Impact of Rest and Secondary Intervention on the Development of and Recovery from Work-related Musculoskeletal Disorders in Human and Rat Models

by

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Abstract

Experts from different fields study the development of Musculoskeletal Disorders (MSDs) from different perspectives: epidemiologists identify potential MSD risk factors, traditional ergonomists analyze motions, and medical doctors remedy the pain and sickness symptoms by prescribing medicine, rehabilitation programs, and surgeries. However, an integrated understanding of the injury mechanism of MSDs, especially the early developmental stages, is not well established. Accurate and precise quantitative estimation of exposure to specific risk factors is essential for evaluating worker risk, measuring effectiveness of ergonomic interventions in order to promote worker health and well-being.

One commonly prescribed method of MSD prevention and intervention, rest, has been identified of significant healing and recovery value. In sufficient rest, on the other hand, is identified as a potential significant MSD risk factor. However, the scheduling of work-rest cycles at manufacturing sites is often oriented towards meeting production goals and rarely takes MSD prevention of the workers into consideration. Current literature provides little information for the dose-response relationship between duration and frequency of rest breaks during work-rest cycles or between work-shifts and musculoskeletal injury formation and recovery outcomes. Relevant applicable experiment schemes have not been established to quantify the effect of rest in different stages of MSD development and recovery.

This dissertation was designed to address these issues and expand upon the current scientific literature regarding the impact of rest and secondary intervention on MSD development and recovery. Manufacturing work shifts and work-rest cycles were simulated to induce musculoskeletal injuries; human and animal experiment models were developed to complement each another in order to quantify physiological, behavioral and biological outcomes of the injuries and recovery over repeated work-rest cycles. Previous studies that adopted similar animal models have demonstrated significant translational value of these models in reflecting human MSD

injury pathways. Three specific aims were met through three studies, all under the rest versus MSD development and recovery theme.

The study described in Chapter Three studied human subjects' muscle damage biomarker Creatine Kinase's fluctuating levels post repeated bouts of eccentric exercise of either high or low loading levels and taking either long but infrequent or short but frequent rest breaks between equal total workload. We found that short but frequent rest breaks were associated with greater muscle damage.

The second study described in Chapter Four developed a forced downhill treadmill running model for rats to study physiological adaptation, systemic inflammation, stress, and tissue pathology as outcomes of chronic eccentric exertion with either long but infrequent or short but frequent rest breaks when total workload was equal. We found that rats that followed different work-rest schedules expressed significant difference in physiological adaptations and stress responses. Rats that followed long but infrequent rest scheduling might have experienced greater body weight as well as oxidative stress fluctuations in comparison with ones that followed short but frequent rest scheduling, although both working groups experienced significant deficit in body weight during the experiment period as well as significant pathological changes in Achilles tendon.

The third study described in Chapter Five examined effectiveness of flat treadmill running, a common secondary intervention for MSDs, and rest on rats' task performance, pain behaviors, systemic inflammation, and tissue pathology during and post exposure of high repetition, high force (HRHF) upper extremity tasks over 14 weeks. Six treatment groups: (1) food restricted control (FRC); (2) trained to high force and euthanized (TRHF); (3) trained to high force and rested (TRHF+Rest); (4) trained to high force, rested and ran on treadmill (TRHF+Rest+TM); (5) trained then performed the HRHF task (10wk HRHF); (6) trained, performed the HRHF task and ran on treadmill (10wk HRHF+TM). We did not find treadmill exercise at the chosen speed effective in remedying the rats' voluntary task performance, reflexive grip strength, or mechanical allodynia induced by the high repetition high force reaching task. Instead treadmill running had opposite effects on functional outcomes, likely due to nerve inflammation

and pain occurred during HR training, not recovering during rest, becoming more expressed during HRHF task and even worse when treadmill running was prescribed together with the HRHF task. We found that rest attenuated the mechanical sensitivity and systemic inflammation, remedied tendon's morphological changes, yet did not improve the rats' grip strength.

Overall, these findings hold great promise for future development of guidelines to manufacturing work-rest scheduling in a manner that protects worker's health and well-being while maintains productivity. They also add to the body of existing knowledge of MSD development and recovery mechanism, which can be expanded to meaningful clinical applications and can contribute to ergonomic assessment design and refinement.

Acknowledgments

The past seven years at Auburn has been an incredible experience for me. The journey of pursuing doctoral education started as a promising but somewhat abstract idea, but now has solidified to a pursuit of an academic career, a systematic style of thinking, and a commitment of life-long learning that I carry daily. There are many individuals that I would like to express gratitude to who witnessed and participated in this sometimes electrifying and sometimes arduous process.

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and time to stay in regular contact with me since we first met in 2014. A substantial portion of this dissertation reflects her guidance towards critical thinking, rigorous logic and effective conveyance of information. She has become my mentor, collaborator, friend and fellow female scientist/engineer over the past years. I appreciate her deeply.

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Chapter 1

Introduction

Musculoskeletal Disorders (MSDs) are culprits of repetitive and forceful motions, awkward postures, vibration, cumulative exposure and inadequate recovery times between exposures (Yassi, 1997; Gallagher & Heberger, 2013; D. Xin et al., 2017). MSDs affect the tendons, muscles, joints, nerves generally in the back, neck, upper and lower extremities (Yassi, 1997). Work-related MSDs (WMSDs) are painful for employees and costly for both employees and employers, which accounted for 31% of all workplace injuries and a median of 12 lost work days per case in 2015 (BLS, 2015). There remains a call for effective prevention, intervention and treatment measures for these often debilitating disorders (OSHA, 2014; Bove, Harris, Zhao, & Barbe, 2016).

Unlike non-biological materials that strictly follow a stress versus strain relationship, biological materials have the ability to heal over time, although current literature is scant on the healing process or on the quantification of the healing rate (Gallagher & Schall Jr, 2017). It is suspected that healing and recovery over time does not follow a linear pattern (Gallagher & Schall Jr, 2017). Therefore, scheduling and optimizing the work and rest within work-rest cycles may have an impact on the development of WMSDs. Current literature does not hold definitive evidence of intervention measures that effectively prevent or intervene with the MSD injury process, perhaps partially due to lack of clear identifications of different stages of MSDs. What are needed to expand the current understanding is: (1) a definitive list of MSD risk factors; (2) the dose-response relationship between risk factors and the different MSD stages; and (3) a quantitative measure to take rest, healing and recovery of biological materials into consideration. Current diagnoses of MSDs often only identify fully developed WMSDs cases that

are too severe to recover to tissues' pre-injury state. MSDs at early stages are usually not easily identified nor promptly addressed. As a result, discomfort associated with the disorders continue to develop, leading to worker dissatisfaction, job displacement and cumulative treatment costs.

Aimed at improving safety and health of workers, the National Occupational Research Agenda (NORA) calls for reduction of incidence and prevalence of MSDs, development of intervention strategies, and control of MSD risk factors across different sectors including manufacturing, healthcare and social assistance, agriculture, forestry and fishing, construction, mining and public safety. In-depth understanding of MSD development and healing mechanism is key to fulfill these goals.

1.1 Research Objectives

Research gaps regarding early MSD development from molecular, histopathological and physiological perspectives are identified in Chapter Two's literature review. This dissertation aims to initiate evidence-based efforts aimed at increasing the quantitative understanding of the dose-response relationship between certain risk MSD factors, such as insufficient rest and tissue-specific injuries. Specifically, experiments to examine how exposure to different work-rest cycle scheduling affects tissue trauma formation and systemic responses have been developed. The author believes that the findings presented and the mechanisms developed from this dissertation will serve as precursors of more extensive future research on work-rest scheduling and rescheduling, early-stage MSD prevention and intervention, which are applicable and beneficial to worker's safety and health, as well as human performance in jobs that are physically demanding. This dissertation seeks to address these issues through the following specific aims.

Specific Aim 1:

Investigate the impact of long and short between-work rest breaks on muscle micro-trauma development.

Specific Aim 2:

Develop a forced downhill treadmill running rat model in order to measure long and short

between-work rest breaks' different impacts on Achilles tendon morphology, voluntary task performance, reflexive strength, pain behavior, and systemic inflammation induced by chronic repetitive and forceful exertions.

Specific Aim 3:

Examine the efficacy of commonly touted secondary intervention methods, treadmill running and rest, for MSDs through a voluntary high repetition high force lever-reaching and pulling task for rats.

