 2008; Xin et al, 2011; Abdelmagid et al, 2012; Elliott et al, 2009; Kietrys et al, 2011; Keitrys et al, 2012; Fedoczyk et al, 2010; Rani et al, 2009a,b, 2010; Kietrys et al, 2011; Abdelmagid et al, 2012). Methods not described in these papers are as below.

Bone and nerve morphometry and histomorphometry for ED1+ cells

MicroCT analysis was performed according to recent guidelines (Bouxsein et al., 2010). A Skyscan 1172, 12 MPix model, high resolution cone-beam microCT scanner (Skyscan, Ltd, Antwerp, Belgium) was used to scan a 6 mm length of distal radial and ulna bones, using the following settings: x-ray source spot size of 300 nm, camera pixel size of 8.91 µm, Al 0.5mm filter, voltage of 59 kV, current of 167 µA, rotation step of 0.40^{o}, frame averaging of 4, a ring artifact correction of 10, and a beam hardening correction of 60%. The image slices were reconstructed using cone-beam reconstruction software (Skyscan NRecon) based on the Feldkamp algorithm, a process that yielded 8 µm thick sections in the axial plane, for each radius and ulna. Using the Skyscan CT Analyzer (CTAn) software, regions of interest (ROIs) were drawn by hand a few voxels away from the endocortical surface approximately every 10-20 slices, and then interpolated by the software for the other slices. For the distal metaphyseal trabeculae, morphological traits were assessed starting 1 mm proximal to the growth plates, and then extending proximally from this position for 1 mm (112 slices). The volume of interest for trabecular microarchitectural variables was bounded to a few pixels within the endocortical margin. For trabecular bone, an upper threshold of 255 and a lower threshold of 75 were used to delineate each pixel as bone or non-bone, and simple global thresholding methods were used. Trabecular morphometric traits were then computed from binarized images using direct 3D techniques that do not rely on prior assumptions from the underlying structures. Trabecular bone volume per total volume (BV/TV) was measured in 3 dimensions (3D).

Statistical Analyses

Two-way ANOVAs were used to assess the interaction between force and repetition, in which the factors repetition (low rep, high rep) and force (low force, high force) were compared. Also, two-way ANOVAs were

Table 1. Force-repetition interaction statistics[1] for Week 12 of repetitive task performance, organized by sensorimotor function and tissue type.

Sensorimotor motor assay or analyte tested	Force p values	Repetition p values	Interaction p values	Low Force vs. High Force Bonferonni Posttests
Motor Performance				
Voluntary loads per week (force*repetition)	p<0.0001	p<0.0001	p=0.001	Low Rep p<0.05 High Rep p<0.001
Neural Response – Peripheral and Central				
Palmar Mechanical Sensation (von Frey assay)	n.s.	p=0.0002	n.s.	Low Rep n.s. High Rep p<0.001
Median Nerve Conduction Velocity	p<0.0001	n.s.	n.s.	Low Rep p<0.01 High Rep p<0.001
Macrophages (ED1+) in median nerves	n.s.	p=0.01	p=0.05	n.s.
Substance P in dorsal horns	p=0.02	n.s.	n.s.	n.s.
[2]**Muscle & Tendon Responses: Grip Strength, inflammatory cytokines, injury marker hsp70**				
Forearm grip strength	n.s.	p<0.0001	n.s.	n.s.
TNFalpha muscle	n.s.	p<0.0001	n.s	n.s.
TNFalpha tendon	p=0.003	p=0.0005	n.s	Low Rep n.s. High Rep p<0.05
IL-1alpha muscle	p=0.02	p=0.03	p=0.002	Low Rep n.s. High Rep p<0.001
IL-1alpha tendon	p=0.007	n.s.	n.s.	n.s.
Hsp70 muscle	p=0.008	p=0.03	p=0.002	Low Rep n.s. High Rep p<0.001
Hsp70 tendon	p<0.0001	p=0.0008	n.s.	Low Rep p<0.01 High Rep p<0.001
Serum Inflammatory Cytokine Response				
TNFalpha serum	p=0.001	p=0.0009	p=0.003	Low Rep n.s. High Rep p<0.001
IL-1beta serum	p<0.0001	p<0.0001	p<0.0001	Low Rep n.s. High Rep p<0.001
IL-1alpha serum	p<0.0001	p<0.0001	p<0.0001	Low Rep n.s. High Rep p<0.001
Bone response: Forelimb bone and Serum bone turnover biomarker levels				
Osteoclast numbers in radius (N.Oc/BS)	p=0.0003	p=0.02	n.s.	Low Rep p<0.05 High Rep p<0.01
Serum CTXI (Collagen type 1 degradation in bone)	p=0.04	n.s.	p=0.04	Low Rep n.s. High Rep p<0.01
Serum C1,2C (Collagen type I & II degradation marker)	p=0.009	n.s.	n.s.	n.s.
Serum Osteocalcin (Bone formation marker)	p=0.56	n.s.	p<0.0001	Low Rep p<0.05 High Rep p<0.001
Bone mass (% BV/TV)	p=0.01	n.s.	p=0.006	Low Rep n.s. High Rep p<0.01

[1] Results of individual two-way ANOVAs shown. n.s. = not significant; [2] Flexor Digitorum muscle and tendons.

used to determine the differences between groups when multiple factors were used: week and group. One-way ANOVAS were used when only 12 week data was compared to NC, FRC or naïve data. The Bonferroni post-hoc method for multiple comparisons was used and adjusted p-values are reported.

Results

Mean Voluntary Reach Performance

The ability of the rats to maintain target loads per week (force * repetitions) reduced over time in all but the LRLF rats (Fig. 1), with a positive interaction between force and interaction by week 12 between the groups (Table I). Specifically, in week 1 of task performance, LRHF rats were below target levels ($p<0.05$, adjusted posthoc p values will be indicated in figure panels hereafter; Fig 1.A), and analysis of force x repetition showed variations in each ($p=0.0006$ and $p<0.0001$, respectively) but no significant interaction. In week 6, all but the LRLF group were below target levels (Fig. 1B), and analysis of force * repetition showed variations in each ($p<0.0001$ and $p=0.0007$, respectively), but no significant interaction. By week 12, again, all but the LRLF group were below target levels (Fig. 1C), and analysis of force x repetition showed significant variation in force, repetition and their interaction (Table 1).

Neural Response – peripheral and central

Signs of repetition and force-induced neuritis, injury and spinal cord sensitization were observed with training and task performance in the forepaw, median nerve and dorsal horns of cervical segments of the spinal cord (Fig 2; Table 1). Specifically, forepaw withdrawal threshold increased after mechanical stimulation with von Frey hairs (a decrease is a sign of mechanical allodynia) was observed by in week 0 in LRHF and HRHF rats, compared to NC/naïve data, and also in 12 week HRHF rats compared to LRLF rats (Fig. 1A). Each task group developed further increases in forepaw withdrawal thresholds by week 6 that were still evident by week 12, compared to NC/naïve data (Fig. 2B,C). Analysis of force x repetition effects of palmar sensation showed a significant difference only for repetition (Table 1). Nerve conduction velocity testing of the median nerve shows a slowing of the conduction in both high force groups by week 12, compared to NC and rats trained to the LRLF task (TRLF, aa'; Fig. 2C), with analysis of force x repetition showing a significant difference for force (Table 1), suggestive of HF task-induced nerve compressive injury. Neuritis, in the form of increased activated macrophages in the median nerve, was increased in the high repetition groups at week 0, compared to NC/FRC values (Fig. 2E). By week 12, each group had increased macrophages in the median nerve (Fig. 2F). Analysis of force x repetition effects of macrophages in the median nerve showed significant differences for repetition and the interaction of force and repetition (Table 1). There was also an increase in substance P immunoreactivity (a nociceptor related neurochemical) in the dorsal horns of the spinal cord by week 12 in HRLF, LRHF and HRHF, compared to LRLF rats, and in 12 week HRHF rats compared to NC/Naïve data (Fig. 2G,H). Analysis of force x repetition effects showed significant effects of force on spinal cord Substance P immunoreactivity (Table 1).

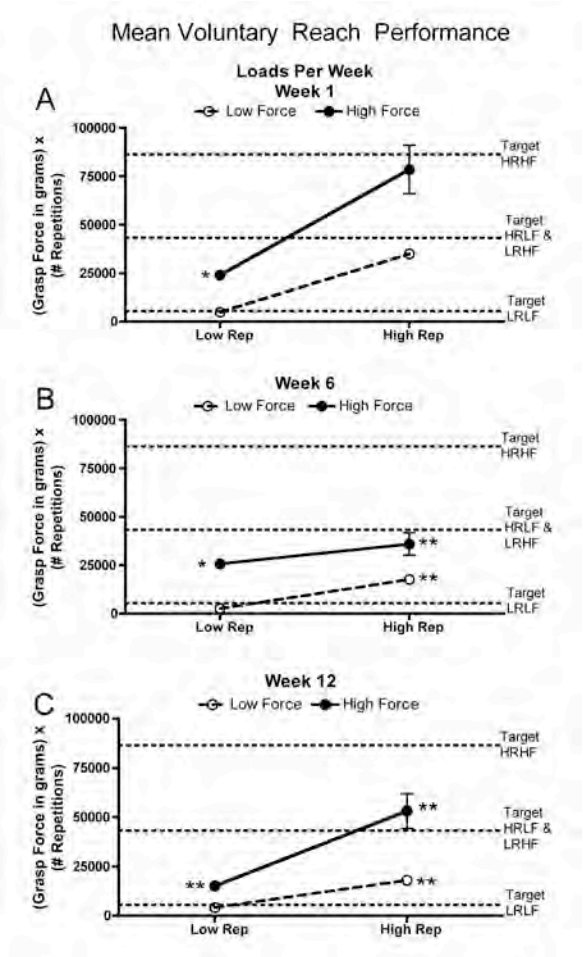


Figure 1. Estimated mean load per week in weeks 1, 6 and 12 of task performance (A-C). Estimated by multiplying mean grasp force in grams by number of repetitions (reaches/min x minutes/day x 3 days/week). Dashed lines show target loading levels for each of the four task groups per week, as indicated: low repetition low force (LRLF), high repetition low force (HRLF), low repetition high force (LRHF) and high repetition high force (HRHF). Symbols: * and **: $p<0.05$ and $p<0.01$, compared to target levels.

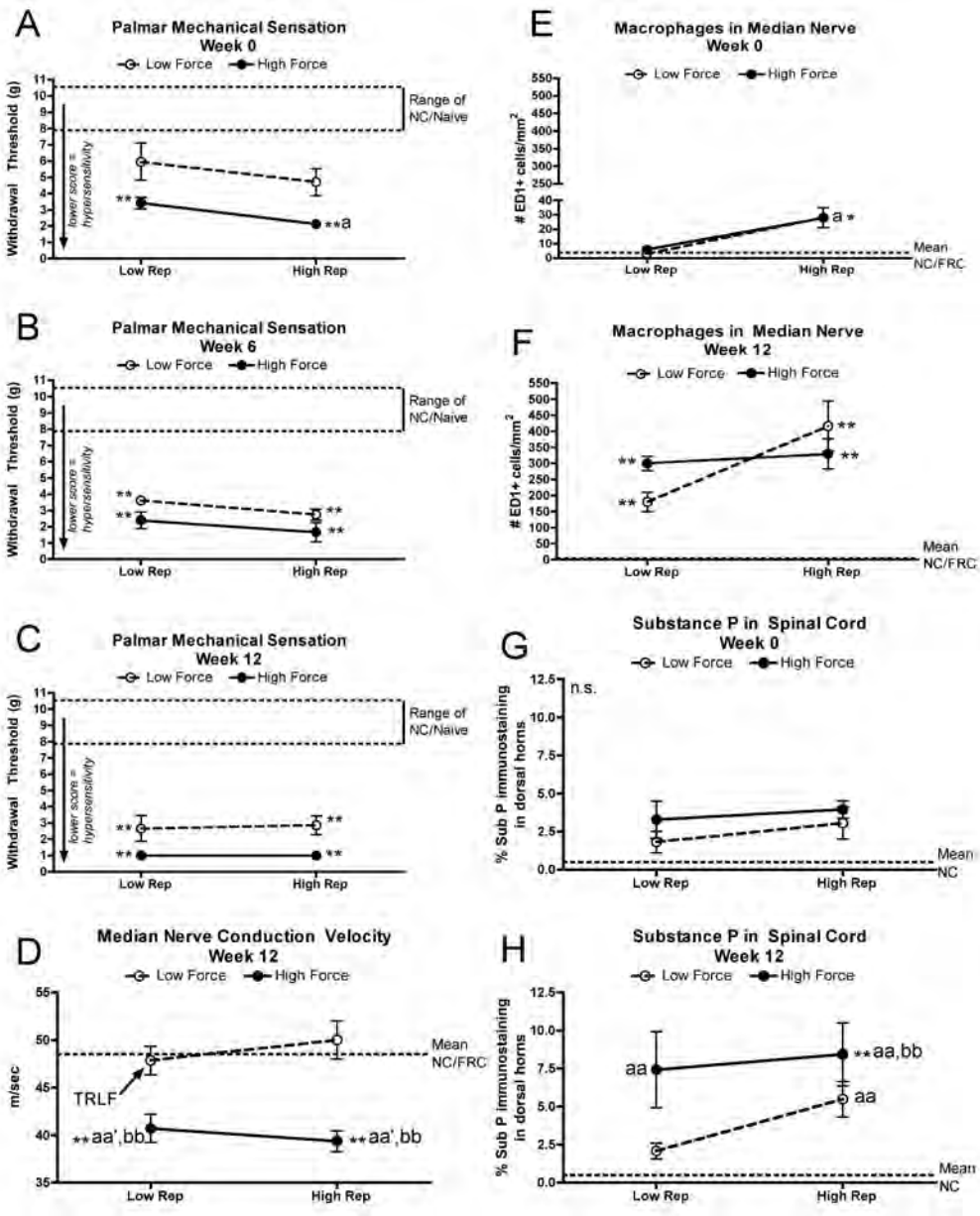


Figure 2. Sensory and neural responses to performance of one of the following tasks for 12 weeks: low repetition low force (LRLF), high repetition low force (HRLF), low repetition high force (LRHF) and high repetition high force (HRHF). (A-C) Palmar mechanical sensation, tested as withdrawal thresholds to mechanical stimulation with a series of von Frey hairs, in weeks 0 (end of training), 6 and 12. The range around the standard error of the mean is indicated by dashed lines. (D) Nerve conduction velocity (NVC) of the median nerve in the reach forelimb. (E &F) Numbers of ED1-immunoreactive macrophages in the median nerve at the level of the wrist, in task weeks 0 and 12. (G&H) Percent area of substance P immunoreactivity in the dorsal horns of lower cervical spinal cord segments, in weeks 0 and 12. Data for lamina I and II of the dorsal horns are presented. Symbols: a and aa: $p<0.05$ and $p<0.01$, compared to LRLF rats; aa': $p<0.01$, compared to TRLF rats (panel D only); bb: $p<0.01$, compared to HRLF rats; * and **: $p<0.05$ and $p<0.01$, compared to the mean for normal controls (NC), food restricted controls (FRC) rats, or mean naïve data.

Musculotendinous Responses

Repetition and force-induced forearm grip strength, flexor digitorum muscle and tendon inflammation, and injury were observed with both training and task performance to high force levels (Fig. 3 and 4). Specifically, grip strength was significantly reduced be 12 weeks in HRLF and HRHF rats, compared to naïve levels and to LRLF rats, but not at 0 weeks or in LRLF or LRHF rats (Fig. 3A,B). Analysis of force x repetition effects on grip strength at 12 weeks showed a significant difference for repetition level (Table 1). With regards to inflammatory cytokine production, TNF-alpha was increased in the muscles and tendons of 0 week HRHF rats, as well as in 12 week HRHF rats, compared to NC, LRLF and HRLF rats (Fig. 3C-F). TNFalpha was also increased in the muscles of 12 week LRHF rats, compared to NC, LRLF and HRLF rats (Fig. 3D). In both tissues, analysis of force x repetition effects showed a significant effect of repetition level on TNFalpha; force also had an effect on TNFalpha levels in in tendons (Table 1). IL-1alpha levels were similarly increased in 0 and 12 week HRHF rat muscles (Fig. 3G,H), and in 0 week HRHF tendons (Fig. 3I), although a resolution was seen by week 12 (Fig.

3J). In both tissues, analysis of force x repetition effects showed a significant effect of repetition and force level, and their interaction, on IL-1alpha; force also had an effect on IL-1alpha levels in tendons (Table 1).

Levels of MMP2 and TGFB1 were increased, although not significantly in 12 week HRHF tendons (Fig. 4A-B). PDGFab and PDGFbb were not above NC values in any group (data not shown). In contrast, hsp 70 levels were increased in the tendons of 12 week HRHF rats, compared to NC and each of the other groups (Fig 4C), and in the muscles of 12 week HRHF rats, compared to NC and LRLF rats (Fig. 4D), suggestive of tissue injury and indicative of the need for protein repair. Interestingly, hsp70 levels were significantly lower in the tendons of 12 week LRLF rats, compared to NC and LRHF rats (Fig. 4C), and in the muscles of 12 week HRLF rats, compared to the other groups (Fig. 4D). Analysis of force x repetition effects showed a significant effect of both force and repetition on hsp70 on flexor digitorum tendons and muscles, a significant interaction was also seen in the

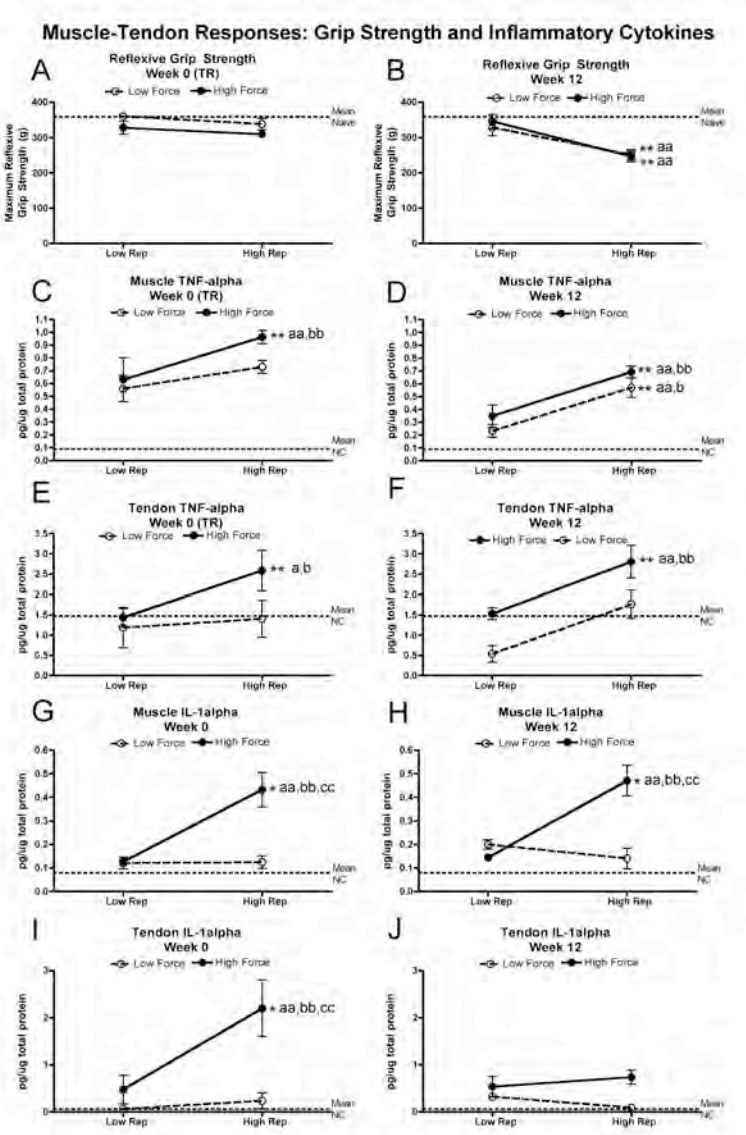


Figure 3. Maximum grip strength in the preferred reach limb, and inflammatory cytokines in flexor digitorum muscles and tendons tested using ELISA. (A&B) Maximum reflexive grip strength in grams (g) in week 0 (after training) and after performance of one of the 4 task for 12 weeks. (C&D) Muscle TNFalpha in week 0 and 12. (E&F) Tendon TNFalpha in week 0 and 12. (G&H) Muscle IL-1alpha in week 0 and 12. (E&F) Tendon IL-1alpha in week 0 and 12. Symbols: a and aa: $p<0.05$ and $p<0.01$, compared to LRLF rats; b and bb: $p<0.05$ and $p<0.01$, compared to HRLF rats; c and cc: $p<0.05$ and $p<0.01$, compared to LRHF rats; * and **: $p<0.05$ and $p<0.01$, compared to normal controls (NC) rats or naïve data.