1.2 Dissertation Organization

The organization of this dissertation follows the Auburn University Dissertation Guide (AU, 2015). The dissertation consists of six chapters. Chapter One introduces necessary definitions and the most recent statistics on the key concepts of the dissertation topic, lays out foundation for future chapters, identifies research gap and objectives. Chapter Two provides a comprehensive literature review of current understanding of how different types of rest and intervention measures impact the development and recovery of WMSDs in human and rat models. Each of the three following chapters is a stand-alone manuscript addressing one or a few the research objectives identified in Chapter One by describing the purpose, methods, results, discussion and conclusion of an experiment. The experiment in Chapter Three measured degrees of bicep muscle micro-trauma when human subjects were exposed to different load-repetition regimens and rest schedules over time. Chapter Four reports physiological adaptation, systemic inflammation, tendon histopathology and task performance outcomes of the rats post exposure to long-term repetitive loading when given different rest schedules using a novel forced downhill treadmill running rat model. Chapter Five validates the efficacy of rest and treadmill running, which are commonly prescribed secondary intervention methods, in alleviating MSDs using a high force high repetition lever-pulling upper extremity rat model and identifies the limitations of this method. Overall conclusions, limitations and suggestions for future work are presented in Chapter Six.

Chapter 2

Review of the Literature

2.1 Classifications of MSDs

MSDs can be classified based on the specific tissues of the body that are affected. Tendon-related MSDs include tendonitis, epicondylitis, De Quervain's disease, Dupuytren's contracture, trigger finger, and ganglion cyst. Nerve-related MSDs include carpal tunnel syndrome, cubital tunnel syndrome, Guyon canal syndrome, pronator teres syndrome, radial tunnel syndrome, thoracic outlet syndrome, cervical syndrome, and digital neuritis. Muscle-related MSDs include tension neck syndrome, muscle sprain and strain, and myalgia and myositis, circulatory type disorders (including hypothenar hammer syndrome) and Raynaud's syndrome. Joint-related MSDs include osteoarthritis, and bursa-related disorders such as bursitis. The relationships between the MSD symptoms and functional impairment and disability remain unclear due to different bodies of knowledge the clinicians and researchers have used to develop the assessment criteria. Therefore, understanding these relationships and establishing standardized criteria remain research priorities (Buckle & Devereux, 2002).

Although both involve tissue rupture, MSDs are fundamentally different from acute tissue traumas where a significant external force injures a tissue. Although MSD-related tissue trauma may appear to be a sudden event, evidence support that there is usually an accumulation of loading and subsequent fatigue damage over a relatively long period of time during which early fatigue damage, healing, and repetitive damage occur along with a series of biologic responses. However, little is known about these biologic responses to early fatigue damage accumulation (Andarawis-Puri & Flatow, 2011). Lack of definitive understanding and the ability

to predict when and how the above early-stage responses will occur impedes timely diagnosis, intervention and prevention of MSDs when tissues are most likely to quickly heal and return to its pre-injury state.

2.2 MSD Risk Factors

MSD triggers may include one factor or a combination of risk factors. If not differentiating affected body parts, physical MSD risk factors include forceful exertion, repetition, awkward posture and vibration, while psychosocial risk factors include insufficent rest, intensified workload, time pressure, low job control, monotonous work and low support from coworkers and management (Devereux, Vlachonikolis, & Buckle, 2002). It is worth noting that the range of attributable fraction (AF), an estimate of the proportion of disease that would be reduced in the exposed population if exposure was eliminated, for insufficient rest (33%-70%) is nearly as high as the physical risk factors (44%-95%), although sources that contributed to calculating the AF of insufficient rest were scarce. There remains a need for more and further studies to confirm these findings (Punnett & Wegman, 2004).

If affected body parts are differentiated, the risk factors have some similarities and some differences. Common risk factors that are reported to affect MSDs in most body parts are heavy physical work, repetition, awkward posture, and high BMI. Physical risk factors of neck MSDs are identified as heavy physical work, awkward posture, and frequent lifting; psychosocial risk factors are low work satisfaction and support and high stress; individual risk factors are older age, female gender, sedentary lifestyle, high BMI, comorbidity, and smoking. For low back MSDs, physical risk factors identified are heavy physical work, awkward static or dynamic postures, and lifting; psychosocial factors are negative affectivity, low job control, high psychological demands, and low job satisfaction; individual risk factors include younger age, female gender, African American race, smoking, high BMI, and co-morbidity (Punnett & Wegman, 2004; da Costa & Vieira, 2010). Physical risk factors for upper extremity MSDs include heavy physical work, repetition, awkward static or dynamic postures, and prolonged

computer work; its psychosocial risk factors include fear avoidance, high stress, high job dissatisfaction, low level of job control, and monotonous work; individual risk factors include older age, smoking, high BMI, and co-morbidity. Lower extremity MSDs' physical risk factors include heavy physical work, repetition, prolonged standing, kneeling, squatting, or climbing; psychosocial risk factors are high stress and fear avoidance; individual risk factors are previous knee injury, smoking, high BMI and concurrent chronic disease (Bernard & Putz-Anderson, 1997; Devereux et al., 2002; Punnett & Wegman, 2004; da Costa & Vieira, 2010).

2.3 Early Stage MSD Development

MSDs start as micro-trauma to muscle, connective tissue, and/or bones and joints through eccentric muscle action, concentric muscle action or joint action that lead to adaptive microtrauma (AMT). AMT refers to the microtrauma, or the initial pphase along an "injury continuum" to muscle, connective tissue, and/or bones and joints. With the ultimate purpose of healing injured tissues, AMT results in a mild inflammatory response. The healing process requires absence of persistent injury stimulus and sufficient rest time, otherwise the injury would continue to progress to a sub-acute phase, which results in the release of cytokines (local inflammatory factors) (L. L. Smith, 2000). As tissue injury is dose dependent when repetitively strained with force, three possible outcomes of acute inflammation are: (1) restoration to pre-injury state; (2) healing with scar tissue formation; and (3) chronic fibrosis. Continued exacerbation from the risk factors will cause the injuries to progress to a chronic inflammation and/or fibrosis phase and eventually stimulate systemic immune and/or inflammatory responses. The chronic inflammation process can begin 2-4 days after the onset of acute response and may last for weeks, months, even years if the normally healing process is continuously interrupted by the initiating stimuli, leading to recurring acute inflammation (Fig. 2.1) (Barr, Barbe, & Clark, 2004; Barr & Barbe, 2004).

Cytokines are protein molecules that are released by a wide range of cells from the immune system. They communicate with cells that function in immune response and signal cell movement to inflammation, infection and trauma sites (Zhang & An, 2007). Responding to

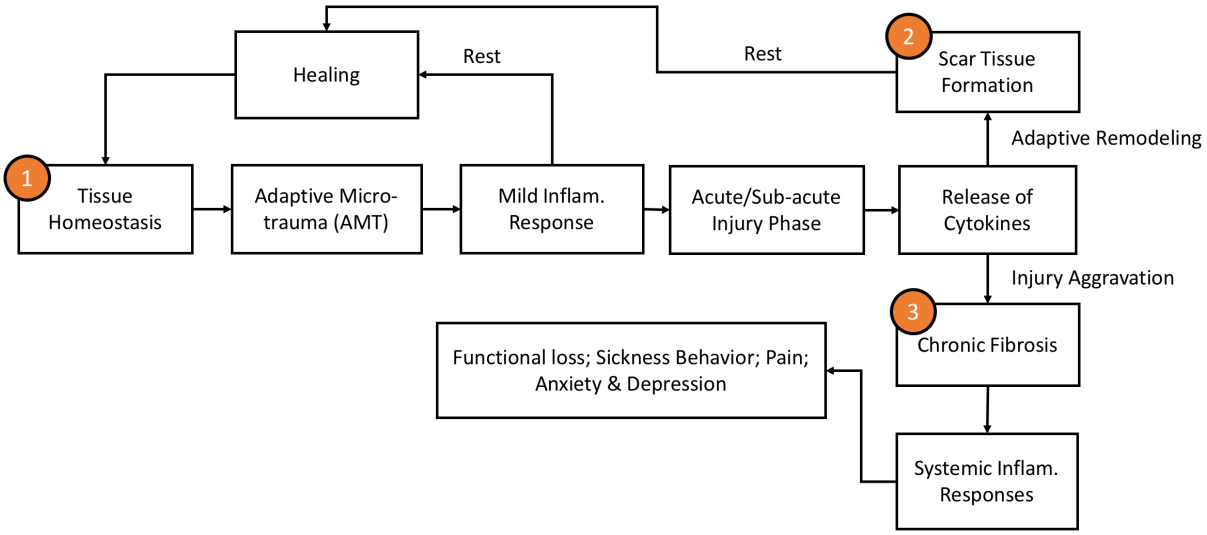

Figure 2.1: MSD Development Cycle

tissue injury stimuli, the inflammation process starts within a few hours to initiate healing. Neutrophils are attracted to the inflammation sites through the chemotaxis process and are the first responders in the "clean up" process. Within 24 hours, however, the neutrophils become inactive. The monocytes then arrive, having moved from the circulation to the tissues, and transform into macrophages, which represent a systemic inflammatory response. Cytokines integrate the systemic inflammation by stimulating surrounding cells (paracrine) or themselves (autocrine), engaging the liver and the central nervous system, leading to amplified inflammation. For example, the pro-inflammatory cytokine Tumor Necrosis Factor-alpha (TNF-alpha) serves key functions in peripheral muscle proteolysis and whole-body immune responses (Cavaillon, 1994; L. L. Smith, 2000; Barr & Barbe, 2004; Barbe & Barr, 2006; Liao, Zhou, Ji, & Zhang, 2010).

2.4 Tissue Fatigue Damage Behavior

After microscopic fissures in tissue (micro-trauma) are developed in the early stage of a MSDs, if tissues are subjected to continued repetitive loading and unloading, new fissures will emerge and the existing ones will enlarge, leading to potential structural damage of the local tissues. The rate and magnitude of fissure expansion are dependent on the magnitude of the load and the

number of loading cycles. Fatigue Failure Theory, applicable to both mechanical and biological materials, suggests that the magnitude of applied stress to the injury site and the injury rate follow a logarithmic relationship. A relatively small increase of the load could significantly accelerate the injury rate. On the other hand, a modest reduction of the of the imposed level of stress could exponentially reduce the injury rate, and therefore increase the number of cycles needed to cause tissue failure (Gallagher & Heberger, 2013).

Most biological tissues, including tendons, muscles and bones, possess viscoelastic properties because the composition of their structures such as cells, extracellular matrices, structural proteins are viscoelastic. Elasticity, one aspect of viscoelasticity, is a solid material property. Elastic materials deform and recover to its original shape instantaneously as stress is being applied and removed. Stress is linearly proportional to strain for a linear elastic material (Fig. 2.2). Viscosity, the other aspect of viscoelasticity, is a measure of resistance to flow and a fluid property. Viscous materials deform and recover to its original shape gradually when subjected to loading and unloading (Sasaki, 2012; Özkaya, Leger, Goldsheyder, & Nordin, 2017). The stress-strain relationship of viscoelastic materials, such as tendon, would therefore display properties of both aspects: stress initially increases faster than strain in the toe region, followed by a linear region, then the failure region marked by sharp decline of stress due to tissue failure (Fig. 2.3). Coinciding with the logarithmic stress versus rate of injury relationship per Fatigue Failure Theory, tendon stress (percentage of ultimate tensile strength) versus the logarithm of corresponding numbers of cycles to failure follow a linear relationship (Fig. 2.4) (Schechtman & Bader, 1997).

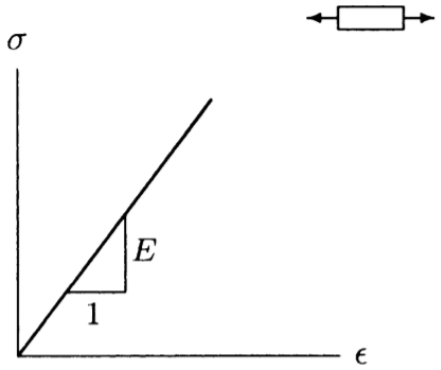

Figure 2.2: Stress vs Strain of Linearly Elastic Meterial. Original image was published in book by Ozkaya et al., 2012.

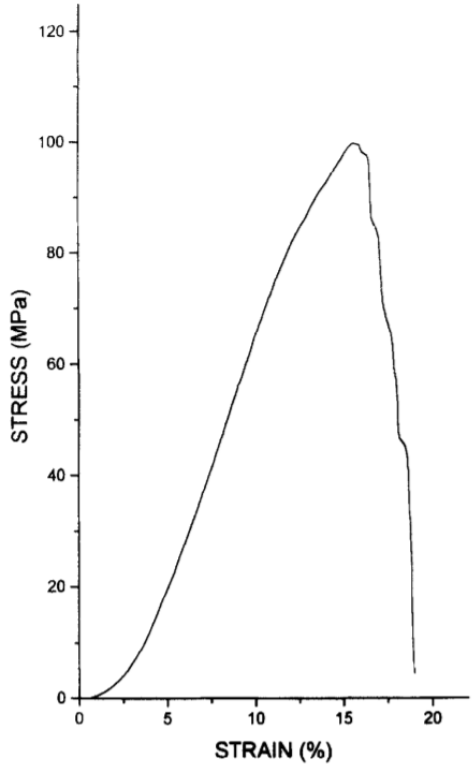

Figure 2.3: Stress vs Strain of Tendon. Original image was published in article by Schechtman and Bader, 1997.

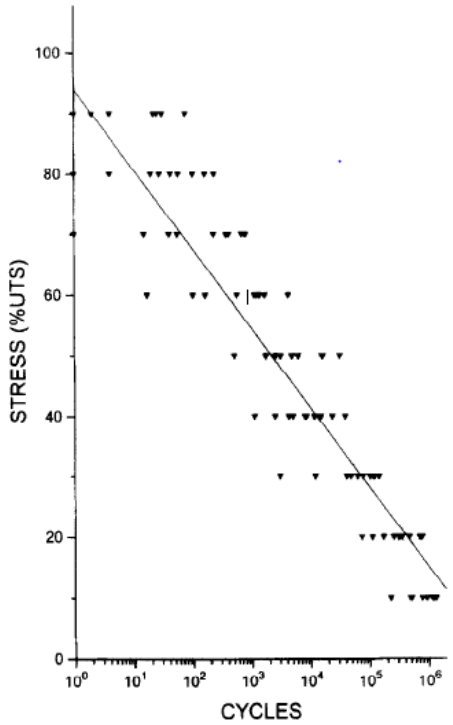

Figure 2.4: Tendon's Ultimate Tensile Strength (UTS) vs Number of Cycles to Failure. Original image was published in article by Schechtman and Bader, 1997.

2.5 Work-Rest Cycle

The consensus of current literature generally suggests that if sufficient rest is not taken in between work bouts, workers can still have significant injury exposure even if appropriate work technique is applied, for example, when repeatedly handling heavy objects. Longer rest periods between work bouts have shown to reduce soreness in humans (Cutlip, Baker, Hollander, & Ensey, 2009). Work-rest cycle needs to be appropriately set according to the injury exposure level to reduce injury susceptibility. However, as the healing mechanism *in vivo* is complex and demonstrates variabilities from injury to injury, no definitive work-rest cycle guideline has been established to counter MSDs. Per NIOSH National Research Council and Institute of Medicine' s recommendation, further investigation is required to understand the effect of work-rest cycle on muscle and other soft tissue injury in order to explicate the etiology of tissue injury and subsequent responses (Council et al., 2001).

Rest is believed to provide important recovery value, which may be a function of injury level when rest starts (dose) and the length of rest required to recover or to reach a homeostatic status (response). Rest is more effective before tissue injury reaches a threshold level; and its effectiveness declines exponentially with time (Mital, Kilbom, & Kumar, 2000). The types of rest can be classified in a few different ways: passive rest features inactivity while active rest includes massage, light stretching, heat and exercises that improve blood circulation. The three common types of rest at work are micro-breaks that are short breaks of a minute or less, lunch breaks or information breaks such as training or work interruptions, and working rest such as switching to a lighter or different task than the workers main job task. In addition, off-work breaks include evenings, weekends, vacations and holidays (Mital et al., 2000).