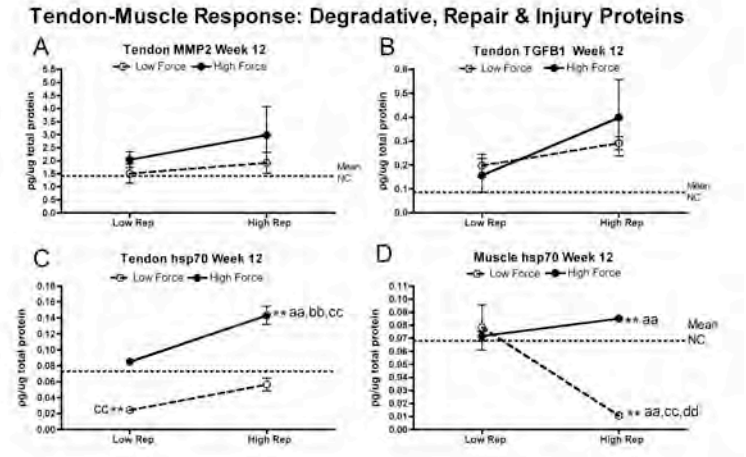


Figure 4. Degradative, repair and injury proteins in flexor digitorum tendons and muscles tested using ELISA after performance of one of the 4 tasks for 12 weeks. (A) Matrix melloproteinase 2 (MMP2) in tendon. (B) Transforming growth factor beta 1 (TGFB1) in tendon. (C&D) The inducible heat shock protein 70 (hsp70) in tendon and muscle. Symbols as shown in Figure legend 3, plus: d and dd: $p<0.05$ and $p<0.01$, compared to HRHF rats.

muscles for hsp70 (Table I).

Serum Responses

Levels of inflammatory cytokines in the serum increased differentially, depending on the cytokine, with training and task performance (Fig. 5). Specifically, serum TNFalpha increased only in the 12 week HRHF rats, compared to NC values and the other groups (Fig. 5A,B). In contrast, serum IL-1beta increased after training, and then again in week 12 in the HRHF rats, yet lower than at week 0, compared to NC values and the other groups (Fig. 5C,D). Serum IL-1alpha increased only in week 12 in HRHF rats, compared to NC values and the other groups (Fig. 5E,F). Lastly, serum MIP2 increased with training in the 0 and 12 week HRHF rats, compared to NC values (Fig. 5G,H). Analysis of force x repetition effects showed significant effects by force, repetition and their interaction for serum levels of TNFalpha, IL-1beta and IL-1alpha (Table 1). There were no significant force x repetition findings for serum MIP2.

Bone Responses

The distal radius showed signs of bone resorption with high force task performance for 12 week, while serum analysis of bone turnover markers indicates a general increase in bone degradation with the high force tasks but bone formation with the LRHF and HRLF tasks (Fig. 6). Specifically, osteoclasts numbers per bone surface (N.Oc/BS) were increased in 12 week LRHF and HRHF distal radial metaphyseal trabeculae, compared to NC, LRLF and HRLF rats, but not in 0 week or 6 week rats (Fig. 6A,B; 6 week data not shown). Analysis of force x repetition effects showed significant effects by force and repetition on N.Oc/BS (Table I). Serum CTX1, a key bone marker of bone degradation by osteoclasts, was increased in 12 week HRHF rats, compared to NC rats and the other groups (Fig. 6C). Also, serum C1,2C a more general marker of collagen type I and II degradation in bone, cartilage, tendon and muscle, was increased in 12 week LRHF and HRHF groups, compared to NC, LRLF and HRLF rats (Fig. 6D). In contrast, osteocalcin, a key serum biomarker of bone formation was increased significantly in the 12 week LRHF and HRLF groups (Fig. 6E), indicative of bone adaptation and growth to these tasks. Analysis of force x repetition effects showed significant effects by force and the interaction of force and repetition on serum levels of CTX1 and osteocalcin, and an effect of force on serum C1,2C (Table I). Lastly, the degradative processes in bones were confirmed using microCT, in which the distal radial

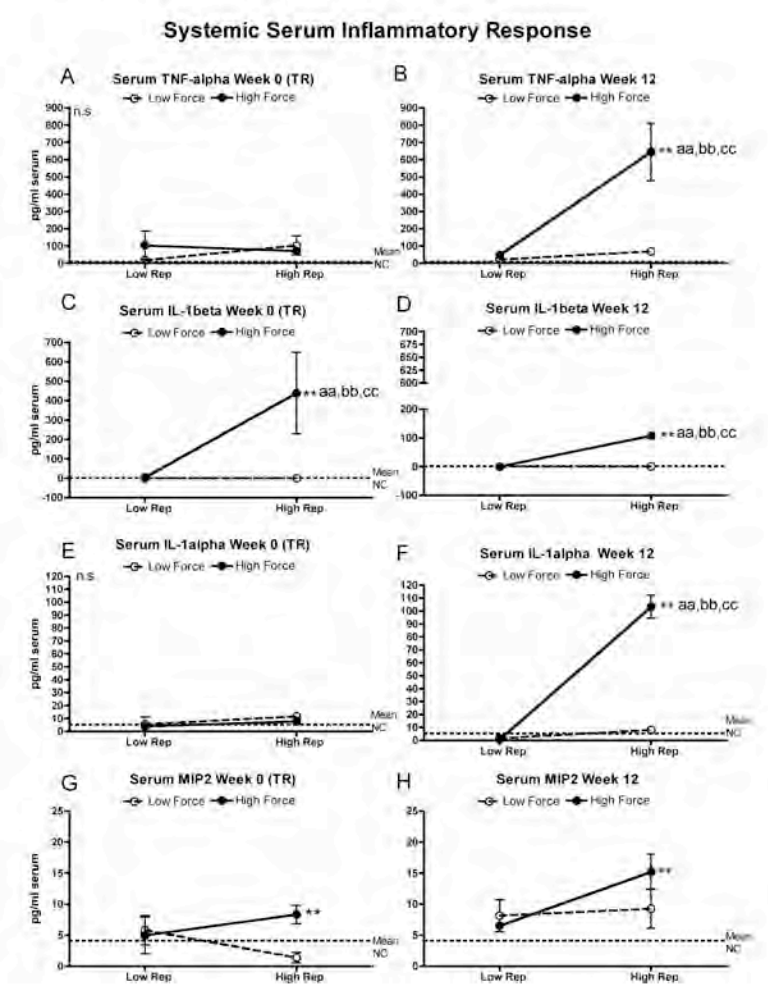


Figure 5. Serum inflammatory cytokine increases tested using ELISA. (A&B) Tumor necrosis factor alpha (TNF-alpha) in weeks 0 and 12. (C&D) Interleukin 1-beta (IL-1beta) in weeks 0 and 12. (E&F) IL-1alpha in weeks 0 and 12. (G&H) Macrophage inflammatory protein 2 (MIP2) in weeks 0 and 12. Symbols as shown in Figure legends 3 and 4.

metaphyseal trabeculae showed reduced bone volume fraction (BV/TV), compared to NC rats and the other groups (Fig. 6F). We did not perform microCT analysis on bones of LRHF before sectioning these bones; therefore, HRHF-to-LRLF rats were substituted, rats, which showed similar cumulative loads as LRHF rats (estimated cumulative load, mean ± sem: HRHF-to-LRLF 217,372 ± 3,280 versus LRHF 259,373 ± 2,985; p=n.s.). Analysis of force x repetition effects showed significant effects by force and the interaction of force and repetition on bone mass (Table 1).

Discussion

We found significant interactions of force x repetition for several behavioral and tissue outcomes, including mean voluntary loads per week, the number of macrophages in the median nerve, levels of an inflammatory cytokine and repair/injury protein in flexor digitorum muscles (IL-1alpha and hsp70), serum levels of 3 inflammatory cytokines (IL-1alpha, IL-1beta and TNFalpha), two serum markers of bone turnover (CTX1 and osteocalcin), and distal radial bone mass. However, not all tissues responded in this manner. Some peripheral nerve and musculoskeletal responses were a consequence of high repetition (mechanical sensation and grip strength), while others were more a consequence of high force (median nerve conduction velocity and substance P in the spinal cord), or with both force and repetition but not their interaction (tendon TNFalpha and hsp70, and bone osteoclasts). Thus, our fatigue hypothesis was supported at least partially. In tissues in which it was not supported, perhaps differential tissue physiological responses to repetition or loading, vascularization differences, or healing properties (e.g. complete versus fibrotic types of tissue healing) lead to individual outcomes observed.

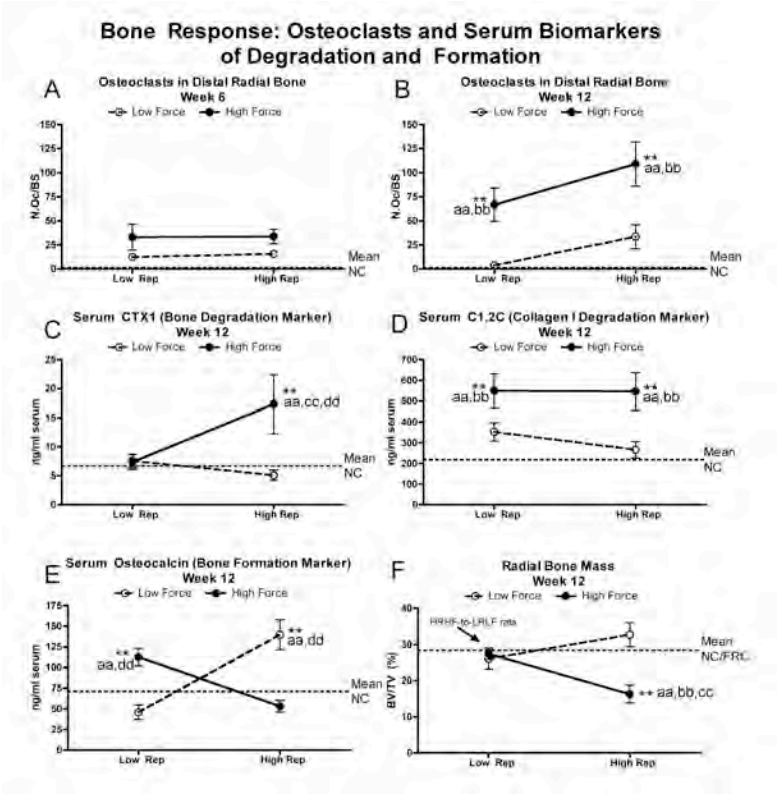


Figure 6. MicroCT analysis of trabeculae of the distal radial (A-D) and ulnar epiphyses (E-H). Results for trabecular bone volume (BV/TV), are shown. Serum bone turnover markers, and bone mineral density and cellular histomorphometry of distal radial trabeculae. (B-D) Serum levels of Trap 5b, CTX1 and osteocalcin, assayed using ELISA. (E&F) Cellular density of osteoclasts (N.Oc.), normalized to bone surface (BS), in the distal radial metaphyseal trabeculae. Symbols as shown in Figure legends 3 and 4.

Neural responses and sensorimotor declines

Neural tissues were affected during the training period in which rats were ramping upwards to high repetition or high force levels, and with continued performance of each task, but particularly in HRHF rats. By week 0, rats learning the high force tasks had mechanical allodynia, and rats learning the HRLF and HRHF tasks had increased nerve macrophages, changes that increased in rats of each task group by week 12, each indicative of inflammatory neuritis. We have reported that macrophages increase in intraneural and extraneural tissues of this nerve at wrist level in rats performing repetitive tasks with or without force (Clark et al, 2003; Clark et al, 2004; Elliott et al, 2008, 2009a). The increased substance P in dorsal horns of cervical spinal cord segments of week 12 HRLF, LRHF and HRHF rats further confirms the presence of peripheral inflammatory processes in not only the median nerve but also in the musculotendinous tissues (Elliott et al, 2009). We have reported that substance P increases both in peripheral tendons and in lamina 1 and 2 of lower cervical segments of task rats (Elliott et al, 2008, 2009a,b; Fedorczyk et al, 2010), regions receiving nociceptor afferent input from the forelimb. Discomfort from nerve inflammation likely contributed to the difficulty of HRLF,

LRHF and HRHF rats in maintaining target load levels in weeks 6 and 12. The slowed median nerve conduction velocity in 12 week LRHF and HRHF rats suggests that a compression injury was also present and contributing to the sensorimotor declines. This is substantiated by a study using a pinching task in primates in which motor performance declines coincided with a diagnosis of carpel tunnel syndrome (Sommerich et al., 2007). Injuries to peripheral nerves often result in axonal degeneration and removal of their surrounding myelin sheaths by macrophages (Bruck, 1997; Perry et al., 1987), and eventually, perineurial and epineurial thickening from increased deposition of collagen and other extracellular matrix components (Siironen et al., 1992, 1996), changes reported in our model (Clark et al, 2003, 2004; Elliott et al, 2010; Rani et al, 2009a).

Muscle and tendon responses and motor declines

Muscle, tendons and grip strength were affected during training to high force, and with continued performance of the HRHF task, and partially by the HRLF task, suggesting that musculotendinous tissues adapt to the LRHF task over time, partially adapt to the HRLF task, but not the HRHF task. The decrease in grip strength in week 0 and 12 in the 12 week HRHF rats is most likely due to the increased inflammatory cytokines in muscles and tendons at these time points. This is supported by our prior findings that treatment of HRHF rats with ibuprofen or anti-TNFalpha attenuates grip strength declines, declines in voluntary grasp force, and reduces muscle TNFalpha levels (Rani et al, 2010; Kietrys et al, 2011; Abdelmagid et al, 2012). We have also shown that muscle and tendon inflammatory cytokine levels negatively correlate with grip strength in a dose dependent manner (Elliot et al, 2010; Coq et al, 2009). Although the increase in a degradative collagenase, MMP2, and a fibrotic repair cytokine, TGFB1, in 12 week HRHF tendons was not significant, we have previously shown increases in other fibrosis-related proteins in tendons and muscles, and increased muscle TGFB1, with HRHF task performance and concomitant motor declines (Rani et al, 2009a; Fedorczyk et al, 2010; Abdelmagid et al, 2011). We are currently exploring if longer studies result in greater increases in MMP2 and TGFB1. It was interesting to find that hsp70, a repair protein and tissue injury marker, increased in HRHF muscles and tendons, but decreased with the LRLF task in tendons and with the HRLF task in muscles. We hypothesize that these musculotendinous tissues accommodated to the demands of the lower level tasks, but were unable to accommodate to the HRHF task.

Serum responses

We have previously shown dose response changes in serum inflammatory cytokines in rats performing HRLF versus LRLF tasks (Barbe et al, 2008), so it was not a surprise to observe the greatest increases in HRHF trained and task rats. We have previously shown that grip strength correlates negatively with serum levels of inflammatory cytokines in young rats performing the HRLF and LRLF task short term, and in aged rats performing a HRLF task for 12 weeks (Barbe et al, 2008; Xin et al, 2010). Several studies have shown that patients with work-related repetitive strain injuries (RSIs) have increased serum inflammatory cytokines, including IL-1β, IL-1RII, IL-1ra, IL-6, IL-18, and TNFα (Gotoh et al, 2001; Carp et al, 2007; Rechardt et al, 2011). In the study by Carp, we found that serum TNFalpha and IL-1beta levels were moderately correlated with symptoms of pain and weakness in these patients, and that TNFalpha was predictive of the severity of their symptoms (Carp et al, 2007).

Bone responses

Similar to musculotendinous tissues, bone responded catabolically to long term HRHF loading with increased osteoclasts in the distal radial metaphyseal trabeculae, decreased bone mass, and increased serum biomarkers of bone resorption. The HRHF results match our prior reports of increased Trap5b, a serum biomarker indicative of osteoclast numbers, decreased epiphyseal plates, cortical bone thickness, radiocarpal joint degradation in 12 week HRHF rats (Rani et al, 2009a; Driban et al, 2011). We were please to see signs of bone formation in the form of increased serum osteocalcin in LRHF and HRLF rats, and even a small increase in bone mass in the LRHF rats. These latter two findings suggest that the radii are adapting positively to the LRHF and HRLF loading, the latter are supported by prior findings showing qualitative signs of cortical bone

adaptation in 12 week HRLF rats (Barr et al, 2003). We have also shown increased serum osteocalcin in 6 week HRHF rats that declined with continued task performance (Rani et al, 2009b, 2010). The increase of both serum osteocalcin and CTXI in the 12 week LRHF rats indicates that bone resorption and formation processes are keeping the bone in homeostasis. We hypothesize that longer work periods will lead to even greater gains of bone in the HRLF rats, continued homeostasis in the LRHF rats, but more loss in the HRHF rats.

Conclusion

It is clear from these results that performance of a high repetition high force tasks for prolonged periods leads to chronic inflammatory and concomitant catabolic effects on many tissues types, even those at a distance from the localized injury site in involved tissues. Treatments needs to take into account the differential healing characteristics of each affected tissue.

3. In the next study, we examined the following aims: To examine if long-term exposure to a high repetition-low force (HRLF) regimen causes persistent tissue injury and inflammation in musculoskeletal tissues (aim 2), pathological tissue reorganization and degeneration in musculoskeletal tissues (aim 3), or declines in motor performance (aim 4).

Gao H, Paul F, Wade C, Barr AE, Barbe MF. Chronic low grade inflammation precedes increased musculotendinous and serum TGFB1 and MMP2, increases that accompany persistent grip strength declines. Submitted to Archives of Physical Medicine, Oct 2012. Published as a proceedings paper, Proceedings of the Human Factors and Ergonomics Society 56th Annual Meeting, 2012, pg 743-747; Presented at HFES October 2012.

Rationale

We have shown that continued performance of repetitive tasks induces grip strength declines despite anti-inflammatory treatments. We hypothesized that one cause may be musculotendinous fibrosis and degeneration.

According to the Bureau of Labor Statistics report entitled Nonfatal Occupational Injuries and Illnesses Requiring Days Away from Work for 2010, work related musculoskeletal Disorders (WMSDs) account for 29% of lost workday injuries and illnesses in the US (BLS, 2010). Studies in humans with long-term chronic overuse syndromes find evidence of inflammation, fibrosis and degeneration of musculoskeletal tissues. However, the factors underlying these pathophysiological responses are still under investigation. This impedes progress towards their prevention.

Several studies have detected biomarkers of inflammation in serum of patients with diagnosed WMSDs, including TNF-α and members of the interleukin 1 (IL-1) family (Carp et al, 2007; Rechardt et al, 2011). Although not yet assessed in patients with WMSDs, serum MMP2 is a sensitive biomarker of extracellular matrix turnover and collagen degradation in animal models (Garner et al, 2011). It would be of interest to identify biomarkers of inflammation and degeneration of musculotendinous tissues, so that treatment of WMSDs can be targeted appropriately.

In our rat model of WMSDs, performance of a high repetition negligible force (HRNF) food-retrieval task for 8 weeks induced increased inflammatory cytokines in muscles and tendons that persisted across the 8 weeks of task performance (Barbe et al, 2008). The increase in tissue inflammatory cytokines was matched by increases of similar cytokines in serum, increases that correlated with grip strength declines. In a recent study, we observed that performance of a high repetition low force (HRLF) lever-pulling task for 12 weeks induced a serum cytokine response in week 6 that was resolved by week 12 in young adult rats, yet the grip strength declines persistent (Xin et al, 2011). We have also reported persistent grip strength declines in task rats treated with either anti-TNFalpha or ibuprofen (Abdelmagid, et al, 2012; Rani et al, 2010; Kietrys et al, 2011), albeit the treated task rats had improved forelimb strength compared to untreated task rats. We hypothesize that there is either a persistent low-grade inflammatory response due to continued performance of the repetition task or that there or fibrotic degradative changes in the involved muscles or tendons that are contributing to the motor deficits. The latter part of this hypothesis is partially supported by findings of

increased fibrotic proteins and collagen deposition in muscles of rats performing a high repetition high force (HRHF) task for 6 weeks, although since that was a short work task protocol, longer experiments are needed to fully address this hypothesis.

Aims:

Therefore, our goal here was to determine if declines in forearm grip strength occurring with long-term reaching and pulling at moderate task levels are associated with persistent inflammatory or with degenerative responses in forearm muscle and tendons. We also sought to identify serum biomarkers indicative of underlying tissue processes. We assessed forearm grip strength, serum for biomarkers of inflammation or tissue degeneration, and flexor digitorum muscles and tendons for indicators of inflammation, degradation and fibrosis in rats performing a voluntary HRLF lever-pulling task for 18 weeks.

Methods

Subjects. 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. Seventy young adult, female Sprague-Dawley rats (3 mo of age at onset) were used. Rats were divided into one of 5 groups as shown in Figure 1A: age-matched normal controls (NC, n=15); age-matched food restricted controls (FRC, n=23), trained-only rats that underwent the initial training, and that were then either euthanized after the training period (TR0: n=14), or rested for 21 weeks (n=16, TR21); and rats that trained to learn the task, and then performed the high repetition low force task (HRLF) task for 12 (n=10), 18 (n=12) or 24 weeks (n=12) before euthanasia. Adult female rats were used for several reasons: (1) Human females have a higher incidence of work-related MSS/MSDs than males [32]; and (2) for inclusion of data from our past studies on female rats. Rats were housed in a central animal facility in separate cages with a 12-hour light: dark cycle and free access to water. They were weighed weekly and their food was adjusted to maintain 95% body weight of age-matched controls.