2.6 Theories on Cyclic Loading's Healing Process in Different Tissue Types

The ability to heal is a unique property of living tissues. Extending the Palmgren-Miner's cumulative damage model 2.1, where n_i and N_i are number of loading cycles at stress level S_i and number of cycles to failure at this stress level respectively, for metallic structures, Nash

accounted for structures that have self-healing abilities and proposed an updated model 2.2, where $D_S(t)$ is the damage associated with mechanical stress, $D_A(t)$ is the damage associated with aging, $D_D(t)$ is the damage associated with disease, and $H(t)$ is damage repaired by healing. (Nash, 1967)

$$\sum_{i=1}^{n} \frac{n_i}{N_i} \tag{2.1}$$

$$D(t) = D_S(t) + D_A(t) + D_D(t) - H(t) \tag{2.2}$$

A healing rate of 1% per day is sufficient to repair any fatigue damage in tendon incurred by 20MPa of cumulative stress during normal daily locomotion (less than 20% of tendon's ultimate stress) in 20 hours (Fig. 2.5) (Schechtman & Bader, 1997). The healing rate and appropriate duration of rest for other types of soft tissues injuries that occur under different stress levels, however, remain unexplored, affecting the usability of Nash's cumulative damage model of self-healing structures.

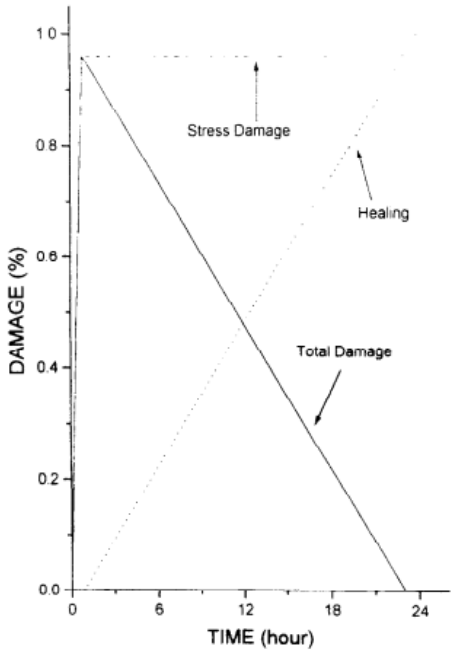

Figure 2.5: Tendon % Damage and Healing by Hour. Original image was published in article by Schechtman and Bader, 1997.

Following cyclic loading, the injury and healing processes of tendon lead to a series of mechanical and biological responses. Maximum tensile strength decreases within the first day. Subsequently, tendon cross-sectional area increases; Young's modulus (slope of the "linear" region of the stress-strain curve) decreases; allowable maximum stresses decrease; cellularity increases; tendon collagen organization is disrupted; and cell morphology is changed (Fig. 2.6) (Fung et al., 2009, 2010). Biological responses following cyclic loading induced tendon injury is listed in Table 2.1 (Nakama, King, Abrahamsson, & Rempel, 2005; Galloway, Lalley, & Shearn, 2013).

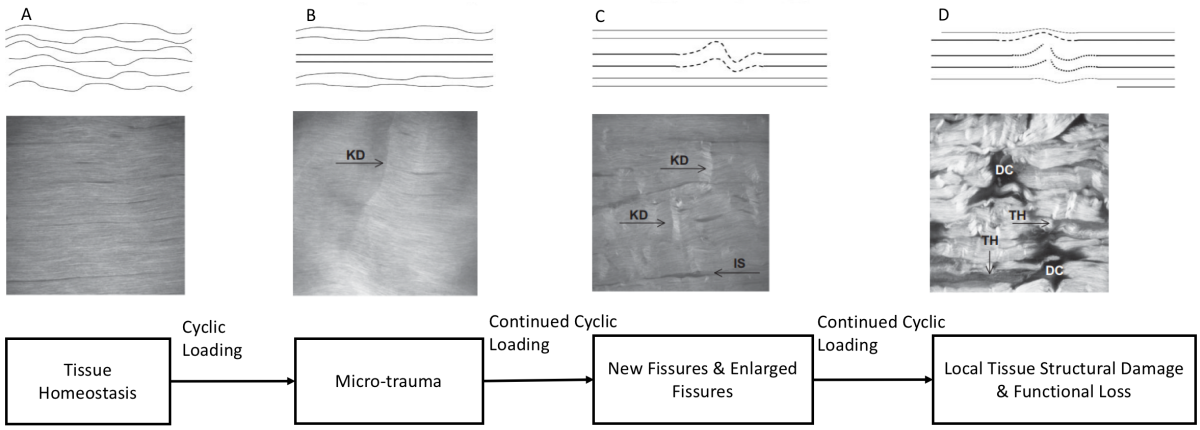

Figure 2.6: Important stages of tendon trauma from histomorphological and clinical perspectives. (A) Control, aligned collagen fibrils. (B) Low level fatigue loaded tendons, kinked fiber deformations (KD). (C) Moderate level loaded tendons, kinked fiber deformations with widening of the inter fiber space (IS). (D) High level loaded tendons, severe matrix disruption with fiber thinning (TH) and matrix discontinuities (DC). Original images were published in articles by Fung et al., 2009, 2010.

Following a similar three-stage healing process, muscle and bone healing progresses with first, the cellular and vascular response to injury, or inflammation; second, new cell replacement of the necrotic ones, or repair; and third, reorganization of the newly repaired tissues, or remodeling. Following muscle injury, pro-inflammatory cytokines are released; and phagocytosis of the injured tissue takes place. In addition, serum creatine kinase level increases; satellite cells are activated to fuse and repair the damaged myofibers, and fibroblasts enhance satellite proliferation yet may deposit scar tissue that can later impair muscle function (Hawke & Garry, 2001; Tsivitse et al., 2003).

Table 2.1: Post-injury Tissue Healing Process

Stage	Time Frame	Process
Inflammation	Week 1	Fibrin clots are developed to stablize the injury site.
		Homeostasis.
		Migration of neutrophils, macrophages and erythrocytes.
		Subsequent neovascularization.
Matrix Production	Week 1-4	Matrix producing fibroblasts synthesize disorganizaed collagen and other extracellular matrix proteins.
		Macrophages release pro-inflammatory cytokines and metalloproteinases.
		Degradation of collagen matrix; formation of micro-tears cellular proliferation.
		Tendon fibrillation.
		Matrix production.
Remodeling and Maturation	Week 4 and after	Collagen turnover to create more organized extracellular matrix;
		Cell density and vascularity decrease.
		Tendon thickening; scar tissue formation.

Bone healing is initiated with an immune response demonstrated by haematoma formation and inflammation. Cytokines including IL-1, IL-6 and TNF-alpha directs movement and recruitment of mesenchymal cells, which differentiates into different mesenchymal lineages whose end-stage cells fabricate bone and other types of tissues (J. Gao & Caplan, 2003). Meanwhile, the platelets release growth factors PDGF and TGF-b, which facilitates mesenchymal cell activation, proliferation, migration, angiogenesis, inflammatory cell chemotaxis and further platelet generation. The mesenchymal cells continue to proliferate and differentiate to osteoblasts, which forms woven bone (hard callus) and endochondral bone (soft callus). Eventually, the endochondral bone is replaced by marrow-filled woven bone, capable of bearing weight (Dimitriou, Tsiridis, & Giannoudis, 2005).

Ambiguity and contradictions exist in loading related to the tissue healing process. In 1892, Julius Wolff stated that healthy animal or human's bones will remodel to adapt to the load that they are placed under over time. Later studies showed that tissues other than bone such as connective and muscular tissues also follow Wolff's Law (Frost, 1990). Responding to loading, cellular detection of tissue strains is followed by tissue modification. Matrix molecules and prostaglandins are synthesized due to mesenchymal cell stretching or compression and the

resultant realignment of cytoskeletal elements. Such matrix deformation could lead to changes in macro-molecular organization, fluid flow, and cell function (Galloway et al., 2013). Some claim that during persistent cyclic loading of the bone, especially those rest-inserted loading at low load magnitudes, bone formation exceeds its absorption rate, and therefore leads to increasing bone volume, density, matrix organization, collagen insertion into the bone and bone strength (Buckwalter & Grodzinsky, 1999; Srinivasan et al., 2003). Similar theory also gained some popularity in post-injury healing of muscle. Some studies have shown that repetitive exercise training such as treadmill running activates satellite cell proliferation and mitotic activity, indicating myofiber repair and regeneration. In addition, muscle force deficit is shown to be attenuated after repeated lengthening contractions in some cases (Gosselin, 2000; H. K. Smith, Maxwell, Rodgers, McKee, & Plyley, 2001).

2.7 Eccentric Exercise in MSD Research

Eccentric contractions are known to induce inflammation and skeletal muscle damage. Serum or plasma creatine phosphokinase, also known as creatine kinase (CK), has been previously used as effective markers to evaluate muscle damage during and after eccentric exercise in both human and rat models (Schwane & Armstrong, 1983; Liao et al., 2010).

2.8 Limitations of Current Literature

Although the early mechanism of cyclic loading related injuries have been explored from molecular and biomechanical aspects, the exact process of injury formation remain unclear. Conflicting theories exist regarding the natural healing process and the mechanisms contributing to tissues' course of decrease in mechanical properties (Buckwalter & Grodzinsky, 1999; Fung et al., 2009; Galloway et al., 2013).

Development of methods and models to identify and quantify injury and healing are necessary in advancing understanding the etiology of tissue injury and its sequential responses in order to develop more definitive and effective plans to help early diagnosis, early intervention,

work-rest cycle scheduling, and job risk assessment (Cutlip et al., 2009). The motivation and purpose of this dissertation were derived from the above needs identified.

Chapter 3

Impact of Rest and Loading on Muscle Micro-trauma in A Human Model

3.1 Introduction

The United States Department of Labor defines work-related Musculoskeletal Disorders (WMSDs) as musculoskeletal system and connective tissue diseases and disorders, involving overexertion, repetitive motion and vibration that lead to living tissue sprains, strains, tears, as well as pain, swelling, and numbness. MSDs represent one of the leading causes of lost workdays in industry and are associated with major economic costs. The Occupational Safety and Health Administration (OSHA) estimated that "work-related MSDs in the United States account for over 600,000 injuries and illnesses and 34 percent of all lost workdays reported to the Bureau of Labor Statistics (BLS). These disorders now account for one out of every three dollars spent on workers' compensation. It is estimated that employers spend as much as 20 billion dollars a year (U.S.) on direct costs for MSD-related workers' compensation, and up to five times that much for indirect costs, such as those associated with hiring and training replacement workers". (OSHA, 2014) In addition, MSDs require long recovery time and pose significant challenges to affected workers personal lives.

Several known MSD risks include high force demands, high repetition rates, the interaction between the two, awkward postures, and long durations (Bernard & Putz-Anderson, 1997; Hoogendoorn, van Poppel, Bongers, Koes, & Bouter, 1999; Gallagher & Heberger, 2013). Several well advocated treatment approaches include thermotherapy (usage of ice or heat at the site of pain) (Wyss et al., 2012), manual therapy (Marcus, 1998; Bove et al., 2016), medications and

dietary supplements such as protease enzyme that modulates the inflammatory response. Existing preventative measures include stretching and warm-up programs (Choi & Woletz, 2010). However, the efficacy of these therapeutic and preventative approaches has not been convincingly validated (Marcus, 1998; Choi & Woletz, 2010; Wyss et al., 2012). It would also be costly to implement such program in a manufacturing facility. Compared to post hoc remedies, designing jobs with MSD prevention in mind would be an effective and affordable alternative (T. G. Smith & Gallagher, 2015).

The MSD development process starts with tissue micro-traumas occurring as a consequence of performing repetitive and/or forceful tasks, leading to local and maybe systemic inflammation, followed by structural tissue damage and eventually MSDs (Barbe & Barr, 2006). This study examined the beginning stage of MSD development muscle micro-trauma, aiming to test the hypotheses that rest interval plays a significant role in MSD prevention and development, in addition to the known risk factors. Previous studies have demonstrated subjects assigned with longer rest intervals (3 minutes) between eccentric exercise work periods were able to perform a significant larger total work volume than the ones assigned with shorter rest intervals (1 minute). Quantitative marker for skeletal muscle micro-trauma, Creatine Kinase (CK) level was significantly more elevated 48 hours and 72 hours post-experiment in the longer rest interval group than the shorter rest interval groups (Machado & Willardson, 2010; Evangelista, Pereira, Hackney, & Machado, 2011). Such results indicated that rest intervals played a significant role in muscle strength endurance in eccentric exercise. Different duration of rest intervals could also lead to different levels muscle micro-trauma, although not all subjects compared in these previous studies performed an equal workload. To provide common ground for comparisons of different subjects' muscle micro-trauma, the authors of this study designed the experiment so that all subjects share the same total work volume (equal number of multiples of their maximum voluntary isometric contraction of the non-dominant biceps, which are explained in the Methods section), total work time and total rest time. The findings of this study may serve as initial evidence that select rest intervals could be implemented at

manufacturing jobs to serve as an affordable MSD preventive measure without impeding the production rate or having to recruit additional employees.

3.2 Methods

3.2.1 Subjects

After acquiring approval from Auburn University Institutional Review Board (IRB), 24 healthy men between the ages of 19 and 50 (mean: 24.1 years; standard deviation: 3.61 years) were recruited to participate in this study. Male subjects were selected because 70.1% of current 15,338 manufacturing workers in the U.S. are men (BLS, 2015). In addition, significantly higher CK levels were reported in men than women despite their racial diversities (Wong et al., 1983). The age limit was based upon a previous study, which concluded that the healthy elderly (64 - 84 years) demonstrated a significant CK decline compared to its younger counterpart (24 - 47 years) (Steinhagen-Thiessen & Hilz, 1976).

Subjects submitted written confirmation for not using medical drugs, dietary supplements, or anabolic steroids, and being free of joint, muscular or cardiovascular diseases within the prior 6 months or during the week of the experiment (Evangelista et al., 2011). Subjects had also confirmed to have not performed eccentric, concentric, isometric or other forms of weight training six months prior to the experiment. Participants agreed not to perform weight training or strenuous physical activities during the week of and a week after the experiment, as significant increases of CK occurred after exercise are usually lower in healthy trained subjects compared to healthy untrained subjects (Brancaccio, Maffulli, & Limongelli, 2007).

Qualified subjects of the above criteria filled out a medical screening form to ensure that no pain or discomfort was present in the non-dominant limb. Subjects were then randomly assigned to one of the four treatment combinations using a random number generator: “High Load, Low Repetition, High Rest” (n=6), “High Load, Low Repetition, Low Rest” (n=6), “Low Load, High Repetition, High Rest” (n=6), or “Low Load, High Repetition, Low Rest” (n=6) (Table 3.3). All 24 subjects completed the experiment. Subjects were compensated for their participation.

Table 3.1: Treatment Combinations

Treatment (a) High Load; High Rest	Treatment (b) Low Load; High Rest	Treatment (c) High Load; Low Rest	Treatment (d) Low Load; Low Rest
5 min, 90% MIVC, 2 rep/min	5 min, 22.5% MIVC, 8 rep/min	3 min, 90% MIVC, 2 rep/min	3 min, 22.5% MIVC, 8 rep/min
		1min Rest	1min Rest
2 min Rest	2 min Rest	3 min, 90% MIVC, 2 rep/min	3 min, 22.5% MIVC, 8 rep/min
		1min Rest	1min Rest
5 min, 90% MIVC, 2 rep/min	5 min, 22.5% MIVC, 8 rep/min	3 min, 90% MIVC, 2 rep/min	3 min, 22.5% MIVC, 8 rep/min
		1min Rest	1min Rest
2 min Rest	2 min Rest	3 min, 90% MIVC, 2 rep/min	3 min, 22.5% MIVC, 8 rep/min
		1min Rest	1min Rest
5 min, 90% MIVC, 2 rep/min	5 min, 22.5% MIVC, 8 rep/min	3 min, 90% MIVC, 2 rep/min	3 min, 22.5% MIVC, 8 rep/min

3.2.2 Eccentric Exercise

Eccentric exercise was selected because it provided a basis for examining the impact of load-repetition and rest frequency on muscle tissue fiber damage and micro-trauma (Komi & Buskirk, 1972; Prasartwuth, Taylor, & Gandevia, 2005; Liao et al., 2010). The symptoms of such micro-trauma include muscle soreness and tenderness, known as delayed-onset muscle soreness (DOMS) (Schwane & Armstrong, 1983; Ebbeling & Clarkson, 1989; Jones, Newham, & Torgan, 1989; Cleak & Eston, 1992; Clarkson, Nosaka, & Braun, 1992). Although DOMS can be healed within weeks upon sufficient rest, its process and symptoms resemble the beginning stage of MSDs. As the micro-trauma prolongs and worsens, MSDs start to develop over time (Komi & Buskirk, 1972; Newham, Jones, & Clarkson, 1987; Newham, 1988; Clarkson & Tremblay, 1988; Whitehead, Allen, Morgan, & Proske, 1998).

Each subject's maximum voluntary isometric contractions (MVICs), peak force produced by a muscle or mucle group, of the non-dominant elbow flexors were measured before the

actual experiment to establish eccentric contraction exertions for different individuals. Each MVIC measurement was separated by a rest period of 2 minutes (Caldwell et al., 1974). The greatest MVIC of the three was used to determine the eccentric load levels for each and every subject.