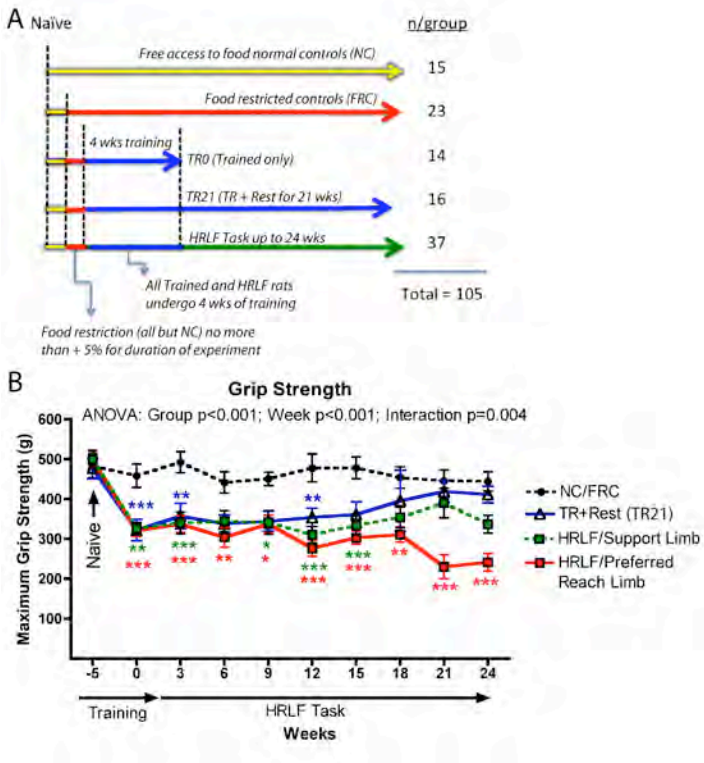


Figure 1. Design of experiments and maximum grip strength. (A) Experimental design. (B) Maximum reflexive grip strength in normal controls (NC), TR+Rest (trained rats that rested after training for 21 weeks; TR0 rat data was incorporated into 0 week timepoint), and high repetition low force (HRLF) rats. Preferred reach limbs and contralateral, support limbs were examined. Symbols: *:p<0.05, **p<0.01, and ***p<0.001, compared to NC.

Training and the Repetitive Task. The trained only and HRLF rats underwent a 4 week training period of 10 min/day, 5 days/week, to learn the task. Subsets of these rats were randomly chosen to become HRLF rats. The HRLF rats reached and pulled a lever at a rate of 8 reaches/min at 15 ± 5% of maximum voluntary force, for 2 hrs/day in 30 min sessions, for 3 days/wk, for up to 24 wks. Details of this training and task are as described previously (Xin et al, 2011). Rats were allowed to use their preferred limb to reach (the ‘‘reach’’ limb), and their contralateral limb as support against the operant chamber wall while pulling (the “support” limb).

Motor function assay: Reflexive grip strength was measured in all animals bilaterally at baseline, after training, and every 3 weeks thereafter, using a rat grip strength recording unit, (Stoelting, Wood Dale, IL). The test was repeated 3-5 times/limb/trial, and maximum grip strength per trial reported and used for statistical comparisons.

Serum assays. Following euthanasia (Nembutal, 120 mg/kg body weight), 18 hours after completion of the final task session, blood was collected from all rats by cardiac puncture using a 23-gauge needle and centrifuged immediately at 1000 g for 20 min at 4^{o}C. Serum was collected, flash-frozen, and stored at -80^{o}C until analyzed. Serum was assessed for: C-reactive protein (CRP), interleukin (IL)-1α and β, IL-6, IL-10, macrophage inflammatory protein (MIP) 2 and 3; matrix metalloproteinase 2 (MMP2), platelet derived growth factor ab and bb (PDGFab and PDGFbb), transforming growth factor beta 1, and tumor necrosis factor-alpha (TNF-α), using ELISA (Aushon Biosystems). All samples were analyzed in duplicate, and presented as pg/ml serum.

Tissue biochemical assays. Following euthanasia and after serum collection, one half of the animals/group were used for biochemical assays, using previously described methods (Barbe et al, 2008)]. Homogenates were assayed for:IL-1alpha, IL-1beta, IL-6, IL-12, TNF-α, MIP2, PDGFab, PDGFbb, TGFB1, and MMP2 using multiplex or single ELISA, following manufacturers' instructions (Aushon Biosystems for multiplex ELISA and Biosource for single plex ELISA). Each sample was run in duplicate. ELISA assay data (pg cytokine protein) were normalized to µg of total protein, determined using a bicinchoninic acid (BCA) protein assay kit. Western blot analysis of CTGF was performed, using anti-CTGF (Cat# sc-14939, Santa Cruz Biotechnology, Santa Cruz, CA) and previously described methods (Abdelmagid et al, 2012). TGFB1 and collagen type 1 western blots were performed similarly using anti-TGFB1 (Cat# MAB240, R&D Systems) and anti-collagen type I (Cat# C2456, Sigma-Aldrich, St. Louis, MO), although a quantitative Licor detection system was used. For TGFB1, fluorescent secondary antibodies were used for visualization. For collagen type I western blot analysis, two lanes of gels were also loaded with purified rat tail collagen type I (Sigma-Aldrich, St. Louis, MO) and used to show that proteins of the correct molecular weight were immunodetected with the collagen type I antibody in skeletal muscle. Gels were striped and also probed with GAPDH, which was used as a loading control. Western blots were repeated at least three times for each analyte examined.

Immunohistochemical Analyses. The remaining animals/group were used for histological analysis. Following euthanasia and after serum collection, animals were perfused transcardially with 4% buffered paraformaldehyde. Forearm musculotendinous tissues were collected and sectioned longitudinally, as described previously (Barbe et al, 2003). Sections were immunostained with the antibodies for CTGF and TGFB1 as described above, and with specific antibodies for MMP1, MMP2, MMP8, and MMP13 proteins using previously described immunohistochemical methods (Abdelmagid et al, 2012). In addition to the western blot analysis to determine antibody specificity for CTGF and TGFB1, negative control staining was performed by omission of either primary or secondary antibodies. Also, gelatinase gels were stained with anti-MMP2 antibody to verify its specificity and to determine if the MMP2 protein was active (it was, data not shown), using homogenized liver as a loading control. Percent area with MMP1, MMP2, MMP8, and MMP13 immunostaining in flexor digitorum tendons at the level of the wrist was assessed using previously described methods (Al-Shatt et al, 2005; Fedorczyk et al, 2010).

Assessment of Tendon Histopathology. A series of adjacent sections from above were stained with hematoxylin and eosin (H&E), dehydrated and coverslipped with DPX mounting medium. These sections were examined by two naive examiners for histopathological changes in tendons of TR21 and 18-week HRLF rats (n=3 each). Tendons were assessed for histopathological changes using the Bonar scale, which assesses 4 factors on a 4-point (0-3) scale: cell shape and cellularity, collagen organization, and vascularization (Cook et al, 2004). Each of these determinations was made in the flexor digitorum tendons at the level of the wrist joint, at 3 microscope fields/rat, in two to three separate sections per rat.

Statistical Analyses. All data are expressed as mean ± SEM. Grip strength was analyzed by 2-way ANOVA with the factors week and limb. Biochemical assays were analyzed using one-way ANOVAs. In each statistical analysis, the Bonferroni method for correcting for multiple comparisons was used for post-hoc analyses, with results compared to NC and/or TR data. A Kruskal-Wallis nonparametric test was used to determine differences in tendon pathology scores. Adjusted p values are reported, and after adjustment, p values of < 0.05 were considered significant.

Results

Grip Strength declined progressively in the HRLF reach limb.

Forearm grip strength was reduced compared to NC immediately after training and then was persistently decreased in the preferred reach limbs of HRLF rats through the 24 weeks of task performance (two way ANOVA: group $p<0.001$, week $p<0.001$, interaction $p=0.004$) (Fig. 1B). Specifically, post hoc analysis showed that grip strength was significantly reduced, bilaterally, in TR0 and HRLF rats immediately after the initial training period. These declines continued in the reach limb throughout this 24 week study, compared to NC (See Fig. 1B for p values). In contrast, grip strength declines recovered with rest in trained-only rats (TR+Rest), and in the contralateral support limb of the HRLF rats.

Cyclical low-grade musculotendinous inflammation. There were low grade but significantly increased inflammatory cytokines in flexor digitorum muscles and tendons of HRLF rats' reach limbs: IL-1beta after training (week 0); TNFalpha cyclically in weeks 0, 12 and 24; and IL-6 and IL-10 in week 24. (Fig. 2; posthoc p values shown in individual figure panels). Specifically, in HRLF reach limbs, IL-1beta and TNFalpha were increased in reach limb muscles and tendons after training (Fig. 2A,C,E,G), persistent increases of TNFalpha in reach limb tendons through week 12 and in muscle in week 24 (Fig. 2C,G), increased IL-6 bilaterally in muscles and tendons at 24 weeks (Fig. 2B,F), and increased IL-10 in muscles in week 24. Note that TNF-α was increased seven-fold higher in flexor digitorum tendons than in muscles in 12-week HRLF reach limbs. IL-12 and MIP2 were not significantly increased in these tissues, compared to NC (data not shown).

Cyclical low-grade systemic inflammation.

Several inflammatory cytokines had low grade but significantly increases in serum in week 12 HRLF rats: IL-1alpha, MIP2, MIP3, TNFalpha, as well as an anti-inflammatory protein, IL-10 (Fig. 3A-F). Each resolved by week 18, with resurgence of serum TNFalpha as well as IL-6 by week 24 (Fig. 3E,F). IL-12 was also increased in 18-week

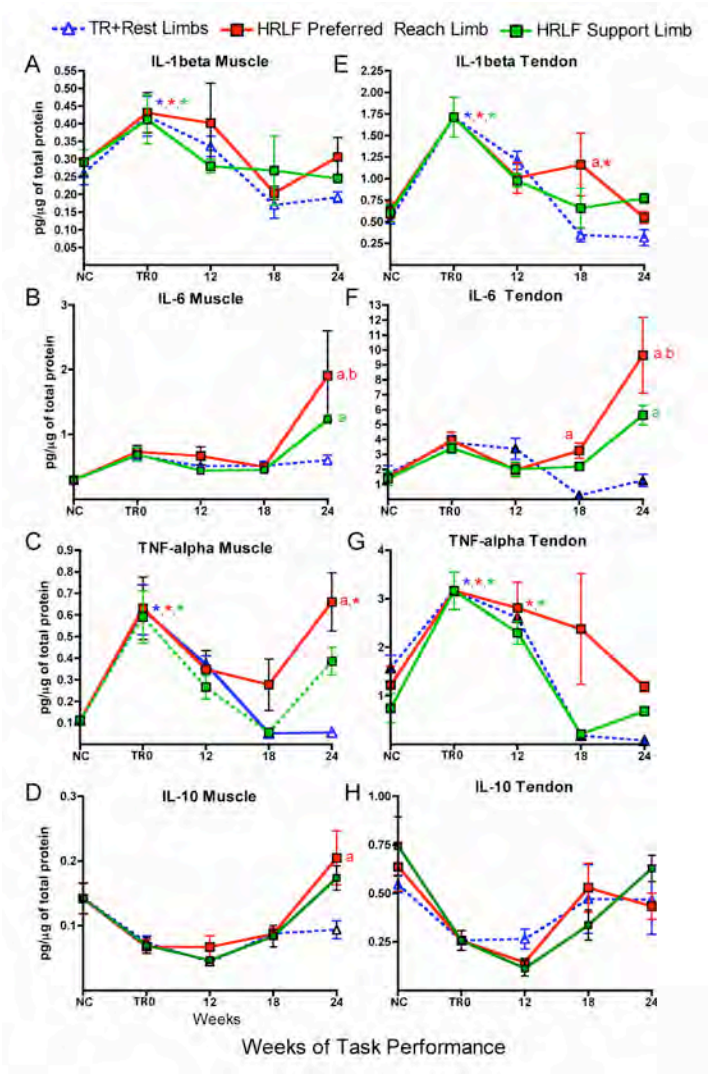


Figure 2. Tissue inflammatory cytokines. Pro- and anti-inflammatory cytokines in flexor digitorum muscles and tendons of normal controls (NC), TR+Rest rats (trained rats that rested for 21 weeks, shown as 24 wks for statistical purposes), TR0 rat data at 0 week time point (trained only), and high repetition low force (HRLF) rats at 12, 18 and 24 weeks of task performance with data from reach and contralateral support limbs examined separately. (A) Muscle IL-1beta. (B) Muscle IL-6. (C) Muscle TNF-alpha. (D) Muscle IL-10. (E) Tendon IL-1beta. (F) Tendon IL-6. (G) Tendon TNF-alpha. (H) Tendon IL-10. Each analyte assayed via single-plex ELISA. Symbols: * and **: $p<0.05$ and $p<0.01$, compared to NC; a: $p<0.05$, compared to TR0 rats; b<0.05, compared to TR21 rats (trained rats that rested to 21 weeks).

HRLF rats, compared to NC rats ($p<0.05$; data not shown). C-reactive protein and Rantes were not significantly increased in serum (data not shown).

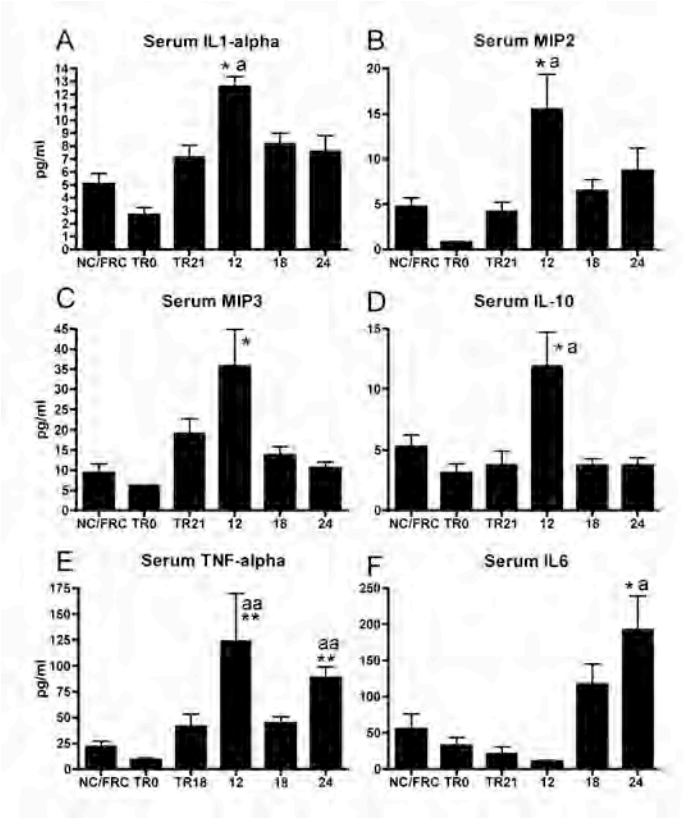


Figure 3. Serum inflammatory cytokines. Serum levels of pro- and anti-inflammatory cytokines in normal + food-restricted controls (NC/FRC), TR0 (trained only rats), TR21 (trained rats that rested for 21 weeks), and high repetition low force (HRLF) rats that performed the task for 12, 18 or 24 weeks. (A) Serum Interleukin 1-alpha (IL-1alpha). (B) Serum macrophage inflammatory protein (MIP2). (C) Serum MIP3. (D) Serum IL-10. (E) Serum tumor necrosis factor alpha (TNF-alpha). (F) Serum IL-6. Each analyte assayed in batches using multi-plex ELISA. Symbols: * and **: $p<0.05$ and $p<0.01$, compared to NC/FRC; a and aa: $p<0.05$ and $p<0.01$, compared to TR0 rats.

Collagen degradation with long-term task performance.
Matrix metalloproteinase-2 (MMP2) was increased in serum of 18 and 24 week HRLF rats, compared to NC and TR0 rats ($p<0.05$ and $p<0.01$, respectively) (Fig. 4A), and in reach limb tendons of 18 and 24 weeks, compared to TR21 rats (Fig. 4B,D,E,H,I), as assayed using ELISA. Immunohistochemistry was used to show localization of MMP1, 2, 13 and 8 in TR21 and HRLF task rats in flexor digitorum tendon fibroblast (Fig. 4C-K). No increase was observed for any of these MMPs in TR21 rat tendons (Fig. 4C-G). However, increased immunoreactivity for MMP1 and MMP2 was observed in 24 week HRLF rats (Fig. 4C,H,I).

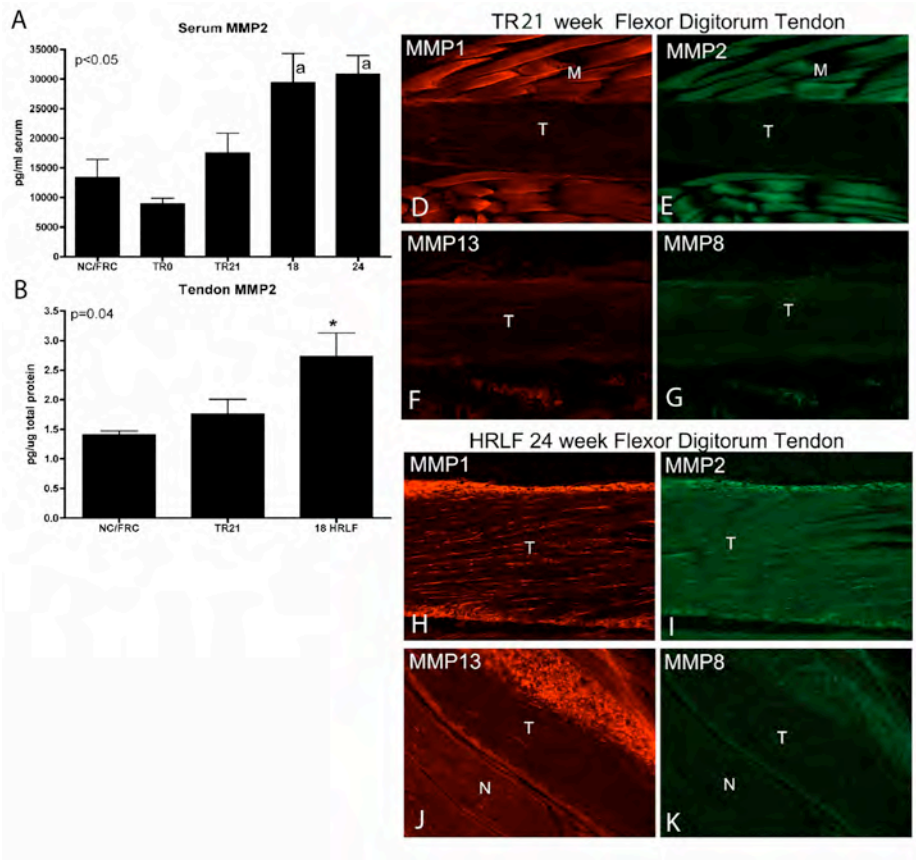


Figure 4. Serum and tendon matrix metalloproteinase 2 (MMP2). Serum and flexor digitorum tendon levels of MMP2 in normal + food-restricted controls (NC/FRC), TR0 (trained only rats), TR21 (trained rats that rested for 21 weeks), and high repetition low force (HRLF) rats that performed the task for 18 or 24 weeks. (A) Serum MMP2 levels, tested using ELISA. (B) Reach limb flexor digitorum tendon MMP2 levels, tested using ELISA. (C) Graph showing quantification of MMP1 and 2 immunoreactivity in flexor digitorum tendons, at or near the level of the wrist. (D-G) Flexor digitorum tendons from reach limb TR21 rats immunostained for MMP1, MMP2, MMP3 and MMP8. (H-K). Flexor digitorum tendons from reach limb 24-week HRLF rats immunostained for MMP1, MMP2, MMP3 and MMP8.. Symbols: M= flexor digitorum muscle. N = median nerve; T = flexor digitorum tendon. * $p<0.05$, compared to NC; a: $p<0.05$, compared to TR0 rats.

Increased fibrotic proteins and collagen with long-term task performance.

Using ELISA, western blots and immunohistochemistry, we found increased fibrogenic proteins TGFB1 and CTGF, as well as increased mature collagen type I, in the flexor digitorum muscles and tendons of reach limbs of long term HRLF task rats (Figures 5 and 6). TGFB1 increased in serum of 18-week HRLF rats, compared to NC rats ($p<0.05$; Fig. 5A). It also increased in reach limb tendons of 18-week HRLF rats, compared to NC rats ($p<0.01$, Fig. 5B). The 50Kda molecular weight TGFB1 protein increased in reach limb muscles of 18 week task rats, compared to both NC and TR21 ($p<0.05$ each; Fig. 5C,F; 24 weeks not examined), although no increase was seen in the 12.5 kDa subunit of TGFB1 (Fig. 5D,F).