After MVIC measurement, each subject performed eccentric exercise with the elbow flexors of the non-dominant arm on a Biodex dynamometer (Fig. 3.1) to induce micro-traumatic reactions in the non-dominant arm's biceps. The non-dominant arm was chosen to ensure that the subject's daily personal tasks were not affected in case of DOMS.

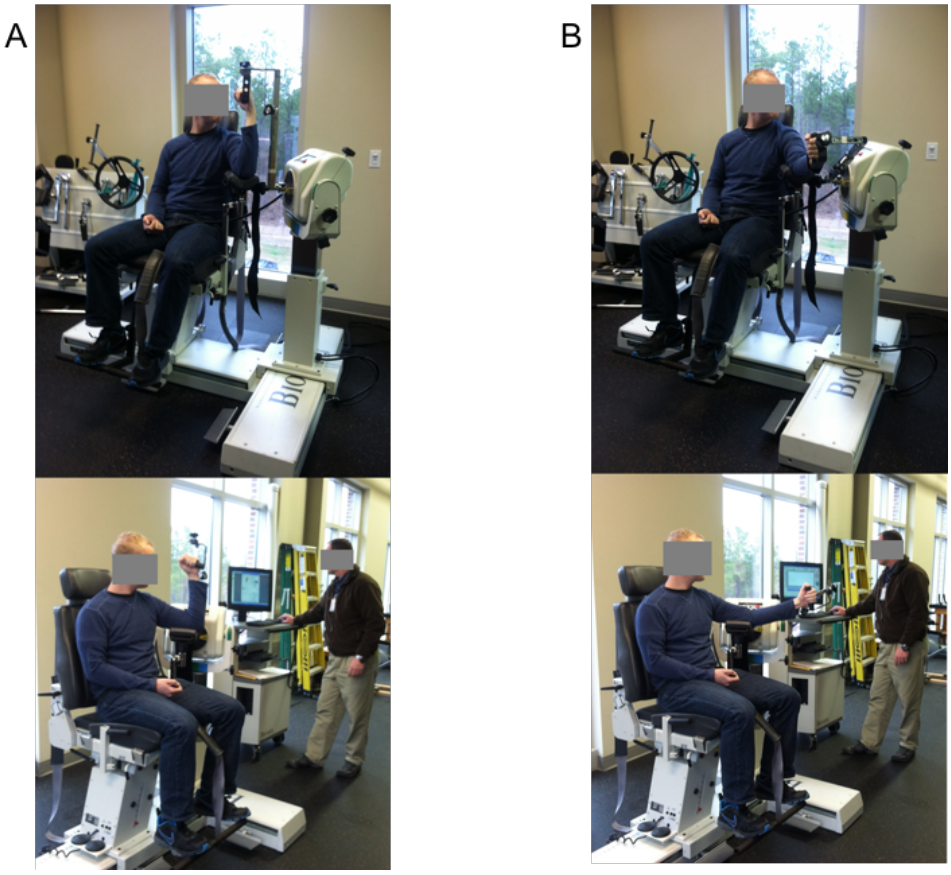

Figure 3.1: Eccentric Exercise. (A) Flexed Posture. (B) Extended Posture.

The exercise period was 19 minutes, including 4 minutes of rest and 15 minutes of work. "High Load" was determined at 90% subject's bicep MVIC to induce CK contrast to the "Low Load" condition. "Low Load" was selected at 22.5% bicep MVIC (Barr & Barbe, 2002). "High Repetition" was set at 8 reps/min. "Low Repetition" was set at 2 reps/min. The "High/Low Load, Low/High Repetition" was considered a combined treatment factor as the "High Load,

Low Repetition" and "Low Load, High Repetition" conditions share an equal total work volume per minute: 1.8 MVIC/min, providing ground for comparison to muscle micro-traumatic reaction under different rest schedules. In order to yield statistically distinctive and meaningful results, "High Rest" interval was set to be 2 minutes between 5-minute work intervals in reference to past relevant experiments. "Low Rest" interval was set to be 1 minute between 3-minute work intervals (Evangelista et al., 2011). A timeline diagram was created to demonstrate the experiment plan for the "High Rest" and "Low Rest" groups (Fig. 3.2). Total work volume for each subject was 27 times his bicep MVIC. The total rest time for each subject was 4 minutes. Each subject practiced the timing of contractions with the non-dominant arm prior to the experiment.

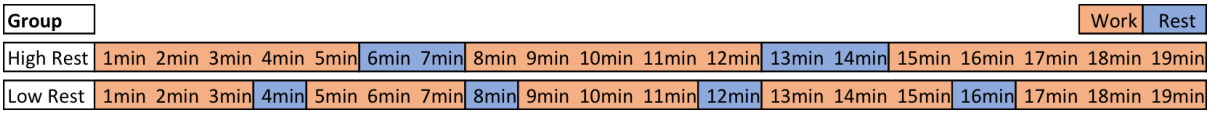

Figure 3.2: Time Diagram of "High Rest" and "Low Rest" Groups' Experiment Plan.

During a repetition, the subjects began with their non-dominant arm flexed 90° at the elbow and ended with the elbow fully extended (Fig. 3.1), resisting the dynamometer handle bar from descending in an arc with a radius of subject's forearm length and a focus at his elbow. Each extension took 2 seconds. The experimenter monitored the subject's muscle activity on the Biodex Dynamometer monitor and provide verbal feedback to encourage the subject to exert and to maintain the assigned percentage of MVIC during the repetition. The dynamometer automatically returned the bar back to the starting position after each repetition, getting ready for the next. Subjects were given permission to terminate the exercise at any point of the exercise if discomfort arose.

3.2.3 Serum Creatine Kinase Sample Collection and Analysis

Serum CK activity has been extensively studied and considered a quantitative biomarker for skeletal muscle micro-trauma (Machado & Willardson, 2010; Evangelista et al., 2011). Serum CK measurement is an important aid in the diagnosis of skeletal disorders (Wong et al., 1983). It has been reported that post-exercise CK elevates in the following 24 and 48 hours, peaks

at 96 hours and decreases between days 4 and 10 (Brancaccio et al., 2007). Therefore, CK was selected as a quantitative biomarker for muscle-trauma in this study and was measured pre-exercise and post-exercise at days 0, 1, 2, 4 and 8.

Five-milligram of blood were obtained from the subject's non-dominant arm's antecubital vein while they were in a seated position. Samples were collected immediately prior to exercise (day 0) as baseline, then post-exercise on days 1, 2, 4 and 8 (Chiang et al., 2009; Evangelista et al., 2011). The blood samples were then sent to the Laboratory Services at East Alabama Medical Center (Opelika, AL) on the same day as the samples were collected for CK measurements. CK levels pre- and post-exercise were recorded for comparison within and between different treatment groups.

3.2.4 Statistical Analysis

The number of replicates for each treatment combination was determined with the assistance of the operating characteristic curve for this two-factor factorial design (Montgomery, 2014). Previous studies that used CK as muscle micro-trauma indicator were referenced to decide population standard deviation: 500 $U \cdot L^{-1}$ (Chiang et al., 2009; Evangelista et al., 2011). The desirable power for this study was decided to be 0.70. The number of replicates to achieve a power of 0.70 would be 5 replicates per treatment combination. This study used 6 replicates per treatment combination due to budget permission. With 6 replicates per treatment combination, statistical power was boosted to close to 1.00.

Statistical analysis of the change and trending of serum CK level over time were conducted to examine the significance of loading-repetition combination, and rest. A double natural logarithmic transformation (Ln(Ln(Original Data))) was conducted on the original CK records due to the non-normal characteristic of the original datas residuals. Residuals of the transformed data demonstrated normal pattern (Shapiro-Wilk test: p=0.1460). Two-way ANOVA with repeated measures and Tukey pair-wise comparisons were conducted via Statistix 8.0 to examine significance of different rest and loading patterns, as well as possible interactions between the

two over time. The Type I error rate was set at 0.05 for ANOVA tests and also for post hoc tests. Figures were generated using GraphPad Prism 7.