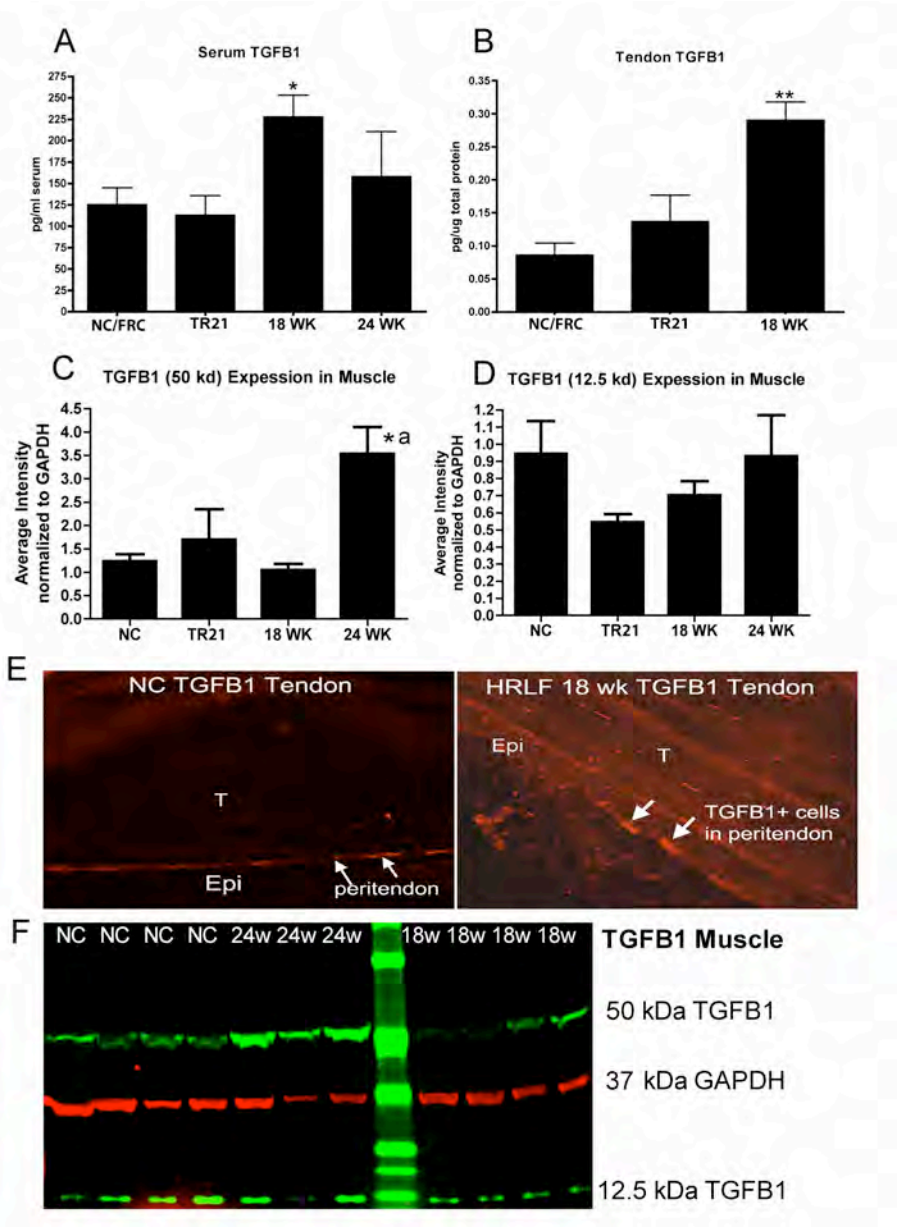


Figure 5. Serum and flexor digitorum tissue transforming growth factor beta 1 (TGFB1). Serum and flexor digitorum tendon and muscle levels of TGFB1 in normal + food-restricted controls (NC/FRC), or just NC, TR21 (trained rats that rested for 21 weeks), and high repetition low force (HRLF) rats that performed the task for 18 or 24 weeks. (A) Serum TGFB1), and (B) tendon TGFB1, assayed using ELISA. (C&D) The results of western blot analysis of muscle TGFB1 in which two bands were detected, 50KDa and 12.5 kDa bands of TGFB1. Average intensity normalized to GAPDH is shown for 3 blots; a representative western blot is shown in panel F. (E) Immunohistochemical staining for TGFB1 in tendons of a NC and a 18 week HRLF rat reach limb, showing localization of TGFB1. (F) A representative western blot of reach limb muscles from NC and 18- and 24-week HRLF rats probed with anti-TGFB1 and GAPDH. Bands in green were detected with an anti-TGFB1 antibody and a secondary antibody tagged with Cy2. Bands shown in red were detected with an anti-GAPDH antibody and a secondary antibody tagged with Cy3. Symbols: * and **: $p<0.05$ and $p<0.01$, compared to NC/FRC or NC only, as indicated; a: $p<0.05$ compared to TR21. Epi = epitendon; T = flexor digitorum tendon.

There was also an increase in CTGF immunopositive cells in reach limb flexor digitorum muscles, tendons and tendon sheaths of 18-week HRLF rats (Fig. 6A-C). This was matched by an increase in mature collagen type I in 18 week muscles (Fig. 6E). Increased Collagen type I precursor submits were also present in flexor

digitorum muscles of TR0 rats, proteins not present in 18-week HRLF muscles (Fig. 6E). Signs of tendon pathology was also assessed analyzed histologically using the Bonar scoring method. The epitendon of 18-week HRLF rats was thickened ($p<0.05$; Fig. 6D).

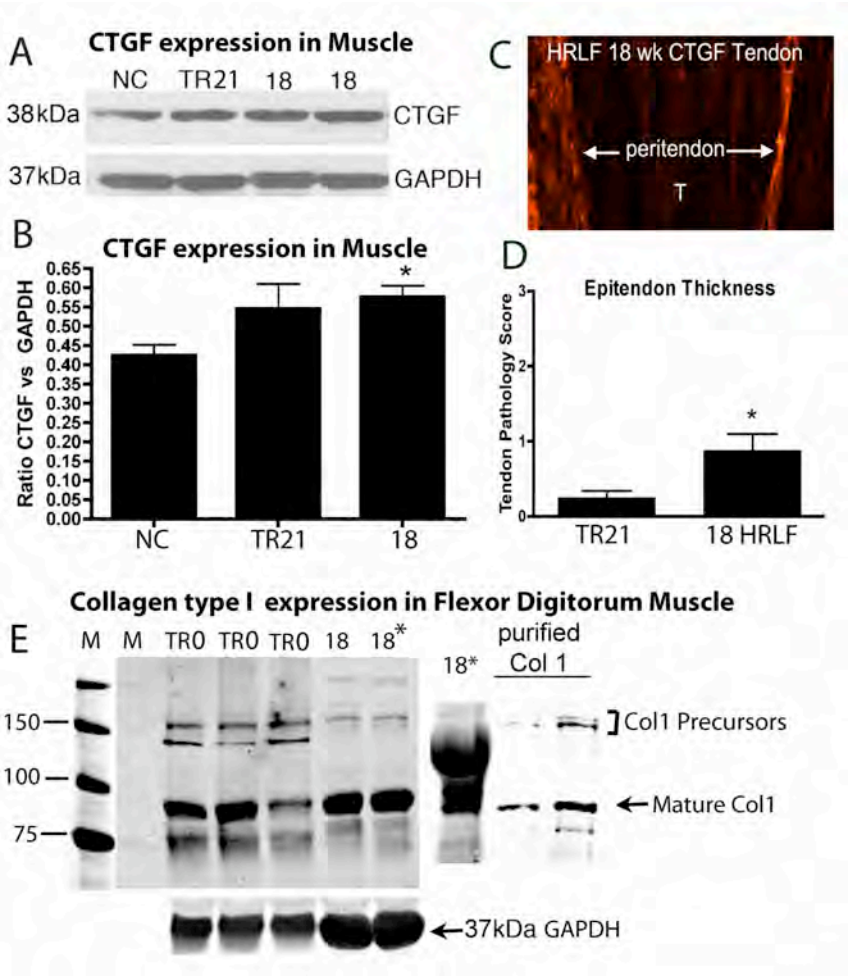


Figure 6. Flexor digitorum muscle and/or tendon connective tissue growth factor (CTGF) and collagen type I. CTGF levels are shown in NC, TR21, and 18 week HRLF rats. (A) Western blot of muscles from NC, TR21 and 18-week HRLF forelimb muscles probed with anti-CTGF. GAPDH was used as a loading control. (B) The ratio of CTGF/GAPDH is shown for three replicates of the western blot. (C) Immunohistochemical staining for CTGF in a tendon of an 18 week HRLF rat reach limb showing the location of CTGF in the epitendon. (D) Bonar scoring for tendon pathology was performed, and an increase in epitendon thickness was observed. *:$p<0.05$ and **:$p<0.01$, compared to TR21. (E) Western blot of muscles from TR0 and 18-week HRLF rats probed with anti-collagen type I. Lanes of left blot include M (Markers), 3 different TR0 samples, and two different 18 wk HRLF reach limb muscle sampes; 18* indicates sample sample on two gels. The blot on the right shows an overloading problem with sample 18*. However, it also shows two lanes in which purified rat tail collagen was loaded and probed with the anti-collagen type I as positive loading controls (L). Two bands of collagen type I precursor proteins are shown in both blots at approximately 140kDa, and bands for mature collagen type I between 75 and 90kDa. GAPDH was used as a loading control.

Increased repair proteins in serum but not in tendons

Lastly, there was an increase in serum levels of PDGFab and PDGbb in 18 week HRLF rats, compared to to (Fig. 7A,B). However, tendons levels were not increased (Fig. 7C.D), indicating that they were not the source of PDGF.

Discussion

We observed persistent declines in forearm grip strength with long-term overuse activity at a moderate task level. These declines were associated initially with a low-grade inflammatory response in forearm flexor digitorum muscles and tendons (evidenced by a small but significant increase in IL-1beta and TNF-α in the muscles and tendons immediately after training). As the task continued, serum levels of TNF-α rose significantly above baseline levels in week 12, an increase that was matched temporally by significant increases in TNF-α in flexor digitorum tendons. By week 18 of task performance, the serum inflammatory response had resolved, only to resurged\ in serum, tendons and muscle in week 24, albeit a key anti-inflammatory protein, such as IL-10 also increased in tissues in week 24. At the later time points of 18 and 24 weeks, serum biomarkers of degradation (MMP2), fibrosis (TGFB1), and repair (PDGFab and bb) also

increased. The increased serum MMP2 and TGFB1 was coincident with increased MMP2 and TGFB1 in tendons.

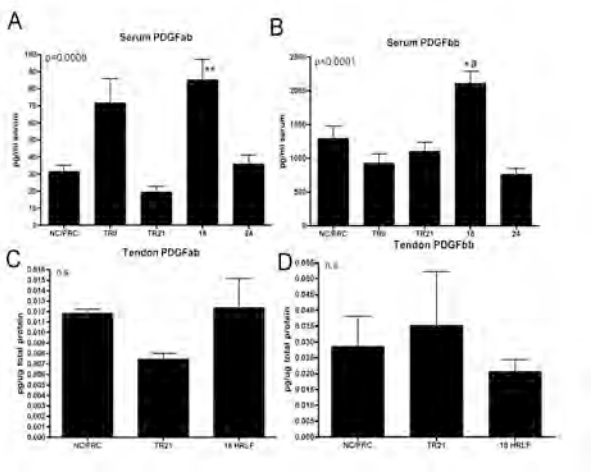


Figure 7. Serum and tendon levels of platelet derived growth factor ab (PDGFab) and PDGF bb. ELISA data for normal controls combined with food restricted controls (NC/FRC), TR0 (trained only rats), TR21 (trained rats that rested for 21 weeks), and high repetition low force (HRLF) rats that performed the task for 18 or 24 weeks. * and **:p<0.05 and p<0.01, compared to NC/FRC; a: p<0.05, compared to TR21.

Regarding the motor changes, declines were present immediately after the training period (TR0 time point of TR+Rest and HRLF rats). Grip strength resolved with rest in the trained-only rats and by week 12 in the support limbs of HRLF rats. This matches findings in a recent paper from our lab showing a reduction in grip strength after training in rats performing the HRLF task (Kietrys et al, 2011). The recovery of grip strength with rest in the TR+Rest rats is suggestive of tissue healing as a consequence of rest. The recovery of grip strength in the support limbs of HRLF rats may be due to adaptation of the tissues to the demands of providing support against the chamber wall. In contrast, the continued use of the reach limb to pull on the lever across the 18 weeks led to persistent grip strength declines with no sign of resolution.

The serum inflammatory cytokine response was low but still significantly increased for TNF-α, MIP 2 and MIP3 with performance of this moderate demand task. Several inflammatory cytokines tested were not above detectable levels and there was no increase after training. In fact, in this study, different and more sensitive ELISA assay kits were used than in our recent Xin et al, 2011 study. In that study, no changes in serum cytokines were detected in young adults rats that had performed the HRLF task for 12 weeks, although the significant increase in serum MIP2 levels were evident in that study as in this one. The levels of serum TNF-α, MIP2, and MIP3 here are similar to those observed in a prior study examining the effects of performance of a high repetition negligible force, food retrieval task (Barbe et al, 2008).

We also observed a low-grade tissue inflammatory response in musculotendinous tissues with long-term performance of this moderate demand reaching and lever-pulling task. This TNF-α inflammatory response was cyclical in nature in muscle, in that it was evident after the initial training session (albeit low grade), not present in 6-week HRLF rats, but then reappeared in 12-week HRLF rats. Tendon seemed more effected, with increases after training (TR0), and in 6- and 12-week HRLF rats. However, TNF-α again resolved in 18-week HRLF. The initial peak of tissue inflammation after training is likely due to training-induced tissue injury. The resolution phase in trained animals and in the HRLF support limbs is due to tissue repair as a consequence of rest. In the HRLF task rats, the resolution of inflammation by week 12 is probably due to tissue adaptation to the demands of the task. However, the reappearance of the tissue inflammatory response thereafter suggests that tissue repair and growth processes did not keep pace with tissue degenerative processes.

The tissue inflammatory response was considerably lower in this study than in past studies from our lab in which rats performed tasks with higher force levels or with more fine-manipulative requirements (Fedorczyk et al, 2010; Barbe et al, 2008). We have also already shown correlations between increased inflammatory cytokines in forearm muscles and tendons and decreased forearm grip strength (Barbe et al, 2008; Coq et al, 2009). Reduced grip strength can decline as a result of muscle inflammation (Kehl and Fairbanks, 2003). Kehl and Fairbanks reported reduced forelimb grip force following i.m. injection of carrageenan, an agent used to stimulate cutaneous inflammation and activate muscle nociceptors. Baker et al. (2007) have also reported declines in isometric force production with inflammation and damage to myofibrils postulated as injury mechanisms. Thus, even though at low levels, the increased TNFalpha in muscles and tendons of HRLF reach limbs at 0, 12 and 24 weeks likely contributed to grip strength declines in these same limbs.

In contrast to the low-grade inflammatory responses, *collagen degradative changes* were evident in both

serum and forearm tissues. MMP2 was increased in serum and forearm tendons. We also observed increases in other MMP1 in 18-week HRLF reach limbs. Each of these MMPs is involved in collagen catabolism, and each increases in tendons after injury (De Mello Malheiro et al., 2009). The observed increase of these MMPs in the tendons support the presence of tendon degradative processes as a result of long-term performance of this moderate demand repetitive task.

We also observed increased TGFβ1, CTGF and collagen type 1, proteins linked to fibrogenic repair. This observation is in line with studies showing that CTGF, collagen type 1, and TGFB1 increase under conditions of muscle overload or injury and are linked to tissue fibrosis (Kjaer, 2004; Abdelmagid, et al, 2012).The increase of each in the tendons and muscles HRLF task rats is more indicative of a fibrotic response than a regenerative repair response. These degenerative changes likely also contribute to the observed declines in grip strength. Our findings show that serum TGFB1 should be monitored in the future as an indicator of fibrosis, as it is a sensitive serum biomarker of fibrogenic diseases (Neuman et al, 2012).

Conclusions

These findings show that even moderate demand tasks can induce tissue degradative changes if the work is performed for long periods of time. Also, the temporal association of grip strength declines with both the low-grade tissue inflammatory cytokine response and the degenerative changes support a contribution from each process to functional declines occurring with overuse. Lastly, serum biomarkers MMP2 and TGFB1 were pinpointed as markers of underlying tissue degenerative and fibrotic changes. Serum levels of CTGF levels were not tested in this study, but may also serve as a biomarker of tissue degradative processes induced by long-term performance of repetitive tasks.

4. In the next study, which we are entirely proud to present based on its novelty as well as importance in the field of work related musculoskeletal disorders, we examined the following aims: To examine if long-term exposure to a high repetition-low force (HRLF) regimen causes persistent tissue inflammation in musculoskeletal tissues (aim 2), or declines in neurological associated behavior (a modification of aim 4).

Xin DL, Massiocotte V, Harris MY, Amin M, Barbe MF. Sickness behaviors, serum and brain IL6, increase with performance of high demand tasks in a rat model of overuse. Submitted to Brain Research, Oct 2012. Presented at Society for Neuroscience, October 2012.

Overview of study

We have shown in a rat model of work-related overuse that prolonged performance of repetitive tasks increases serum inflammatory cytokines that correlate with sensorimotor declines. Since virally induced increases in serum inflammatory cytokines also correlate with increased brain cytokines and sickness behaviors, e.g. decreased social interaction and increased aggression, we now examined these in our rat model. Young adult and aged female Sprague-Dawley rats were trained for 4-5 wks for 10 min/day, 5 days/wk at either a low force (TRLF; 15% max force) or a high force (TRHF; 60% max force) task level. The rats then performed a high repetition low force task (HRLF) of 8 reaches/min, 15% max force, for 2 hrs/day, 3 days/wk, for 9-24 wks. Duration of social interaction and incidence of aggression with novel juvenile rats were assessed every 3 wks. Results were compared to age-matched food-restricted controls (FRC). Following euthanasia, serum and brains were collected and analyzed for inflammatory cytokines. Behaviorally, at end of initial training period, both aged TRHF and TRLF rats had decreased social interaction with juvenile rats than at naïve, and FRC. Both aged and young TRHF rats had increased displays of aggression towards the juveniles than at naïve, behavior not observed in aged or young TRLF, NC or FRC rats. These behavioral changes in aged TRHF rats did not resolve until week 9 of the HRLF task (termed TRHF-HRLF), but resolved by week 3 in the young adult TRHF-HRLF and in aged TRLF-HRLF rats. These behaviors were not observed in young adult

TRLF-HRLF rats, even those performing the HRLF task for 24 weeks. There was an increase of serum interleukin 6 (IL-6) in aged and young TRHF ($p=0.01$ and $p=0.05$, respectfully) and in aged TRHF-HRLF rats that performed the task for 3 weeks; this increase resolved in these aged rats thereafter. No increase in serum cytokines was detected in young adult TRLF or TRLF-HRLF rats. Increased IL-6 and IL-6 receptor was also detected immunohistochemically in brains of aged rats with these sickness behaviors, specifically in the anterior cingulate cortex and paraventricular nuclei ($p<0.05$ each). No increase of IL6 or IL-6 receptor was observed in aged TRLF-HRLF rats or any young adult rats. Thus, training to high force, even for a short time period, induced increased serum IL6 and sickness behaviors. Aging also contributed to increased sickness behaviors and brain IL6 and IL-6 receptor.

Rationale

Repetitive strain injuries (RSIs) of the upper extremity are commonly reported occupational illnesses that are painful, potentially disabling and costly. These disorders are also known as work-related musculoskeletal disorders (WMSDs). They include peripheral neuropathies (e.g. carpal tunnel syndrome), tendinopathies and myopathies (Barr et al., 2004; Barr et al., 2003; Clark et al., 2004; Fedorczyk et al., 2009; Rempel and Diao, 2004; Sommerich et al., 2007; Szabo, 1998; Szabo, 2006; Topp and Byl, 1999; van Tulder et al., 2007).

Our group and others have reported that patients with RSIs of the upper extremity have increases in several serum pro-inflammatory markers and mediators, including C-reactive protein (CRP), IL-1, IL-1RII, IL-1ra, IL-6, IL-18, and TNF (Carp et al., 2007; Gotoh et al., 2001; Rechardt et al., 2011). In the Carp study, increased inflammatory markers correlated with the severity of upper-extremity signs and symptoms in patients short-term RSIs, including increased pain and an increase in pain frequency at multiple anatomical sites (Carp et al., 2007). Levels of CRP and TNF were predictive of RSI severity. Gotoh found that differential regulation of two forms of IL-1 receptor antagonist (IL-1ra) mRNAs also appear to play important roles in shoulder pain in rotator cuff diseases (Gotoh et al., 2001). Rechardt's group showed that levels of circulating soluble IL-1RII and IL-18 were associated with incipient upper extremity soft tissue disorders, suggesting an important role for IL-1 family members in these disorders (Rechardt et al., 2011).

Barbe and Barr have developed a rat model of voluntary repetitive reaching and grasping with or without force, in which performance of repetitive tasks induced similar behavioral and physiological tissue changes seen in humans with repetitive strain injuries. In this model, pathological tissue changes are associated with dose-dependent responses in inflammatory cytokines in serum, peripheral forelimb musculoskeletal and nerve tissues (Al-Shatti et al., 2005; Barbe et al., 2003b; Barbe et al., 2008; Barr et al., 2003; Cattano et al., 2011; Elliott et al., 2009a; Elliott et al., 2008; Fedorczyk et al., 2010; Rani et al., 2010; Xin et al., 2011). For example, increased serum levels of tumor necrosis factor alpha (TNFα), macrophage inflammatory protein 2 (MIP2) and MIP3 correlated significantly with reduced grip strength in young rats that had performed a high repetition, negligible force food retrieval task for 8 weeks (Barbe et al., 2008). These findings suggest that serum biomarkers indicative of underlying tissue inflammation may serve as effective biomarkers of RSIs for diagnostic purposes.