3.3 Results

Table 3.2 summarizes average observed CK levels and standard deviations by treatment group by day. HLLR group's average CK level climbed up on Day 1 and 2, compared to Day 0, peaked on Day 4, then sharply declined on Day 8, although CK on Day 8 still was higher than Day 0, 1, and 2's values. HLHR group's average CK level experienced a slight decline on Day 1 and 2, compared to Day 0, drastically increased to a peak value, then declined to a level that was still higher than Day 0, 1, and 2's values. HLLR group's peak value on Day 4 is higher than HLHR's, while HLLR's CK on Day 8 dropped to slightly lower than HLHR's (Fig. 3.3A). Standard deviation of CK remained low in all treatment groups from on Day 0, 1, and 2 ranging between 37 $U \cdot L^{-1}$ and 198 $U \cdot L^{-1}$, but increased to above 1000 $U \cdot L^{-1}$ on Day 4 and 8, particularly in the High Load, Low Repetition groups (Table 3.3; Fig. 3.3B, C).

Table 3.3 summarizes transformed CK averages and standard deviations by treatment group by day. Graphic illustrations are shown in Fig. 3.4. Table 3.4 presents the results of two-way ANOVA with repeated measures based on the transformed data. "Day" was a significant factor (p=0.0115), which confirms a previous study's findings that post-exercise CK fluctuates significantly, peaking in the following 96 hours and declining between Days 4 and 10 (Brancaccio, Maffulli, and Limongelli, 2007). Significant disordinal interaction was discovered

Table 3.2: Original CK Average Levels and Standard Deviation in $U \cdot L^{-1}$ within Each Treatment Group

Treatment Group	Day 0 CK Average	Day 0 CK Stdev	Day 1 CK Average	Day 1 CK Stdev	Day 2 CK Average	Day 2 CK Stdev	Day 4 CK Average	Day 4 CK Stdev	Day 8 CK Average	Day 8 CK Stdev
(a) High Load; High Rest	174.67	155.35	163.00	132.92	155.83	133.30	682.67	1200.47	536.50	1026.35
(b) High Load; Low Rest	90.50	37.13	119.83	37.59	234.00	197.96	931.50	1278.06	470.00	496.71
(c) Low Load; High Rest	195.50	193.00	149.50	119.20	106.17	59.77	107.50	60.81	107.67	65.35
(d) Low Load; Low Rest	99.83	44.81	115.83	81.66	107.50	48.03	96.00	33.45	117.00	53.25

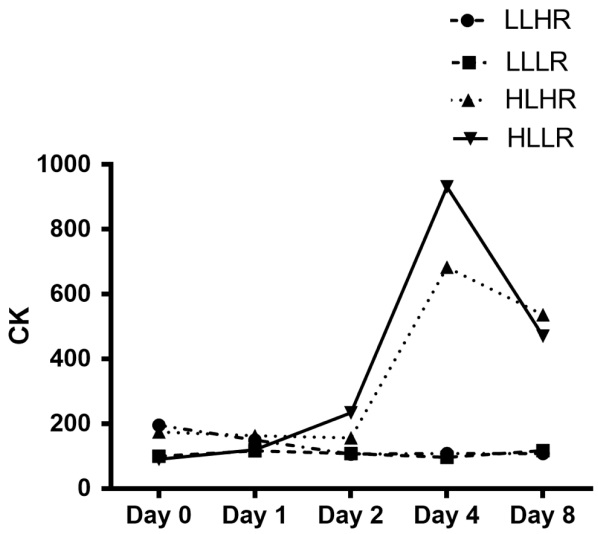

Figure 3.3: Original CK levels in $U \cdot L^{-1}$ by treatment group by day; HLLR: High Load, Low Repetition, Low Rest; HLHR: High Load, Low Repetition, high Rest; LLLR: Low Load, High Repetition, High Rest; LLLR: Low Load, High Repetition, Low Rest; LLHR: Low Load, High Repetition, High Rest.

between load-repetition combination and "Day" (Table 4, p=0.0000; Fig. 3.5) with respect to CK expression (Kirk, 1995). Tukey pairwise comparisons of CK levels on different days were conducted for load-repetition-combination by Day. Results showed that for the "High Load, Low Repetition" groups, CK level on Day 4 was significantly different than the ones on Day 0 and 2; CK level on Day 8 was significantly different than Day 0. Figure 3.5 demonstrated disordinal interaction between load-repetition-combination and Day. Statistically significant interaction between Rest and Day in Table 4 (p=0.0322). Tukey pairwise comparisons of CK levels on Rest by Day was conducted. Results indicated that CK levels on days 4 and 8 are significantly higher than Day 0 under "Low Rest" conditions. Disordinal interaction between Rest and Days was observed from Figure 3.6.

The results of the study indicated that shorter but more frequent rest intervals (Low Rest) led to greater CK response, indicating greater muscle micro-trauma and MSD risk, than the longer but less frequent ones (High Rest), when subjects' non-dominant biceps underwent eccentric exercise and were given equal total work time, total rest time and total work volume,

Table 3.3: Transformed CK Average Levels and Standard Deviation in $U \cdot L^{-1}$ within Each Treatment Group

Treatment Group	Day 0 CK Average	Day 0 CK Stdev	Day 1 CK Average	Day 1 CK Stdev	Day 2 CK Average	Day 2 CK Stdev	Day 4 CK Average	Day 4 CK Stdev	Day 8 CK Average	Day 8 CK Stdev
(a) High Load; High Rest	1.59	0.15	1.58	0.64	1.57	0.13	1.69	0.23	1.64	0.21
(b) High Load; Low Rest	1.49	0.08	1.55	0.33	1.63	0.17	1.73	0.28	1.70	0.23
(c) Low Load; High Rest	1.58	0.17	1.56	0.63	1.52	0.09	1.51	0.11	1.51	0.10
(d) Low Load; Low Rest	1.51	0.08	1.52	0.52	1.53	0.08	1.51	0.07	1.54	0.08

Table 3.4: Two-way ANOVA with Repeated Measures on Days 0, 1, 2, 4 and 8

SV	SS	df	MS	F	P
Between Blocks	1.9268	23	0.0838		
Load	0.2230	1	0.2230	2.62	0.1209
Rest	0.0006	1	0.0006	0.01	0.9343
Load * Rest	0.0039	1	0.0039	0.05	0.8325
Error (Between Blocks)	1.6993	20	0.0850		
Within Blocks	0.8244	96	0.0086		
Day	0.0849	4	0.0212	3.47	0.0115*
Load * Day	0.1776	4	0.0444	7.26	0.0000*
Rest * Day	0.0681	4	0.0170	2.78	0.0322*
Load * Rest * Day	0.0043	4	0.0011	0.17	0.9513
Error (Within Blocks)	0.4895	80	0.0061		

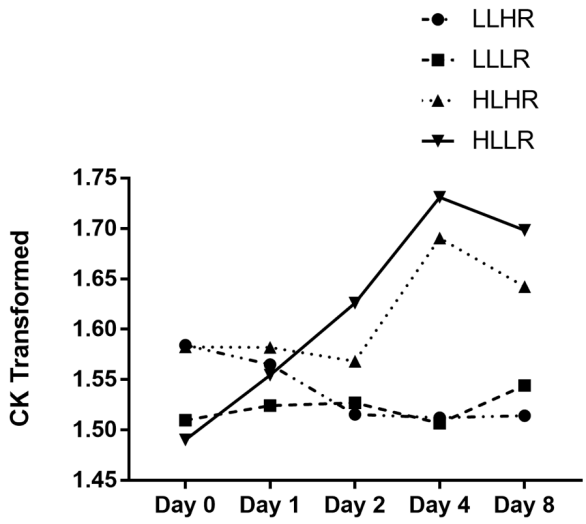

Figure 3.4: Transformed CK levels in $U \cdot L^{-1}$ by treatment group by day; HLLR: High Load, Low Repetition, Low Rest; HLHR: High Load, Low Repetition, high Rest; LLLR: Low Load, High Repetition, High Rest; LLLR: Low Load, High Repetition, Low Rest; LLHR: Low Load, High Repetition, High Rest.

especially when loading level was high (High Load, Low Repetition). CK level remained relatively low and steady when load level was low (Low Load, High Repetition) and rest level was high (High Rest).

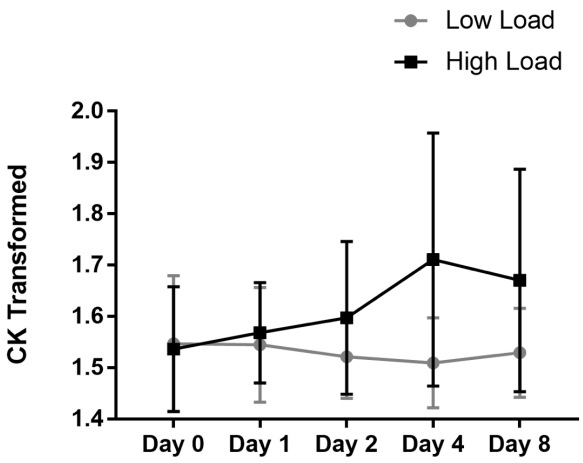

Figure 3.5: Transformed CK levels by Day: High Load vs Low Load in $U \cdot L^{-1}$.