Levels of serum inflammatory cytokines, such as IL-6, also appear to contribute to a constellation of physiological and behavioral responses known collectively as the "sickness response", "sickness behaviors", or the Systemic Inflammatory Response Syndrome (SIRS) (Bluthe et al., 2000a; Bluthe et al., 2000b; Cartmell et al., 2000). (Clark et al., 2008; Kelley et al., 2003). These responses include weakness, lethargy, hyperalgesia, mechanical allodynia, decreased social interaction and exploration, fatigue and social withdrawal (Capuron and Dantzer, 2003; Dantzer, 1994; Dantzer, 2004; Dantzer et al., 1998; Dantzer et al., 1999a; Dantzer et al., 1999b; Dantzer et al., 2002; DeLeo and Yezierski, 2001; Kelley et al., 2003; Teeling et al., 2007). These behaviors are a normal response to infection or injury and are responsible for re-organizing perceptions and actions to enable individuals to respond appropriately to the illnesses.

Several sources of cytokines leading to sickness behaviors have been postulated, including humeral. Circulating cytokines either cross the blood brain barrier or activate signaling cascades in the circumventricular organs of the brain by activating cytokine receptors on astrocytes or other cells of the blood brain barrier (Castanon et al., 2004; Dantzer, 1994; Dantzer, 2004; Dantzer et al., 1999a; Goujon et al., 1996; Goujon et al., 1995; Merlot et al., 2002). Neurons located in the circumventricular organs then signal neurons in the hypothalamus and pituitary gland, which may drive physiological changes, such as fever, but also several

behavioral changes via connections between the hypothalamus and frontal cortical and limbic brain regions. Brain regions in which cytokines increase after injection of rodents with lipopolysaccharide include the nucleus tractus solitarius (NTS), ventrolateral medulla (VLM), hypothalamic paraventricular nucleus (PVN), ventromedial part of the preoptic nucleus (VMPO) and anterior cingulate cortex (AntCg).

Although sickness behaviors to different infectious conditions have been widely studied, a link between RSI and sickness response remains to be determined. It has been shown that cumulative exposure to repetitive motion tasks not only causes local tissue damage but also persistent behavior symptoms. Many researchers believe physical and psychosocial factors play important roles in RSI and consequential pain or emotional distress (Cleland, 1987; Macfarlane et al., 2000; Tyrer, 1994). It has been demonstrated that individual psychosocial factors are important predictors of the onset of the symptoms (Macfarlane et al., 2000; Theorell et al., 1991) (Shaw et al., 2002) (Harkness et al., 2004). However, it is important to note that anxiety and depression are more frequent in patients diagnosed with RSI than in control subjects, although the changes are not very different from patients with other pain conditions or chronic diseases (Marinus and Van Hilten, 2006).

Numerous epidemiological studies have demonstrated a relationship between advancing age and susceptibility to risk factors for RSIs. The incidence rate of lost workday injuries and illnesses due to repetitive motion is 1.6 times higher in workers aged 55 - 64 compared to those aged 25 – 34 (BLS, 2009). Studies indicate that older workers may be more susceptible to WMSDs than younger workers because of decreased physical capacity or a greater propensity for injury (Akrouf et al., 2010; McDermott et al., 2010; Nunes, 2009). This may be due to an increased incidence of chronic, low grade, inflammatory status that is a typical feature of the aging, a process termed “inflamm-aging” that can occur in otherwise healthy individuals and in the absence of acute infection (Franceschi, 2007; Franceschi et al., 2000; Franceschi et al., 2007; Singh and Newman, 2011). In the elderly, increased serum cytokines is associated with increased frequency of behavioral and cognitive complications (Evans et al., 2005) (Perry et al., 2003). In several rodent models of aging, an excessive neuroinflammatory cytokine response in brain tissues is coupled with a myriad of complications, including cognitive impairment (Barrientos et al., 2006; Chen et al., 2008) and exaggerated sickness behaviors (Abraham et al., 2008; Godbout et al., 2005; Huang et al., 2008).

Aims

Therefore, the specific aims here were to determine if: (1) the performance of repetitive upper extremity tasks results in exposure-dependent induction of sickness behaviors (social interaction and aggression), (2) if aging enhances these responses, and (3) if they correlate with increased serum or brain inflammatory cytokine levels. The hypotheses were that performance of repetitive upper extremity tasks would induce greater incidence of sickness behaviors in aged than in young rats, and that performing high force tasks would enhance this response due to an increase in serum inflammatory cytokine levels compared to lower force tasks. We further hypothesized that the increased serum inflammatory cytokines would correlate with increased brain cytokines and increased sickness behaviors.

Methods

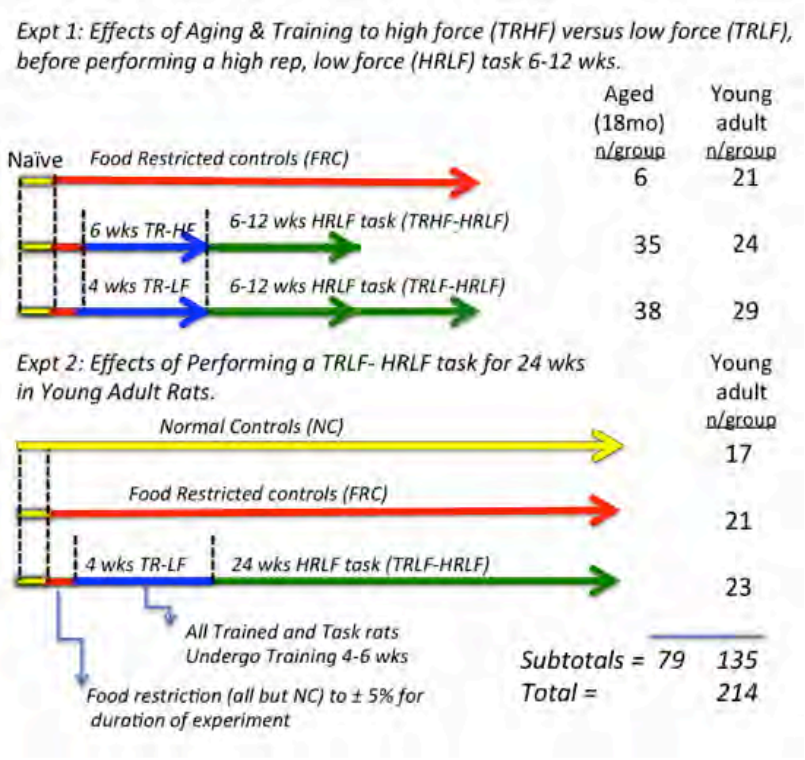
Animals

All experiments were approved by the Institutional Animal Care and Use Committee in compliance with NIH guidelines for the humane care and use of laboratory animals. Studies were conducted on aged and young adult female rats. A total of 228 rats were used in two separate experiments. Experiment 1 examined the effects of aging (aged versus young) and training to high force (TRHF) versus low force (TRLF), before each group performed a high repetition low force task (HRLF) for 6 or 12 weeks as shown in Fig. 3-1. In this first experiment, there were 79 aged, female Sprague-Dawley (15 mo of age at onset of experiment; 17-19 mo of age at euthanasia) and 74 young female Sprague-Dawley rats (3 months of age at onset of experiment; 6-7 mo of age at euthanasia). Experiment 2 examined the effects of performing a HRLF-LRLF task for 24 weeks in young adult rats in order to ascertain if longer task performance by young adult rats would result in equal behavioral problems as shorter task regimens by aged rats (Fig. 3-1). A total of 61 young adult Sprague-

Dawley rats were used in this second set of experiments (3 months of age at onset of experiment; 11 mo of age at euthanasia). In addition, 32 juvenile rats (4-7 weeks of age) were used for the behavioral studies in which the time and type of interaction of the adults with the juveniles was recorded; these juveniles did not perform the repetitive tasks and are not counted in the tally shown in Figures 3-1.

All rats were housed individually in the central animal facility in transparent plastic cages in a 12-hour light: 12-hour dark cycle with free access to water. All rats were inspected weekly and again post-mortem for presence of illness or tumors in order to reduce confounders for serum cytokine increases. As a consequence, an additional 10 of the aged rats were eliminated from the study due to age-related health issues, such as renal failure, presence of tumors, or mortality from unknown causes. To further reduce illness related confounders, additional sentinel rats were maintained and examined for presence of viral infections as part of the regular veterinary care (no viruses were detected).

Overview of experiments



All rats acclimated to the facility and handled for at least 1 week prior to beginning the experiments. Aged rats were 11-12 months of age at the time of purchase and were then aged to 15 months of age. They were multi-housed during that time period. All rats but normal controls were food restricted to 5% of their normal weight or the weight of matched normal controls beginning at the onset of the training period and the duration of the 12-week task. Food restriction was necessary to motivate rats to perform the repetitive task. Cohorts of aged or young adult Sprague-Dawley rats were trained to perform either a high force task (TRHF) or a low force task (TRLF) during an initial training period of 10 min/day for 4-6 weeks. After training, cohorts of the trained rats went on to perform a high repetition low force (HRLF) task for up to 24 weeks. Four comparison groups were included in the design: normal controls (NC = free access to food and rested until euthanasia); food restricted only controls (FRC = rats that were food restricted but were neither trained nor performed the task, but rested until euthanasia); rats that were trained to perform a high force task (TR-HF = trained-only rats that were food restricted, trained for 6 weeks for 10 min/day to learn a high repetition high force task, and then euthanized at the end of the training period); rats that were training to perform a low force task (TR-LF = trained-only rats that were food restricted, trained for 4 weeks for 10 min/day to learn a high repetition low force task, and then euthanized at the end of the training period). The young adult normal control rats were used for weight comparison controls for the young adult food restricted rats, as they were still growing.

The focus of Experiment 1 was to determine the effects of age and training level on sickness behaviors, and serum and brain inflammatory cytokines, in rats performing a HRLF task. In this experiment, a total of 167 rats were food restricted to within 5% of their naïve weights; 78 were aged rats that were 15 months of age at the onset of the experiments; the remaining were young adults rats that were 3 months of age at the onset of the experiments. As shown in Figure 3-1, 6 aged and 21 young adult food-restricted rats were used for comparison purposes. Additional food-restricted rats were trained during a 4 or 5 week training period to perform a reaching and handle-pulling task at one of two force levels: high repetition high force (TR-HF; 13 aged and 10 young adult) or high repetition low force (TR-LF; 16 aged and 20 young adult). These TRHF and TRLF rats were euthanized immediately after the training period. Thirty-six more TR-HF rats went on to

perform the HRLF task for 3-6 weeks (TRHF-HRLF; 22 aged and 14 young adult); while 45 more TR-LF rats went on to perform the HRLF task for 3-6 weeks (TRLF-HRLF; 21 aged and 24 young adult).

The focus of Experiment 2 was to determine if long-term food restriction or continued performance of the HRLF task for 24 weeks by young adult rats induced sickness behaviors or increased serum or brain cytokines, since, as to be shown subsequently, young adult TRLF-HRLF that worked for 6 weeks did not show significant changes in these behaviors. As shown in Fig 3-1, twenty-three TRLF-HRLF rats performed the HRLF task for 24 weeks. Results were compared to 21 age-matched food restricted controls (FRC) and 17 normal controls (NC).

Behavioral Apparatus and Training Period

The behavioral apparatus is as described previously in detail (Clark et al., 2004, Elliott et al., 2010), and as described briefly in Chapter 2 (Xin et al, 2011). The training period is as described in Chapter 2 for the HRLF rats, and as described earlier and previously (Clark et al., 2003; Elliott et al., 2010; Xin et al, 2011).

HRLF and HRHF task regimen

At the end of the training period, subsets of trained animals were randomly selected to begin the HRLF and HRHF task regimens for 2 hrs/day, 3 days/wk, for 6-24 weeks, as shown in Figure 3-1. The task was divided into 4, 0.5-hr sessions separated by 1.5 hrs in order to avoid satiation. The target reach rate for each task was 4 reaches/min, although they tended to over reach (to 8 reaches/min), as shown previously (Elliott et al., 2010; Kietrys et al., 2011). The target force levels were 15% and 60% maximum grasp force for the TRLF and TRHF training period regimens, respectively, and 15% for the HRLF task.

Sickness Behavioral Testing

Sickness behaviors were examined in all rats. Although one measurement of sickness response was body weight, although we attempted to maintain weight to no less than 5% of age-matched normal controls to reduce food restriction-induced catabolic tissue changes that would affect serum cytokine changes. Animals that lost excessive weight were given free access to food as needed. Specifically, we examined the duration of social exploration of the adult rat towards a novel juvenile rat during the first 5 minutes following introduction of the juvenile rat, with behavior assessed at the naïve point (before food-restriction), after food restriction, after training (week 0 of task performance) and then every three weeks thereafter. Adult rats were first placed into clean, clear plastic chambers and allowed to acclimate for 10 minutes. A novel juvenile rat was then introduced, and the interaction of the adult rat towards the novel juvenile rat was measured for up to 5 minutes. The following were counted as positive interactions with the juvenile rat: 1) sniffing of any kind (face-to-face/genitoanal), 2) crawling over or under younger rat, 3) grooming of one another, and 4) licking. Results are presented as seconds spent interacting with the juvenile rat. Aggression by the adult rat towards the juvenile rat was also noted, i.e. boxing, lateral displays, biting, wrestling, and kicking. Such actions ended the social interaction session and the juvenile rat was removed from the cage immediately. The numbers of rats showing aggression per week is presented, as are the numbers of rats showing no aggression per week. Trained observers who were blinded to group assignment obtained all other behavioral data.

Collection of Serum and measurement of serum cytokines and chemokines

Rats were euthanized with an overdose of sodium pentobarbital (Nembutal; 120 mg/kg body weight), and serum collected as described previously (Xin et al, 2011) from the following aged rats: FRC week 3 (n=4) and week 9 (n=5); TRHF (n=8), TRLF (n=6), TRHF-HRLF weeks 3 and 6 (n=6 each), and TRLF-HRLF week 6 (n=6). Serum was also collected from the following young adult rats: FRC (n=7); TRHF (n=10), TRLF (n=7), TRHF-HRLF week 6 (n=4), TRLF-HRLF week 12 (n=7), TRLF-HRLF week 18 (n=11), and TRLF-HRLF week 24 (n=7). Serum was analyzed for 3 cytokines using a customized multiplexed sandwich ELISA system (Aushon Biosystems Searchlight system): IL-1alpha, IL-6, and TNF-alpha, as described previously (Barbe et al, 2008), with data presented as pg/ml serum.

Brain cytokine analysis using immunohistochemistry

Brains were collected from select rats for examination for presence of inflammatory cytokines. One limitation of these experiments is that although behavior data was collected at three week intervals from all rats until euthanasia, not enough animals were generated for euthanasia at each 3 week interval. As a consequence, serum and brain tissues were not available for examination from all rats at each behavioral endpoint. Brains from select rats were collected, sectioned and examined using immunohistochemistry for IL-6, IL-6 receptor, IL-1alpha and TNF-alpha from the following aged rats: FRC week 3 (n=4) and week 9 (n=5); TRHF (n=8), TRLF (n=6), TRHF-HRLF weeks 3 and 6 (n=6 each), and TRLF-HRLF week 6 (n=6); and from the following young adult rats: FRC (n=7); TRHF (n=10), TRLF (n=7), TRHF-HRLF week 6 (n=4), TRLF-HRLF week 12 (n=7), TRLF-HRLF week 18 (n=11), and TRLF-HRLF week 24 (n=7).

For this purpose, rats were euthanized with an overdose of sodium pentobarbital (Nembutal; 120 mg/kg body weight), serum collected as described above, and the rats were then perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were collected, postfixed "en bloc" by immersion overnight, and stored in 30% sucrose for 3 days until cryosectioned into 14 micrometer coronal sections and mounted onto charged slides (Fisher Plus, Fisher Scientific). Brain sections, on slides, were blocked with 4% goat serum in phosphate buffered saline (PBS) and incubated with the following primary antibodies: IL-6R (Santa Cruz Biotechnology, Inc. Catalog no.sc-660), Il-6 (Abcam Inc. Catalog no. abc6672), or TNF (R&D systems, Inc. catalog no. MAB510; 1:250 dilution in PBS), for overnight at room temperature and washed. Cytokine primary antibodies were visualized using appropriate secondary antibodies (all from Jackson Immuno) conjugated to Cy2 (green fluorescence) or Cy3 (red fluorescence), diluted 1:250 in PBS, for 2 hours at room temperature before washing.

To examine the cell types expressing specific cytokines, double-labeling of the cytokine-labeled sections were performed antibodies to specific markers: NeuN (a specific neuron marker; Millipore, Billerica, MA; catalog no. MMB337; 1:200 dilution in PBS), glial fibrillary acidic protein (GFAP; a specific astrocytes marker; clone GA-5 Millipore/Chemicon, 1:250), and Iba (a specific microglial cell marker). These latter primary antibodies were visualized using appropriate secondary antibodies (all from Jackson Immuno) conjugated to Cy2 (green fluorescence) or Cy3 (red fluorescence), diluted 1:250 in PBS, for 2 hours at room temperature before washing. DAPI was also used as a counterstain for several of the stained sections.

Quantitative measurements were made using stereological methods (Bioquant, and Bioquant Stereological Plug-In Tool, Nashville, TN) in a set square area of 13.3 cubic microns/section. Three measurements were made in 3 sections per rat, using an optical dissection, with each section separated by 140 micrometers. Brain regions examined for increased cytokine or receptor immunoreactivity included: hypothalamic paraventricular nucleus (PVN), supraoptic nucleus (SON), ventromedial part of the preoptic nucleus (VMPO), bed nucleus of the stria terminalis (BST), and anterior cingulate cortex. Results were plotted and expressed as the mean number of IL6+ or IL6R+ cells/mm^3 plus SEM. The individuals carrying out the image analyses were blinded to treatment.

Statistical Analyses

To determine the effect of repetitive task performance on duration of social interaction, one-way ANOVAs were used. The Bonferroni post-hoc method for multiple post hoc comparisons was used throughout, and adjusted p values are reported. Adjusted p values are reported. Two-way ANOVA were used to compare social interaction data in aged rats by week of task performance, in young adult rats by week of task performance, and in aged versus young rats in specific weeks of task performance. Chi-Square tests were used to assess for differences in incidence of aggression across groups. Univariate ANOVAs was used to compare differences in serum or brain cytokines across groups. All statistical analyses and calculations were derived using Prism statistical software. A *p*-value of <0.05 was considered significant for all analyses. The relationship between inflammatory cytokine and sickness behavior was analyzed using Pearson's correlation analyses.

RESULTS

Sickness Behaviors

Body weight. Two-way ANOVA showed no significant differences in weight by week (p=0.12) or by group (p=0.20). The mean weight of aged normal control rats and all other rats at the naïve time point (before food restriction or training) was 405.0 ± 5.97 g. No significant declines beyond 5% of normal control values were observed for any group (data not shown).

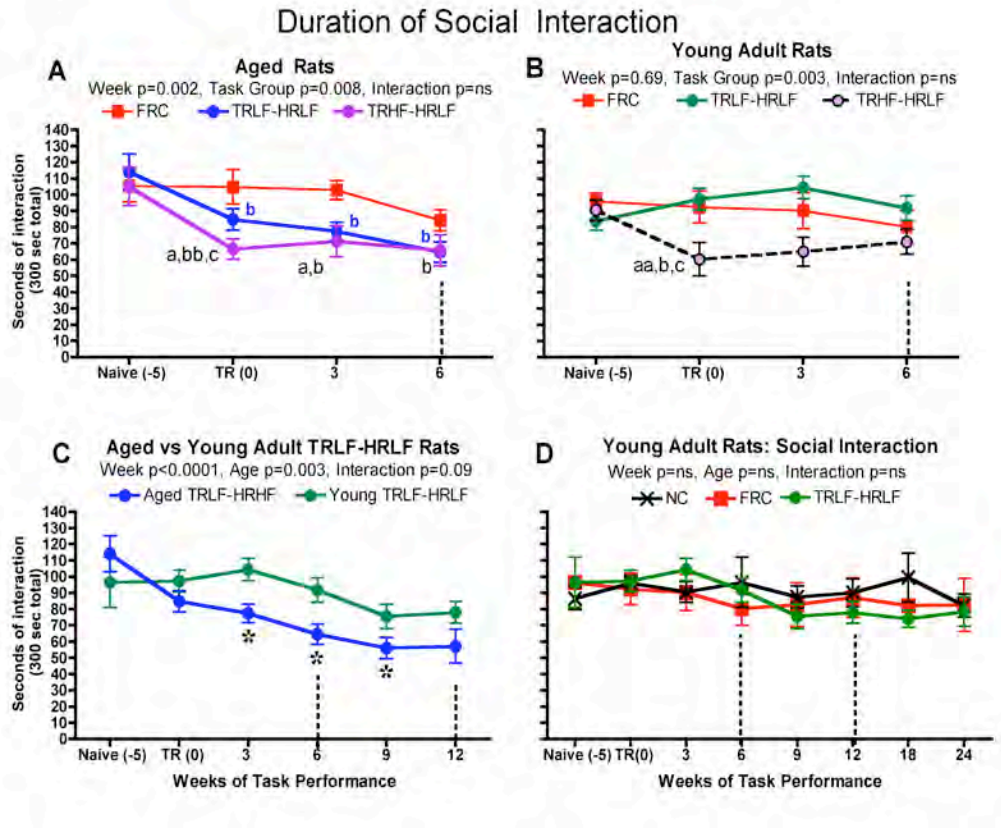


Figure 3-2. Duration of social interaction (in seconds) by adult control or experimental rats towards a novel juvenile rat, observed during a 5 minute observation period. Two-way ANOVA findings and mean + SEM are shown with each individual graph. (A&B) Aged and young adult rats that trained to high force level and then performed a high repetition low force task for 6 weeks (TRHF-HRLF), compared to rats that that trained to high low level and then performed the HRLF task for 6 weeks (TRLF-HRLF). (C) Aged and young adult TRLF-HRLF rats that performed this task for 12 weeks. (D) Young adult TRLF-HRLF rats that performed this task for 24 weeks. Results were compared to food restricted controls (FRC), naïve or normal controls (NC) a and aa: p<0.05 and p<0.01, compared to same aged NC rats or naïve data; b and bb: p<0.05 and p<0.01, compared to same aged FRC; c: p< 0.05 compared to same aged HRLF rats. *, p<0.05 compared to same week of

Social Interaction. Social interaction declined with age and training to high force. Specifically, in each age group, social interaction with novel juvenile rats was lower in both age groups with training to high force (TRHF) than with low force (TRLF), and compared to age-matched naïve data and FRC (Fig. 3-2A,B). Social interaction continued to decrease in aged rats with continued task performance of both the TRHF-HRLF and TRLF-HRLF tasks to 6 weeks, compared to age-matched FRC (Fig. 3-2A). In contrast, social interaction declines recovered over time in young adult rats that continued either task to 6 weeks (Fig. 3-2B). When duration of social interaction was assessed in aged and young adult TRLF-HRLF task that worked for 12 weeks, interaction time decreased more in aged task rats, compared to young adult task rats (Fig. 3-2C). To ascertain if longer performance of the TRLF-HRLF by young adult rats would result in similar declines in social interaction as in aged task rats, we examined this behavior in young adults TRLF-HRLF rats across 24 weeks (Fig. 3-2D). We found no significant changes in TRLF-HRLF rats, compared to age-matched NC and FRC.

Incidence of Aggression.

Both age groups had increased displays of aggression towards juvenile rats after training to TRHF levels than at naïve, behavior not observed after training to TRLF (Fig. 3-3). Specifically, aged rats had increased incidence of aggression after the TRHF training, than at naive, and in TRHF-HRLF aged rats with continued performance to 3 or 6 weeks (Fig 3-4A). No significant increased in aggression was observed in aged FRC or aged TRLF-HRLF rats (Fig 3-4A). Similarly, young adult rats had increased incidence of aggression after the TRHF training, than at naïve, and in TRHF-HRLF aged rats with continued performance to 3 or 6 weeks (Fig 3-4B). No significant increased in aggression was observed in young adult FRC or TRLF-HRLF rats, even in young adult TRLF-HRLF rats that worked for 24 weeks (Fig 3-4B). When comparing the two groups, only a trend towards an increase in aggression was observed in aged TRHF rats, compared to young adult TRLF rats (**: p<0.001, compare Fig 3-3A to Fig 3-3B).

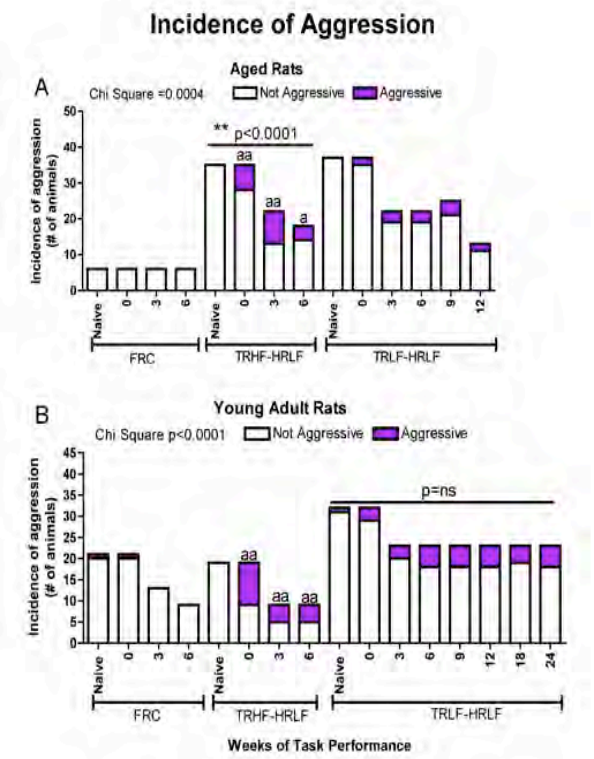


Figure 3-3. Incidence of aggressive behaviors in aged and young adult rats. (A) Chi Square analysis of incidence of aggression in aged food restricted rats (FRC), TRHF-HRLF rats, and TRLF-HRLF rats. (B) Chi Square analysis of incidence of aggression in young-adult food restricted rats (FRC), TRHF-HRLF rats, and TRLF-HRLF rats. Incidence of aggression versus non aggressive is shown. a and aa: p<0.05 and p<0.01, compared to same aged NC rats or naïve data; *, p<0.05 compared to same week of young adult rat;

Serum inflammatory cytokines

Several serum inflammatory cytokines increased with training to the high force level and with aging (Fig. 3-4). Specifically, serum IL-1beta and IL-6 were increased in aged rats that trained to high force, compared to NC/FRC and aged rats that trained to low force (Fig 3-4A,B). Serum IL-6 remained increased in TRHF-HRLF through week 6, while serum TNF-alpha continued to increase in these rats, compared to age-matched NC/FRC and aged rats that trained to low force (Fig. 3-4A-C). Serum IL-1beta and IL-6 also increased in aged TRLF-HRLF rats that worked to week 12, compared to NC/FRC levels (Fig. 3-4B,C). Young adult rats were affected differently. In young adult rats, serum IL-1beta increased after training to high force, and continued to be increased through 6 weeks, compared to age-matched NC/FRC and young adult rats that trained to low force (Fig 3-4D). In contrast, serum IL-6 was increased only after training to high force, compared to young adult rats that trained to low force (Fig 3-4E). TNF-alpha did not increase in young adult rats. Compared across age groups, serum IL-1beta was lower in aged rats than young rats (compare Fig-3-4A to D). In contrast, serum IL-6 and TNFalpha levels were higher in aged rats than young rats performing the same task (compare Fig. 3-4B and E, and Fig 3-4C and F). A Pearson's correlation test showed that serum IL-6 levels correlated negatively and moderately with seconds of social interaction (Pearson's r= -0.33, p=0.01; Fig. 3-5), and positively but weakly with incidence of aggression (Pearson's r= -0.22, p=0.03). Serum IL-1alpha and TNF-alpha levels did not correlate with either duration of social interaction or aggression.

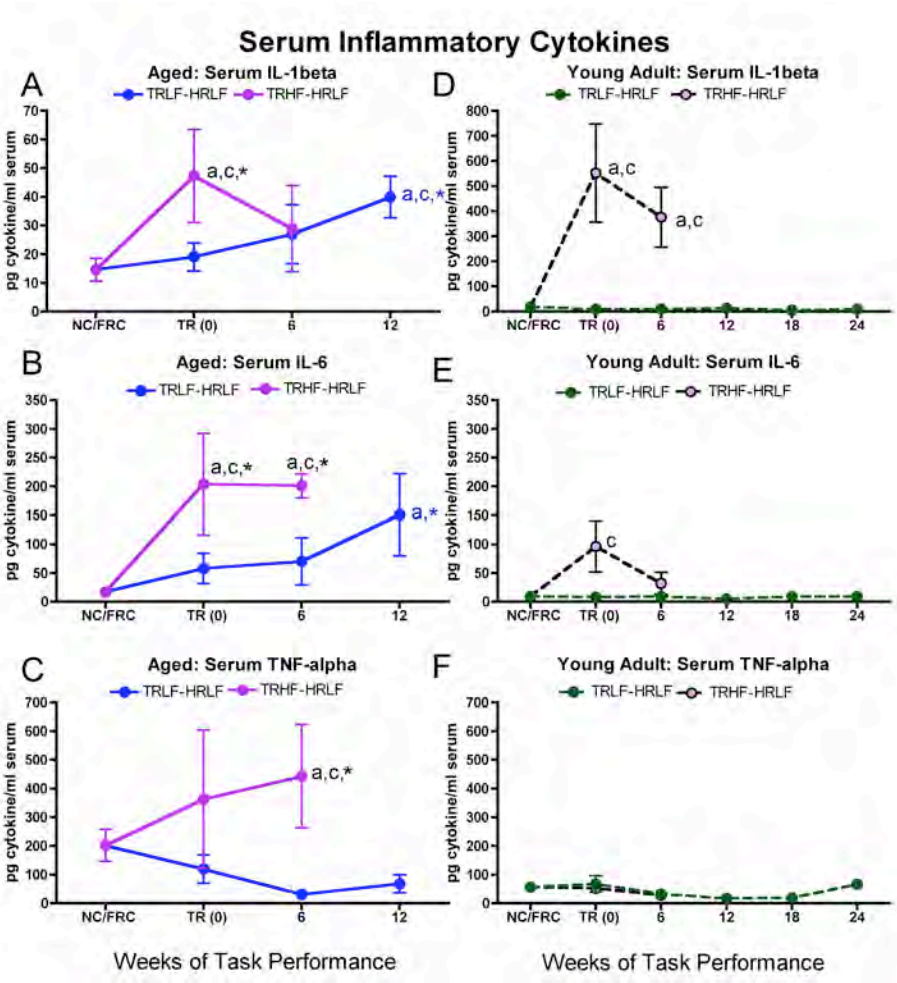


Figure 3-4. Serum inflammatory cytokine levels. Aged and young adult adults rat data is shown for the following groups: Normal control combined with food restricted control rats (NC/FRC), trained-only rats euthanized immediately after training (TR wk 0), rats trained to perform a low force task that then performed a high repetition low force task for 6 or 12 weeks (TRLF-HRLF), and rats trained to perform a high force task that then performed a high repetition low force task for 6 weeks (TRHF-HRLF). Aged rat serum IL-1beta (A), IL-6 (B), and TNF-alpha (C). Young adult rat serum IL-1beta (D), IL-6 (E), and TNF-alpha (F). a: $p<0.05$, compared to same aged NC/FRC; c: $p< 0.05$ compared to same aged HRLF rats. *, $p<0.05$ compared to same week of young adult rats.

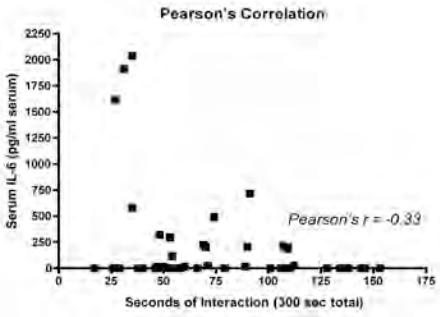


Figure 3-5. Pearson's correlation between serum IL-6 levels and duration of social interaciton, showing a moderate, negative correlation.

Brain Inflammatory Cytokines

Increased IL-6 and IL-6 receptor was detected immunohistochemically in brains of aged rats with sickness behaviors, specifically in the ependymal and endothelial cells, anterior cingulate cortex and paraventricular nuclei ($p<0.05$ each; Fig 3-6, Fig 3-7). Specifically, IL-6 and IL-6R immunohistochemistry was increased above aged NC levels in 6-week aged TRHF-HRLF rats brain in anterior cingulate neurons, Iba+ (microglial cells), and endothelial cells, compared to age-matched NC (Fig. 3-6A,D,F,G; Fig 3-7A-D). Both were also increased in ependymal cells of 6-week aged TRHF-HRLF rat brain (Fig. 3-6H,I), in astrocytes surrounding blood vessels (Fig. 3-6J,K), but not in NC rats (Fig. 3-6B,C). Three of five of the 6-week aged TRHF-HRLF had increased IL-6 staining in the CA3 region of the hippocampus and paraventricular nuclei of the hypothalamus (Fig. 3-6L,M; Fig. 3-7E). No significant increases of IL6 or IL-6 receptor were observed in brains of young adult-TRHF-LRLF rats (Fig. 3-7; not all data shown).

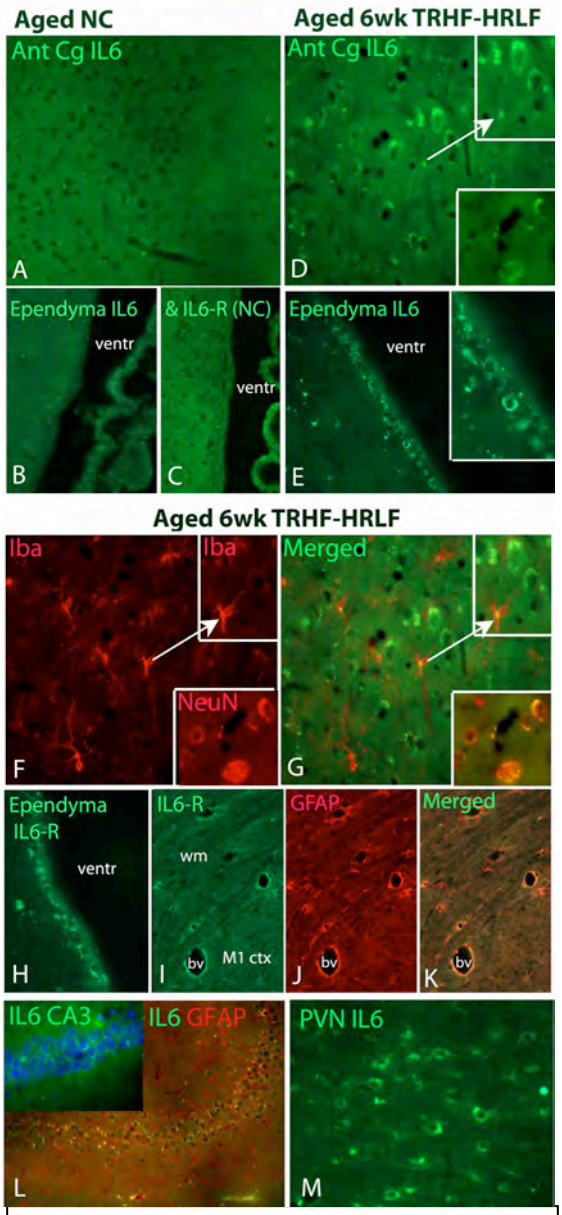


Figure 3-6. Interleukin 6 staining in aged NC and aged TRHF-HRLF rats.

No significant immunohistochemical increases of IL6 or IL-6 receptor were observed in brains of aged or young adult TRLF-HRLF rats (data not shown). Also, no significant increases in IL-1alpha or TNFalpha were observed in brains of any group examined in this study (data not shown). IL-6 levels were measured in brain tissues using ELISA in young adult TRLF rats and 12-week HRLF rats, but no increases were detected in the anterior cingulate cortex, prefrontal cortex, and or hindbrain, compared to NC or FRC levels (data not shown).

Discussion

Behaviorally, at the end of initial training pe had decreased social interaction with juvenile In contrast, only a trend towards decreased interaction was observed in young adult TRHF Both aged and young TRHF rats had increasec displays of aggression towards the juveniles naïve, behavior not observed in aged or TRLF or FRC rats. These behavioral changes TRHF rats did not resolve until week 9 of the task, but resolved by week 3 in the young adult TRHF-HRLF and in aged TRLF-HRLF rats. There was an increase of serum IL-6 in aged young TRHF and in aged TRHF-HRLF rats tha performed the task for 3 weeks that resolved increase in serum cytokines was detected in adult TRLF or TRLF-HRLF rats. Increased IL- IL-6 receptor was also detected in brains of rats with sickness behaviors, specifically, in anterior cingulate cortex and paraventricular nuclei. No increase of IL6 or IL-6 receptor was observed in aged TRLF-HRLF rats or any young adult rats.

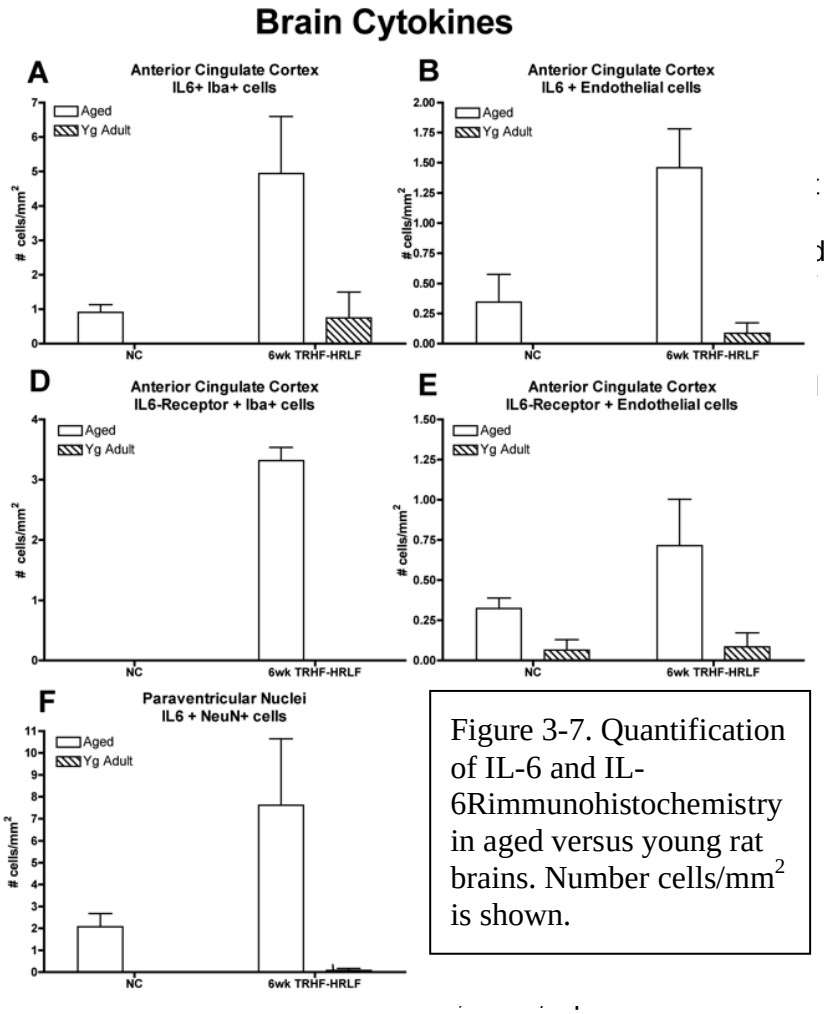


Figure 3-7. Quantification of IL-6 and IL-6Rimmunohistochemistry in aged versus young rat brains. Number cells/mm^2 is shown.

One hypothesis is that the physiological balance between pro- and anti-inflammatory cytokines is lost in the aged brain (Viviani and Boraso, 2011). This translates at the brain level as an enhanced production of pro-inflammatory cytokines, such as IL-6, IL-1, and TNF(Frank et al., 2006; Lukiw, 2004; Maher et Schultzberg, 2010; Streit et al., 2004; Weindruch et al., 2002; Ye and Johnson, 2001a). A second hypothesis is that the increased production of pro-inflammatory cytokines in the aged brain is the result of a “priming” process. Recent evidence indicates there is an increased population of reactive or primed immune cells and glia in the aged brain (Godbout and Johnson, 2009). Once primed, the cells are more sensitive to a triggering stimulus and will react with an exaggerated response characterized by increased cytokine production (Schroder et al., 2006). This “priming” theory in aging is supported by observations that peripheral infection, mimicked by intraperitoneal administration of LPS (Chen et al., 2008; Godbout et al., 2005) (Henry et al., 2009) or inoculation with Escherichia coli (Barrientos et al., 2006), elicits an exacerbated inflammatory response in the aged brain. In the elderly, systemic infection is associated with an increased frequency of behavioral and cognitive complications (Evans et al., 2005) (Perry et al., 2003). In several rodent models of aging, the excessive neuroinflammatory cytokine response is coupled with a myriad of complications including cognitive impairment (Barrientos et al., 2006; Chen et al., 2008), exaggerated sickness behavior (Abraham et al., 2008; Godbout et al., 2005; Huang et al., 2008), and protracted depressive-like behavior.

We observed temporally matched reductions in reduced social interaction skills and task participation in the aged rats performing the HRLF task. While we have previously observed reduced task duration in young rats performing repetitive tasks (Barbe et al., 2003a; Clark et al., 2004; Clark et al., 2003; Elliott et al., 2009b), this is the first study to observe more specific sickness response behaviors, such as reduced social interaction, with repetitive task performance. The concept of serum cytokine-induced sickness response is well established

in clinical and basic science (reviewed in Dantzer et al, 2008). Findings of reduced activity, loss of interest in social activities and even increased aggression in patients with chronic inflammation/infection or receiving cytokine therapies, such as for cancer (Zalcman and Siegel, 2006), have contributed to a hypothesis of inflammation-induced sickness behaviors and aggression. For example, in patients with viral infections, increased serum IL-1 and IL-6 correlate with acute sickness behaviors including fever, malaise, pain, fatigue, mood and depression (Vollmer-Conna et al., 2004). Although the systemic inflammatory response induced with RMI may not be as dramatic as with more severe inflammatory medical conditions, such as sepsis, there is evidence that even low grade systemic inflammatory responses are enough to induce a sickness response. Low dose LPS injection induces small but significant increases in circulating pro-inflammatory cytokines (IL-1 beta and IL-6), which is accompanied by anhedonia (reduced glucose intake) and depressive-like behaviors (Teeling et al., 2007).

Our results are also in agreement with numerous animal studies investigating the role of cytokines in sickness response and depressive behavior (Bluthe et al., 1994; Hayley et al., 2005; Suarez et al., 2002; Swiergiel and Dunn, 2007; Teeling et al., 2007). For example, Merali et al. (2003) found intraperitoneal injection of IL-1 resulted in reduced incentive motivation for a sucrose reward (anhedonia) (Merali et al., 2003), a finding that suggests that our observed reduced task participation is a type of anhedonia. In our model, the source of the circulating cytokines is not by injection, but from task-induced injured musculoskeletal and nerve tissues, as shown in several of past studies (Al-Shatti et al, 2005; Barbe et al, 2008; Elliott et al, 2009).

Behaviors that decline due to maladaptive central nervous system neuroplasticity

Several studies also indicate that maladaptive neuroplasticity in the somatosensory cortex contributes to motor declines (Ballermann, McKenna & Whishaw, 2001; Byl & Melnick, 1997; Byl et al., 1997; Byl et al., 1996; Coq et al., 2009). Disruption of the forepaw map and the emergence of large receptive fields in the somatosensory cortex resulted in involuntary movements characteristic of focal hand dystonia in a monkey model of repetitive prehensile tasks (Byl et al., 1997; Byl et al., 1996). In our model, performance of a repetitive negligible force reaching task for 8 weeks also resulted in disruption of the forepaw map in the somatosensory cortex, as well as the emergence of large receptive fields encompassing several forepaw subdivisions, such as digits-pads and forepaw-forearm (Coq et al, 2009). The larger receptive fields correlated negatively with percent successful reaches. Thus, somatosensory cortical changes likely result in ambiguous interpretation of tactile cues and contribute to declines in grasp control.

Changes in the motor cortex were also detected in the Coq study mentioned above (Coq et al., 2009). These changes included enlarged motor forepaw maps and increased representation of digit and multi-joint movements. Some of these changes seemed more adaptive than deleterious, and likely contribute to our observed skill acquisition. This is supported by studies showing that motor skill learning is associated with expansion of the representation of distal forelimb movements in the motor cortex (Kleim, Barbay & Nudo, 1998; Plautz, Milliken & Nudo, 2000). However, we also observed increased pathological multi-joint movements when stimulating motor cortex neurons in our past study (Coq et al, 2009), and that the amount of current needed to evoke these multi-joint movements was lower than in controls. These changes correlated positively with increased inflammatory cytokines in the forearm muscles, suggestive of a peripheral tissue inflammation influence on the motor cortex. These results combined provide strong evidence that peripheral inflammation, spinal cord neuroplasticity and cortical neuroplasticity contribute to the development of motor declines, and now sickness behaviors, observed with chronic repetitive motion tasks.

Conclusions

Thus, training to high force, even for a short time period, induced increased serum IL6 and sickness behaviors. Aging also contributed to increased sickness behaviors and brain IL6 and IL-6 receptor.In conclusion, our study documents that a robust cytokine and chemokine response pattern is induced by prolonged performance of a repetitive task as well as increased sickness behaviors. The possibility for patients with chronic inflammatory conditions to succumb to the sickness or depressive effects of local and systemic pro-inflammatory cytokines has implications in the management of RMI. A more complete understanding of the relationship between prolonged performance of repetitive tasks and the induction of the sickness response would help direct effective workplace and clinical management strategies. Such findings would also reduce the

negative stigmatization often imposed by health care providers upon patients who present with such vague and apparently psychological complaints.

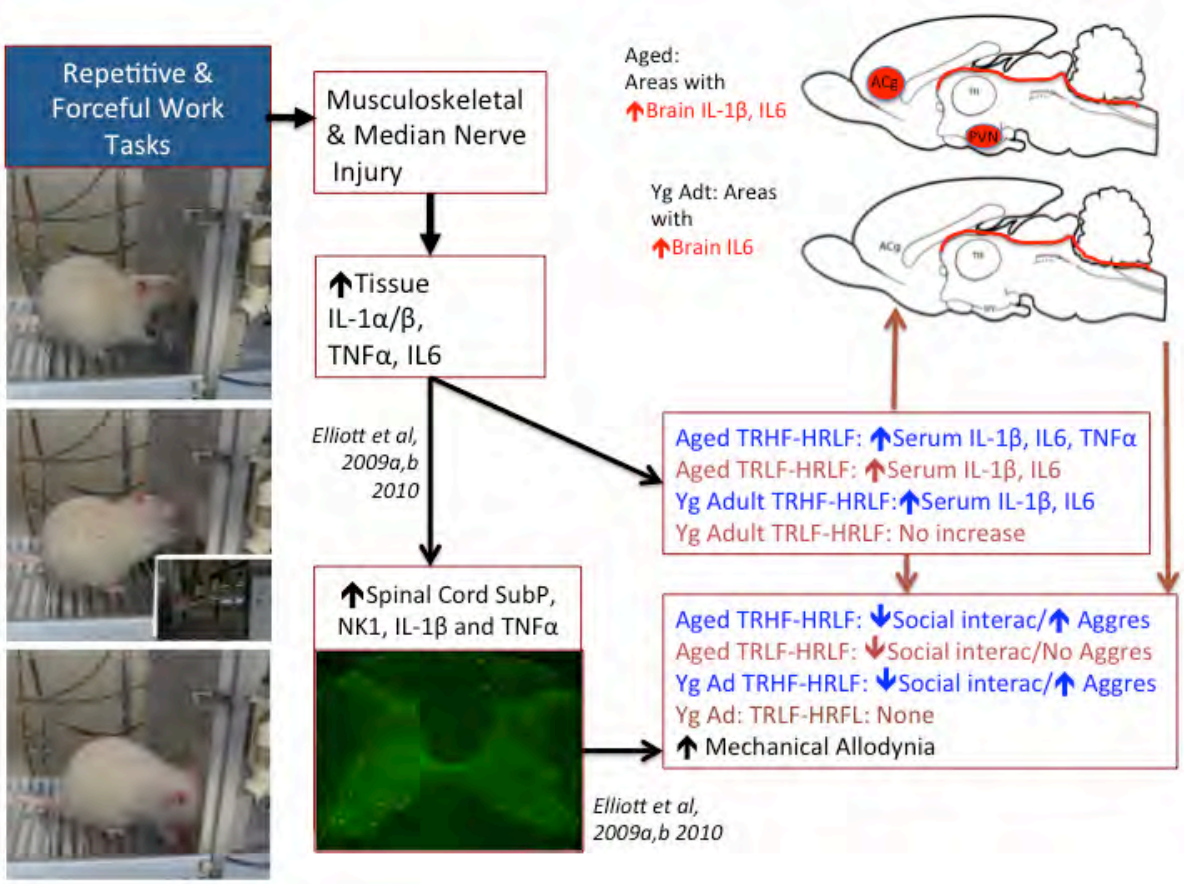


Figure 3-8. Model of WMSDs showing that repetitive and forceful work tasks lead to musculoskeletal and median nerve injury and then induces a release of inflammatory cytokines from injured tissues and infiltrating macrophages. Higher demand tasks induce higher levels of inflammatory cytokines in serum that stimulate increased brain cytokines in ependymal and endothelial cells of young adult and aged rats. Aged rats also have increased inflammatory cytokines in brain regions, such as the anterior cingulate cortex and hypothalamic nuclei. Signaling from these brain regions leads to increased sickness behaviors. Increased pain behaviors may also be exacerbated by the increase serum cytokines, although this has to be explored further in our model.

Primary Outcomes.

1. Peer Reviewed Publications from 2000-2006 (1st funding period)

1. Barr AE and Barbe MF. Pathophysiological Tissue Changes Associated with Repetitive Movement: A Review of the Evidence. Phys Ther 82(2): 173-187, 2002. PMID: 11856068
2. Barbe MF, Barr AE, Gorzelany I, Amin M, Gaughan JP, Safadi FF. Chronic repetitive reaching and grasping results in decreased motor performance and widespread tissue responses in a rat model of MSD. J Orthopedic Res 21(1): 167-176, 2003. PMID: 12507595
3. Clark BD, Barr AE, Safadi FF, Beitman L, Al-Shatti T, Amin M, Gaughan JP, Barbe MF. Median nerve trauma in a rat model of work-related musculoskeletal disorder. J Neurotrauma, 20 (7): 681-695, 2003. PMID: 12908929
4. †Barr AE, Safadi FF, Gorzelany I, Amin A, Popoff SN, † Barbe MF. Repetitive, negligible force reaching in rats induces pathological overloading of upper extremity bones. J Bone and Mineral Research (JBMR) 18 (11): 2023-2032, 2003. PMID: 14606516 *† Equal contributing authors.*
5. Barr AE, Barbe MF. Inflammation reduces physiological tissue tolerance in the development of work-related musculoskeletal disorders. J Electromyography and Kinesiology 14: 77-85, 2004. PMID: 14759753
6. Clark BD, Al-Shatti TA, Barr AE, Amin M, Barbe MF. Performance of a high-repetition, high-force task induces carpal tunnel syndrome in rats. J Orthop Sports Phys Ther (JOSPT) 34(5): 244-253, 2004. PMID: 15189016
7. Barr AE, Barbe MF, Clark BD. Work-related musculoskeletal disorders of the hand and wrist: epidemiology, pathophysiology, and sensorimotor changes. Special Issue on the Hand: Repetitive stress injuries: the pathophysiology. Invited review. J Orthop Sports Phys Ther 34 (10), 610-627, 2004. PMID: 15552707
8. Barr AE, Barbe MF, Clark BD. Systemic Inflammatory Mediators Contribute to Widespread Effects in Work-Related Musculoskeletal Disorders. Invited review. Exercise and Sports Sciences Reviews, 32(4): 135-142, 2004. PMID: 15604931
9. Barbe M, Bradfield J, Donathan M, Elmaleh J. Co-existence of multiple anomalies in the carpal tunnel. Clinical Anatomy 18(4): 251 - 259, 2005. PMID: 15832328
10. Al-Shatti TA, Barr AE, Safadi FF, Amin M, Barbe MF. Increase in pro- and anti-inflammatory cytokines in median nerves in a rat model of repetitive motion injury. J Neuroimmunol. 167 (1-2):13-22, 2005. PMID: 16026858

2. Peer Reviewed Publications from 2006-2011 (2nd funding period):

1. Barbe MF and Barr AE. Inflammation and the pathophysiology of work-related musculoskeletal disorders. Brain, Behavior and Immunity, 20(5): 423-429, 2006. PMID: 16647245
2. Carp SJ, Barr AE, Barbe MF. Serum biomarkers as signals for risk and severity of repetitive stress injuries. Biomarkers in Medicine, 2(1): 67-79, 2008.
3. Elliott MB, Barr AE, Kietrys DM, Amin M, Barbe MF. Peripheral neuritis and spinal cord neurochemicals are induced in a model of repetitive motion injury with minimal force and repetition exposure. Brain Res. 7;1218:103-13, 2008. PMID: 18511022
4. Barbe MF, Elliott MB, Abdelmagid S, Safadi FF, Popoff SN, Amin M, Barr AE. Serum and tissue cytokines and chemokines increase with performance of repetitive upper extremity tasks. J Orthop Res. 26(10):1320-6, 2008. PMID: 18464247
5. Rani S, Barbe MF†, Barr AE, Litvin J†. The Pattern of Periostin-Like-Factor and Periostin in an Animal Model of Work-Related Musculoskeletal Disorder. Bone.44(3):502-512, 2009. PMID: 19095091 † Equally contributing authors.
6. Elliott MB, Barr AE, Clark BD, Amin M, Amin S, Barbe MF. High force reaching task induces widespread inflammation, increased spinal cord neurochemicals and neuropathic pain. Neuroscience. 23;158(2):922-31, 2009. PMID: 19032977
7. Elliott M, Barr AE, Barbe MF. Spinal Substance P and neurokinin-1 increase with high repetition reaching. Neurosci Lett. Apr 17;454(1):33-7, 2009. PMID: 19429049

8. Rani S, Barbe MF †, Barr AE, Litvin J†. Induction of Periostin-Like-Factor and Periostin in Forearm Muscle, Tendon and Nerve in an Animal Model of Work-Related Musculoskeletal Disorder. J Histochem Cytochem. Nov;57(11):1061-73, 2009. PMID: 19620321 † equally contributing authors.
9. Coq JO, Barr AE, Strata F, Kietrys D, Merzenich MM, Byl NN, Barbe MF. Peripheral and central changes combine to induce motor behavioral deficits in a moderate repetition task. Exp Neurol. Dec;220(2):234-245, 2009. PMID: 19686738
10. Fedorczyk JM, Barr AE, Rani S, Amin M, Gao H, Amin S, Litvin J, Barbe MF. Exposure dependent increases in IL-1beta, substance P, CTGF and tendinosis in flexor tendons with upper extremity repetitive strain injury. J Orthop Res. Mar;28(3):298-307, 2010. PMID: 19743505
11. Rani S, Barbe MF †, Barr AE, Litvin J†. Role of TNF alpha and PLF in bone remodeling in a rat model of repetitive reaching and grasping. J Cell Physiol. Oct;225(1):152-67, 2010. PMID: 20458732; NIHMSID # 228971 † equally contributing authors.
12. Xin DL, Harris M, Amin M, Wade CK, Barr AE, Barbe MF. Aging enhances serum cytokine response but not task-induced grip strength declines in a rat model of work-related musculoskeletal disorders. BMC Musculoskelet Disord. 2011 Mar 29;12(1):63. PMID: 21447183
13. Kietrys DM, Barr AE, Barbe MF. Exposure to Repetitive Tasks Induces Motor Changes Related to Skill Acquisition and Inflammation in Rats. J Mot Behav. 2011 Nov;43(6):465-76. Epub 2011 Nov 16. PMID:22087754
14. Abdelmagid SA, Barr AE, Rico M, Amin M, Litvin J, Popoff SN, Safadi FF, Barbe MF. Performance of Repetitive Tasks Induce Decreased Grip Strength And Increased Fibrogenic Proteins in Skeletal Muscle: Role of Force and Inflammation. PLoS ONE 7(5): e38359. doi:10.1371/journal.pone. PMID: 0038359
15. Kietrys DM, Barr-Gillepse AE, Amin M, Wade CK, Popoff SN, Barbe MF. Aging contributes to inflammation in upper extremity tendons and declines in forelimb agility in a rat model of upper extremity overuse. PLoS One. 2012;7(10):e46954. doi: 10.1371/journal.pone.0046954. Epub 2012 Oct 3.
16. Jain NX, Barr AE, Kietrys DM, Litvin J, Popoff SN, Barbe MF. High Repetition High Force Reaching Induces Load and Inflammation-Induced Bone Resorption in a Rat Model of Upper Extremity Overuse. Submitted to JBMR, Oct 2012.
17. Gao H, Paul F, Wade C, Barr AE, Barbe MF. Chronic low grade inflammation preceeds increased musculotendinous and serum TGFB1 and MMP2, increases that accompany persistent grip strength declines. Submitted to Archives of Physical Medicine, Oct 2012.
18. Xin DL, Massiocotte V, Harris MY, Amin M, Barbe MF. Sickness behaviors, serum and brain IL6, increase with performance of high demand tasks in a rat model of overuse. Submitted to Brain Research, Oct 2012.

19. Barbe MF, Gallagher S, Massicotte V, Tytell M, Barr-Gillespie AE. The Interaction for Force and Repetition on Sensorimotor function and biochemical musculoskeletal and neural responses in a voluntary rat model of work. In preparation for submission to Ergonomics, Nov 2012.

3. Conference proceedings (2000-2012)

1. Barr AE, Safadi FF, Garvin RP, Popoff SN, Barbe MF. Evidence of progressive tissue pathophysiology and motor behavior degradation in a rat model of work related musculoskeletal disease. Proceedings of the IEA 2000/HFES 2000 Congress 5: 584 - 587, 2000.
2. Barr AE, Safadi FF, Popoff SN, Barbe MF. Repetitive motion causes systemic inflammation and reach pattern decrements in rats. Proceedings of the joint meeting of the American Society of Hand Therapists and American Society for Surgery of the Hand, #JT06, pp 61-62 in the ASHT program/syllabus, 2000.
3. Barr AE, Amin M, Barbe MF. Dose-response relationship between reach repetition and indicators of

inflammation and movement dysfunction in a rat model of work-related musculoskeletal disorder. Proceedings of the HFES 46th Annual Meeting 1486-1490, 2002.

4. Fedorczyk J, Barr AE, Amin M, Barbe MF. The presence of Substance P in forelimb tendons in a model of upper extremity work-related musculoskeletal disorder. Proceedings of the joint meeting of the American Society of Hand Therapists and American Society for Surgery of the Hand, San Antonio, TX, Sept 22, 2005. *Won "Best Scientific Paper Award" at meeting.*
5. Gao H, Paul F, Wade C, Barr AE, Barbe MF. Increased serum MMP2 accompanies declines in grip strength and degenerative changes in flexor digitorum tendons. Proceedings paper: Proceedings of the Human Factors and Ergonomics Society 56th Annual Meeting, 2012, pg 743-747.
6. Gold JE, Mohamed FB, Ali S, Komaroff E, Barbe MF. Serum and MRI Biomarkers in Mobile Device Texting: A Pilot Study. Proceedings paper: Proceedings of the Human Factors and Ergonomics Society 56th Annual Meeting, 2012, pg 1150-1154.

4. Reports published in Temple University Journal of Orthopaedic Surgery & Sports Medicine (2006-2012)

1. Barbe M, Safadi F, Popoff S, Barr A. Dose-response relationship between reach repetition and indicators of inflammation and movement dysfunction in a rat model of work-related musculoskeletal disorder. Temple University, Journal of Orthopaedic Surgery & Sports Medicine, 2: 67-71, 2007.
2. Barr AE, Hobbs H, Aim M, Safadi F, Barbe MF. Increased tendon calcification and a bone mineralization protein in musculoskeletal tissues with a repetitive reaching task. Temple University, Journal of Orthopaedic Surgery & Sports Medicine, 3: 105, 2008.
3. Kietrys DM, Barbe MF, Amin M, Harris MY, Bempong FK, Barr AE. Upper limb movement degradation with performance of repetitive reaching in a rat model. Temple University, Journal of Orthopaedic Surgery & Sports Medicine, 3: 36-39, 2008
4. Abdelmagid SM, Barbe MF, Rico MC, Arango-Hisijara I, Selim A-H, Anderson MG, John SW, Owen TA, Popoff SN, Safadi FF. Characterization and function of osteoactivin in osteoblasts. Temple University, Journal of Orthopaedic Surgery & Sports Medicine, 3: 40-56, 2008.
5. Carp SJ, Barbe MF, Winter KA, Amin M, Barr AE. Increased Cytokines and C-reactive protein in human serum are associated with severity of musculoskeletal disorders from overuse. Invited Reprint in Temple University, Journal of Orthopaedic Surgery & Sports Medicine, 3: 114-122, 2008.
6. Xin DL, Harris M, Amin M, Wade CK, Barr AE, Barbe MF. Aging enhances serum cytokine response to repetitive motion injury. Temple University, Journal of Orthopaedic Surgery & Sports Medicine, 6:64-69, 2011.

5. Book Chapters (2006-2012) and 1 Book:

1. Byl N, Barbe MF, Barr AE. Evidence based review of epidemiology and management of work related musculoskeletal disorders of the hand and wrist. Repetitive Stress Pathology- Soft Tissue, Chapter 20. In: Scientific Foundations and Principles of Practice in Musculoskeletal Rehabilitation, Volume III: Treatment of Pathology and Injuries. Magee DJ, Zachazewski JE, Quillen WS (Editors), Elsevier, 2008.
2. Barbe MF, Barr AE. Workplace and Other Overuse Injuries. In: Skeletal Muscle Damage and Repair: Mechanisms and Interventions. Peter Tiddus (Editor), Human Kinetics, 147-162, 2008.
3. Safadi FF Barbe MF, Abdelmagid SM, Rico MC, Aswad RA, Litivn J, Popoff SN. Chapter 1: Bone Structure, Development and Bone Biology. In: Bone Pathology, 2nd Edition. Jasvir Khurana (Editor) Humana Press, Philadelphia, PA, pp 5-50, 2009.
4. Barbe MF, Driban J, Barr AE, Popoff SN, Safadi FF. Chapter 2: Structure and Function of Joints. In: Bone Pathology, 2nd Edition. Jasvir Khurana (Editor) Humana Press, Philadelphia, PA, pp 51-60, 2009.
5. Abdelmagid S, Barbe M, Safadi F. Role of Osteoactivin in Bone Formation and Fracture Repair: Role of Osteoactivin in Osteoblast Differentiation and Bone Formation. Lap Lambert Acad. Publ. (April 2010). 144 pages. ISBN-10: 3838354362.
6. Elliott, ME, Barbe MF. Chapter 113: Neuroscience pain chapter. In: Rehabilitation of the hand and

upper extremity. 6th edition. Skirven, Osterman, Fedorczyk and Amadio (Editors). Elsevier. 2011. ISBN: 978-0-323-05602-1.

7. Keitrys D, Barbe MF, Barr AE. 138: Pathophysiology of Work-Related Disorders In: Rehabilitation of the hand and upper extremity. 6th edition. Skirven, Osterman, Fedorczyk and Amadio (Editors). Elsevier. 2011. ISBN: 978-0-323-05602-1.
8. Coq J-O and Barbe MF. Peripheral and central changes combined induce movement disorders on the basis of disuse or overuse. In: Movement Disorders: Causes, Diagnoses and Treatments. Larsen BJ (Editor), Series: Neuroscience Research Progress; Nova Science Publishers, Inc. ISBN 978-1-61209-200-3. Open Access chapter. April 2011. Web site: https://www.novapublishers.com/catalog/product_info.php?products_id=22057
9. Barbe MF and Popoff SN. Chapter 17: Nervous System Diseases, Disorders and Bone. Emerging Therapeutics and Treatment Options. For: Bone-Metabolic Functions and Modulators. Editors: Bronner F, Farach-Carson MC, and Roach HI. Topics in Bone Biology, 2012, Volume 7, 289-308, DOI: 10.1007/978-1-4471-2745-1_17
10. Barbe MF and Barr AE. Pathophysiology of Peripheral Nerve Injury. For: Peripheral Neuropathy. Editors: S Carp. FA Davis. In press for 2012 publication.

6. Presentations/posters (2006-2012)

1. Barbe MF. Feb 2006. Behavioral and pathophysiological neural changes in a rat model of cumulative trauma disorder. Temple University, Dept of Neurovirology/Neuroscience
2. Barbe MF. March 2006. Repetitive upper extremity tasks lead to tissue inflammation, Dept of Pharmacology, Temple University, Philadelphia, PA
3. Barbe MF. Feb 2006. Animal model of repetitive strain injury. Student Science Workshop – Academy of Notre Dame Students, Sponsored by Pennsylvania Society for Biomedical Research, Philadelphia, in cooperation with Temple Univ
4. Barbe MF. May 2006. Elevated Cytokines results as a consequence of performing repetitive tasks. Departments of Radiology/Biophysics, Temple University, Philadelphia, PA
5. Barbe MF. Sept 22, 2006. Repetitive and forceful tasks leads to inflammation with neurological consequences, Grand Rounds for Dept Neurology, Drexel University, Philadelphia, PA
6. Barbe MF. Dec 4, 2006. Animal model of repetitive strain injury. Science Literacy Workshop, Sponsored by Pennsylvania Society for Biomedical Research, Philadelphia, in cooperation with Temple University
7. Barbe MF. Dec 7, 2006. Overload and inflammation in Work-Related Musculoskeletal Disorder. 5th Annual Fisher, DO'52, Distinguished Lectureship. Philadelphia College of Osteopathic Medicine, Philadelphia, PA
8. Barbe MF. Jan 4, 2007. A rat model of overuse injury and its relationship to dance and sports. For "Science in the City" as part of their theme "Science of Sport and Dance" with a focus on the physics of performance and injury. College of Science and Technology, Temple University, Philadelphia, PA
9. Barbe MF. Jan 16, 2007. Repetitive and forceful tasks leads to inflammation with neurological consequences. Jefferson University, Dept Neuroscience and Dept Physical Therapy, Philadelphia, PA
10. Barbe MF. March 30, 2007. Performance of repetitive tasks with or without force induce pathological behavioral and tissue changes. Morgantown, WV, NIOSH
11. Barbe MF. April 9, 2007. Work-related musculoskeletal disorders. Advances in Mineral Metabolism meeting, Snowmass, Denver, CO
12. Barbe MF. Aug 21, 2007. Decreased adaptive and inflammatory responses as a consequence of aging contribute to injury and sickness behavior in a rat model of WMSD. Premus (Prevention of Work-Related Musculoskeletal Disorders) , Boston, MA.

13. Barbe MF. Nov 28, 2007. Central neuropeptide and peripheral inflammatory responses induced by overuse injuries: Differential responses to NSAIDs and ergonomic interventions. Dept of Anatomy and Cell Biology, Musculoskeletal group. Temple Medical School, Philadelphia, PA
14. Barbe MF. Dec 12, 2007. Central neuropeptide and peripheral inflammatory responses induced by overuse injuries: Differential responses to NSAIDs and ergonomic interventions. Neurobiologie Integrative & Adaptative Dept, Marseille Universite. France

15. JM Fedorczyk, AE Barr, M Amin, MJ Handy, Barbe MF. Neurochemical Response in Forelimb Tendons in a Rat Model of Upper Extremity WMSD. AAHS annual meeting, Puerto Rico, January 22 2007.Hand 2:72-73; 2007. Presented Platform.
16. Brown A, Barbe MF, M Handy, Ann E. Barr. High repetition-high force reaching and grasping is associated with motor performance decline in a rat model. Physical Therapy Combined Sections Meeting for Feb 16, 2007. Presented Poster.
17. Barr AE, Kietrys DM, Brown AM, Handy M, Amin M, Barbe MF. Repetitive motion leads to declines in reach motor performance in a rat model. Orthopaedic Research Society 53rd Annual Meeting, San Diego, CA, February 11-14, 2007 *Won the AAOS ORS Best Poster Invitation.* J Urology, 177 (4): 82, 2007. Presented Poster.
18. Hobbs HK, Barr A, Amin M, Safadi FF, Barbe MF. Increased tendon calcification and bone mineralization protein in musculoskeletal tissues with repetitive reaching task. Orthopaedic Research Society 53rd Annual Meeting, San Diego, CA, February 11-14, 2007. Presented Poster.
19. M.B.Elliott, A.E. Barr, M. Amin, H Harris, Barbe MF. The effects of systemic pro-inflammatory mediators on pain sensitization and psychosocial behavior in response to a repetitive motion injury. FASEB J. 2007 21:911.9. Presented Poster.
20. Rani S, Barbe MF, Barr AE, Litvin J. Role of PLF in Bone Remodeling Following Injury. FASEB annual meeting 2007; FASEB J., 6: A1272, 2007. Presented Poster.
21. Rani S, Barbe MF, Barr AE, Litvin J. Role of PLF in remodeling of bone following injury. ASBMR, Sept 2007 annual meeting. Hawaii102; JBMR, 22: S137, 2007. Presented Poster.
22. Barbe MF. Feb 25, 2008. Central neuropeptide and peripheral inflammatory responses induced by overuse injuries: Differential responses to NSAIDs and ergonomic interventions. Temple University, Neuroscience @Temple, Seminar Program, Philadelphia, PA
23. Barbe MF. March 13, 2008. Effects of Repetitive Motion on Peripheral Nervous System, Spinal Cord and Brain. Association of Chiropractic Colleges (ACCR). Washington, DC.
24. Barbe MF. May 10, 2008. Peripheral tendon and spinal cord neurochemical increases are induced in a model of repetitive motion injury with high force and high repetition exposure. O36. Musculoskeletal and Neuronal Interaction international workshop, Cologne, Germany.
25. Elliott MB, A.E. Barr, M. Amin, H Harris, F Bempong, Barbe MF. Increased neuropeptides in cord dorsal horns with performance of repetitive high repetition high force task is attenuated with ibuprofen and ergonomic interventions. FASEB J. 2008 22:945.10. Presented Poster.
26. Barbe MF, J Fedorcyzk, MB Elliott, M Amin, AE Barr. Peripheral tendon and spinal cord neurochemical increases are induced in a model of repetitive motion injury with high force and high repetition exposure. O36. Musculoskeletal and Neuronal Interaction International Workshop, May 10, 2008, Cologne Germany. Presented Platform.
27. Barbe MF, Amin M, Harris M, Driban JB, Safadi FF, Barr AE. Bone resorption and articular cartilage degenerative changes following performance of high repetition high force tasks are attenuated by secondary ibuprofen treatment. ASBMR Annual Meeting, Sept 12, 2008; JBMR, 23: S234, 2008. Presented Poster.
28. Elliott MB, Barr AE, Amin M, Barbe MF. Signs of central sensitization: inflammation induced by high demand task occurs in peripheral nerve and spinal cord tissue. Society for Neuroscience, Nov 15, 2008, #70.13. Presented Poster.
29. Albrecht PJ, Rice FL, BarrAE, Barbe MF. Alterations in cutaneous innervation and epidermal chemistry following repetitive motion injury in rats. Society for Neuroscience, Nov 16, 2008, # 154.6. Presented Poster.

30. Varma D, Barbe MF, Jansen-Varnum S. Quantitative correlation between lipid biomarkers and inflammatory proteins in work- related musculoskeletal disorders (MSDs). Pittcon conference, 2009, #1690-4. Presented Poster.
31. Abdelmagid S, Amin M, Safadi FF , Barbe MF. A bone anabolic factor, osteoactivin, increases in musculoskeletal tissues with performance of a high repetition upper extremity task. ASBMR, Sept 2009, #1159. Presented Poster.
32. Barbe MF. May 9, 2009. Repetitive Motion Disorders and Nerve Inflammation. Workshop Keynote Speaker. American Society of Pain 28th annual meeting, 2009, San Diego, CA.
33. Barbe MF. Oct 28, 2009. Repetitive Motion Disorders and Pain. Invited by Pain Dept, Wyeth Neuroscience, Princeton, NJ
34. Barbe MF. Nov 13, 2009. An animal model of WMSD. Human Factors and Ergonomics Society, Cornell University Chapter, Ithaca, NY
35. Elliott MB, Barr AE, Amin M, Barbe MF. Spinal cord and systemic inflammation are linked with sickness behavior in aged rats performing a repetitive task. Society for Neuroscience, Oct 2009, 268.14/BB20. Presented Poster.
36. Barbe MF. March 8, 2010. Central neuropeptide and peripheral inflammatory responses induced by overuse injuries: Differential responses to NSAIDs and ergonomic interventions. University of New England, Biddeford, ME
37. Barbe MF. Aug 28, 2010. Conservative secondary interventions are effective at attenuating peripheral nerve inflammation but not sensorimotor declines with high repetition high force task. Premus (Prevention of Work-related Musculoskeletal Disorders). Angers, France
38. Barbe MF. Dec 8, 2010. Peripheral and central inflammatory, and nociceptor-related behavioral responses induced by overuse injuries: Differential responses anti-inflammatory interventions. Temple University School of Medicine, MD/PhD seminar series, Philadelphia, PA.
39. Barbe MF, Elliott, ME, Clark BD, Kietrys DM, Wade CK, Duff S, Harris MY, Amin M, Barr AE. Reflexive pain behaviors (mechanical hypersensitivity and decreased reflexive grip strength) can persist even after conservative secondary interventions due to presence of spinal cord sensitization changes. NORA Manufacturing Sector Conference: Partnerships to Improve Occupational Safety and Health Sept 2011. Presented Poster.
40. Abdelmagid SM, Barr AE, Amin M, Rico M, Litvin J, Safadi FF, Barbe MF. Increased anabolic and repair proteins with performance of extended repetitive high force reaching tasks, and the effectiveness of anti-TNFalpha and Ibuprofen as interventions for associated muscle fibrosis NORA Manufacturing Sector Conference: Partnerships to Improve Occupational Safety and Health Sept 2011. Presented Poster.
41. Barbe MF. July 13, 2011. Neuroanatomical and musculoskeletal deficits in adults rats exposed to either sensorimotor restriction or prenatal ischemia. Nemours Biomedical Research, A.I. Dupont Hospital, Willimgton, Delaware.
42. Barbe MF. Sept 27, 2011. Deficits in adults rats exposed to developmental sensorimotor restriction or prenatal ischemia: a rat model of cerebral palsy? Shriners Hospitals Pediatric Research Center, Philadelphia, PA
43. Barbe MF. Oct 20, 2011. Biology of Cumulative Trauma. A keynote speaker at the Cumulative Trauma Injury – Prevention, Care and Disability Management Conference, Canadian Memorial Chiropractic College. Toronto, Canada

44. Massicotte VS, Delcour M, Amin M, Coq JO, Barbe MF. Musculoskeletal alterations in adult rats with white matter damage following prenatal ischemia and developmental sensory motor restriction. P24. Proceedings of the 34rd Annual Meeting of the 20 October 2011. http://pcsfn.com/Documents/PCSFN%202011%20rev%201.pdf
45. Helen Gao, Alex G Lambi, Paul W Fisher, Mamta Amin, Ann E Barr, Mary F Barbe. Persistent low-grade inflammation and degenerative tendon changes parallel grip strength declines in rats

performing a moderate demand repetitive task for 18 weeks. Orthopaedic Research Society Annual Meeting, 2012, PS2 Infection and Inflammation, Poster #: 1905. http://www.ors.org/documents/2012_Program_Book.pdf

46. Xin DL, Massiocotte V, Harris MY, Amin M, Barbe MF. Sickness behaviors (reduced social interaction and increased aggression), serum and brain IL6, increase with performance of high demand tasks in a rat model of overuse. 2012 Society for Neuroscience Annual Mtg, Oct 15 2012.
47. Massiocotte V, Xin DL, Harris MY, Amin M, Barbe MF. Sickness behaviors (reduced social interaction and aggression) induced by in a rat model of overuse are attenuated by anti-inflammatory drugs that reduce overuse-induced systemic inflammation. 2012 Society for Neuroscience Annual Mtg, Oct 15 2012.
48. Barbe, MF. Invited Seminar. November 2011 Biology of Cumulative Trauma, Delaware University, Dept of Kinesiology
49. Barbe MF. Invited Seminar. February 10, 2012 Sickness behaviors are induced by systemic inflammation in a rat model. Temple University. Neuroscience/Psychiatry - Research & Education Initiatives meeting.
50. Barbe MF. Invited Seminar. February 20, 2012 Overuse-induced musculoskeletal degenerative changes attenuate but pain behaviors persist after conservative secondary interventions due to central sensitization. Georgia Health Sciences University, Augusta, GA
51. Barbe MF. Key Note speaker. March 27, 2012. Fascia changes with repetitive motion injury. Keynote speaker. Third International Fascia Research Congress. Vancouver, Canada.
52. Barbe MF. Nanosymposium. Sickness behaviors (reduced social interaction and increased aggression), serum and brain IL6, increase with performance of high demand tasks in a rat model of overuse. 2012 Society for Neuroscience Annual Mtg Nanosymposium.
53. Barbe MF. October 23, 2012 Increased serum MMP2 accompanies declines in grip strength and degenerative changes in flexor digitorum tendons. Platform talk at Human Factors and Ergonomics Society 56th Annual Meeting, 2012. See proceedings papers for listing of this above.
54. Barbe MF. October 26, 2012 Serum and MRI Biomarkers in Mobile Device Texting: A Pilot Study. Platform talk at Human Factors and Ergonomics Society 56th Annual Meeting, 2012. See proceedings papers for listing of this above.
55. Barbe MF. Invited Seminar. Jan 15, 2013 Sensorimotor and serum biomarkers indicative of underlying pathologies in a rat model of upper extremity overuse. U Penn, Penn Center for Musculoskeletal Disorders.
56. Barbe MF. Key Note speaker. Pending. July 8, 2013. Mechanisms of injuries leading to musculoskeletal pathology and behavioral declines in an animal model of repetitive motion injury. PREMUS2013, the 8th International Conference on Prevention of Work-related Musculoskeletal Disorders. Pusan National University School of Medicine in Korea (R.O.K.)

8. Education and training materials

1. Barbe MF, Barr AE, Byl NN, Fedorczyk JM. Work-related health Problems Associated with Repetitive Movement of the Upper Quarter: Evidence for Additional Examination and Treatment. Annual Conference and Exposition of the American Physical Therapy Association (APTA) Course Materials: A Compendium of Conference Handouts: Part 1, Hand Rehabilitation, pp 194-201, 2000. (invited and peer reviewed).
2. Barbe MF. Musculoskeletal Pain Fact Sheet. In: International Association for the Study of Pain (IASP), 2009–2010 Global Year Against Musculoskeletal Pain http://www.iasp-pain.org/AM/AMTemplate.cfm?Section=Home&CONTENTID=9287&TEMPLATE=/CM/ContentDisplay.cfm&SECTION=Home (invited)

E. Intermediate Outcomes

An ***intermediate outcome*** is what a stakeholder does with that knowledge or products generated by the project to contribute to ***end outcomes*** (reductions in workplace illnesses, injuries, fatalities, and/or hazardous

exposures). Intermediate outcomes include public or private policy changes, conduct of training or workshops based on project outputs, self-reported use or repackaging of project data, adoption of technologies or methods developed by NIOSH (or the extramural researcher), implemented guidelines, and changes in workplace behaviors by either employers or employees.

1) The finding of early inflammation that lead to tissue catabolism lead to the adoption of ibuprofen as a secondary preventative treatment for inflammatory immune response and tissue degeneration changes associated with overuse injuries by the extramural researcher (Barbe, MF) in 2 subsequent studies that have recently been published.
   a) Abdelmagid SA, Barr AE, Rico M, Amin M, Litvin J, Popoff SN, Safadi FF, Barbe MF. Performance of Repetitive Tasks Induce Decreased Grip Strength And Increased Fibrogenic Proteins in Skeletal Muscle: Role of Force and Inflammation. PLoS ONE 7(5): e38359. doi:10.1371/journal.pone. PMID: 0038359
   b) Kietrys DM, Barr AE, Barbe MF. Exposure to Repetitive Tasks Induces Motor Changes Related to Skill Acquisition and Inflammation in Rats. J Mot Behav. 2011 Nov;43(6):465-76. Epub 2011 Nov 16. PMID:22087754

2) The finding of early inflammation that lead to tissue catabolism also lead to the adoption of anti-TNFalpha (known as Remicade, Enbrel, and Entanocept for patient treatments) as secondary preventative treatments for the inflammatory immune response and pain behaviors associated with overuse injuries by the extramural researcher (Barbe, MF) in a subsequent study that has recently been completed.

   a) Abdelmagid SA, Barr AE, Rico M, Amin M, Litvin J, Popoff SN, Safadi FF, Barbe MF. Performance of Repetitive Tasks Induce Decreased Grip Strength And Increased Fibrogenic Proteins in Skeletal Muscle: Role of Force and Inflammation. PLoS ONE 7(5): e38359. doi:10.1371/journal.pone. PMID: 0038359

3) Related Peer-Reviewed Publications on Human Subjects with Overuse Injuries (2006-2012):

   a) Carp SJ, Barbe MF, Winter KA, Amin M, Barr AE. Increased Cytokines and C-reactive protein in human serum are associated with severity of musculoskeletal disorders from Overuse. Clin Sci (Lond). 112(5):305-14, 2007. PMID: 17064252
   b) Carp SJ, Barr AE, Barbe MF. Serum biomarkers as signals for risk and severity of repetitive stress injuries. Biomarkers in Medicine, 2(1): 67-79, 2008.
   c) Driban JB, Balasubramanian E, Amin M, Sitler MR, Ziskin MC, Barbe MF.The potential of multiple synovial-fluid protein-concentration analyses in the assessment of knee osteoarthritis. J Sport Rehabil. Nov;19(4):411-21, 2010. PMID: 21116010
   d) Driban JB, Sitler MR, Barbe MF, Balasubramanian E. Is osteoarthritis a heterogeneous disease that can be stratified into subsets? Clin Rheumatol. Feb;29(2):123-31, 2010. PMID: 19924499
   e) Cattano N, Driban JB, Balasubramanian E, Barbe MF, Amin M, Sitler MR, Biochemical comparison of osteoarthritic knees with and without effusion. BMC Musculoskelet Disord. 2011 Nov 28;12(1):273. PMID: 22122951
   f) Muth S, Barbe MF, Lauer R, McClure PW. The Effects of Thoracic Spine Manipulation in Subjects With Signs of Rotator Cuff Tendinopathy. J Orthop Sports Phys Ther. 2012 Aug 17. [Epub ahead of print] PMID: 22951537

4) This extramural researcher, Dr. Mary Barbe, has also conducted many workshops and presentations based on project outputs that were given to ergonomic practitioners and clinicians showing evidence that primary prevention is key to avoiding the inflammation-based tissue degeneration associated with overuse

injuries, but also that early secondary interventions with anti-inflammatory drugs can also be efficacious in some instances. These workshops are listed above in #6 and #7, as well as below:

a) Barbe MF, Barr AE, Byl NN, Fedorczyk JM. Work-related health Problems Associated with Repetitive Movement of the Upper Quarter: Evidence for Additional Examination and Treatment. Annual Conference and Exposition of the American Physical Therapy Association (APTA) Course Materials: A Compendium of Conference Handouts: Part 1, Hand Rehabilitation, pp 194-201, 2000. (invited and peer reviewed).
b) Barbe MF. March 13, 2008. Effects of Repetitive Motion on Peripheral Nervous System, Spinal Cord and Brain. Association of Chiropractic Colleges (ACCR). Washington, DC.
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