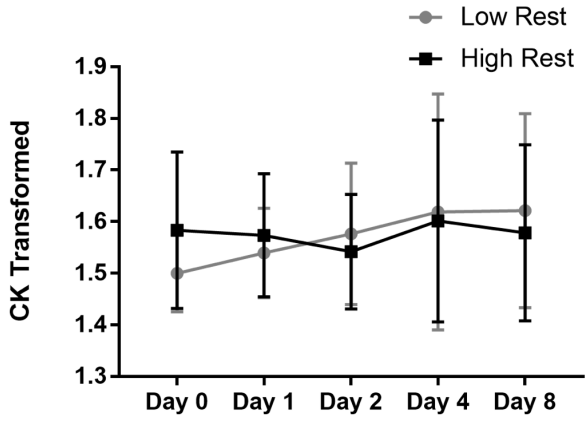

Figure 3.6: Transformed CK levels by Day: High Rest vs Low Rest in $U \cdot L^{-1}$.

3.4 Discussion

Statistical significance of Day, and the disordinal interaction between Load and Day, and Rest and Day respectively suggest muscle micro-traumatic reaction changes significantly over time, especially when load level is high (High Load, Low Repetition, Fig. 3.5) and rest level is low (Low Rest, Fig. 3.6). Had subjects continued to work at the same exertion level and was not given sufficient time to recover, the micro-trauma could accumulate and compound, leading to fatigue, further tissue wear and tear, and possibly eventually MSDs (Finsterer, 2012).

CK on Day 4 of the "High Load, Low Repetition" regimen saw a significant increase (Fig. 3.4A), compared to measurements on Day 0 and 2. Day 8's measurement was also significantly different from Day 0. However, CK level remained low in the Low Load groups throughout the

5 measured days, and even experienced a small drop on Day 4. At low loading level, the muscle was not micro-traumatized, but instead could have been exercised to a greater strength. Ck continued to increase over the 5 observation days in Low Rest groups, leading to a significant difference between Day 4 and Day 0, and Day 8 and Day 0 respectively (Fig. 3.6). In High Rest groups, however, CK mildly fluctuated and its measurements did not display statistically significant difference. Such observation may indicate that duration and frequency of the rest intervals indeed have an impact on muscle micro-trauma development and healing. Shorter but more frequent rest breaks may be more injury conducive than longer but less frequent ones.

According to previous research, if load is not significant enough to cause acute and severe damage, sufficient rest and recovery will repair damaged tissue with fibers almost identical in nature to those damaged, making them more resistant to subsequent eccentric damage. This eccentric training effect has been shown in both human (Komi & Buskirk, 1972; Newham et al., 1987; Newham, 1988; Clarkson & Tremblay, 1988) and animal (J. Faulkner, Opiteck, & Brooks, 1992; J. A. Faulkner, Brooks, & Opiteck, 1993) studies. Therefore, the authors suspect that the "Low Load, High Repetition" condition stimulated CK reaction in the first couple days but, given days to recover, the damaged tissues had been remedied to a stronger state.

Such indications coincide with the Fatigue Failure Theory, which advocates for more repetitions and lower loads to tackle a set total volume, as lower loading per repetition can withstand much greater number of cycles to tissue failure compared to higher loading per repetition (Nash, 1967). Explorations of how and why the Low Load conditions yield statistically non-significant muscle micro-traumatic responses could provide guidance to job allocation at manufacturing plants in regards to MSD prevention.

Significance of Rest and Day interaction confirmed the authors' hypothesis that the healing and recovery that occurred during the rest intervals have an impact on muscle micro-trauma development. The healing rate, as the fatigue failure theory had suggested, may not follow a linear pattern but could be exponential over time, as the CK levels demonstrated significant different post-exercise when subjects were given different rest schedules despite the total rest time being equal. The more subdued expression of muscle damage in in the Long Rest group

could be due to the healing rate accelarating in the latter part of the rest break, which the Short Rest group did not receive. It could also be that the metabolic, molecular and circulatory factors associated with the recovery of viscoelastic material required longer duration of rest time (Gedalia et al., 1999).

Such results might also be explained by considering cyclic loading material failure using the mean stress concept commonly used in materials science and mechanical engineering (Gallagher & Schall Jr, 2017). Mean stress is the time average of principal stress; principal stress is force exerted per unit area. In this study, mean stress for the High Rest groups are 1.285 MVIC per unit area over a work-rest cycle (5-minute work followed by 2-minute rest); mean stress for the Low Rest groups are 1.35 MVIC per unit area over a work-cycle (3-minute work followed by 1-minute rest), greater than the High Rest groups' mean stress. Mean stress is an important factor with respect to the rate of damage accrual in materials fatigue failure (Stephens, Fatemi, Stephens, & Fuchs, 2000). The greater the mean stress on tissues during a loading cycle (assuming an equivalent stress range), the faster damage would accrue. The higher mean stress of the Low Rest group might be able to explain the continuous increase of CK levels and the significant differences between Days 0, 2 and 4, and Days 0 and 8 (Fig. 3.6). A previous study discovered that CK response of eccentric exercise was not as sensitive among trained weightlifters who had a minimum of three years weightlifting experience compared to the untrained weightlifters had not participated in eccentric exercise for the past three years. This previous study also found trained subjects developing severe muscle soreness without displaying matching degree of CK activities compared to the untrained individuals (Vincent & Vincent, 1997). The current study selected subjects who had not been involved in any form of weight training for the previous 6 months due to the above-mentioned reasons, as well as budget restrictions.

Many factors such as ethnicity, muscle mass, and body size could be determinants of CK levels in addition to the age and gender effects that are limited and ruled out in this study respectively. Although all subjects read and signed the agreement of not conducting any form of training and strenuous physical activities immediately 6 months prior, during and after the

experiment, it was impossible for the authors to ensure and confirm that the requirement was fulfilled by all subjects, which would leave a possibility for the data to be unknowingly influenced.

Due to individual MVIC differences, the authors decided to use the predetermined percentages of the individual MVICs to represent the High Load and Low Load conditions as the study was aiming to compare the bicep muscles' micro-traumatic status when similar capacities of the muscles, though from different subjects, were engaged. A predetermined high load for one with a lower bicep MVIC might be too high yet not high enough for one with a higher bicep MVIC.

There are several limitations of this pilot study. The experiment subjects were mostly Auburn University students, who do not perform repetitive and strenuous job tasks on a regular basis. Therefore, characters and conditions of manual material handling workers that may be influential to development of muscle micro-trauma or MSDs, such as work history or injury history relevant to WMSDs, were not captured. Different tissue types, including bones, fibrous tissues, and muscles from different parts of the body, follow similar but different mechanisms of injury and recovery in response to repetitive loading (Buckwalter & Grodzinsky, 1999). These different tissues may react to different work-rest regimens (e.g. of different duration or exertion level) differently.

In the future, different tissue types and durations of rest intervals could be used, and subjects from a real manufacturing setting could be considered to be recruited in order to associate experiment results more accurately to industrial and human performance applications. Future studies could also recruit subjects that carry more homogenous traits such as age, gender, BMI, ethnicity, sleep patterns, diet habits, smoking preferences and occupation, since such individual variability could influence study results (Wong et al., 1983), or to design studies to specifically compare and contrast subject groups that carry two or more distinct traits. Animal studies could be a promising direction to experiment as well because of the much more controlled experiment scenarios. Animal subjects such as mice or rats, could be trained to exercise over a long period time in order to observe long-term effects (Barbe & Barr, 2006). The homogeneity of the

animal subjects will also rule out the above-mentioned individual differences, as well as the possible high responders, which could be a contributor of CK high variances on Days 4 and 8 especially for the "High Load, Low Repetition" condition (Machado & Willardson, 2010). In addition, future studies could look into more types of different loading and work rest patterns. Researchers could also expand the difference between high and low levels of MVIC, as well as the length of high and low levels of rest intervals to explore possible significant impacts.

3.5 Conclusion

To the authors' knowledge, this was the first time for rest interval to be taken into consideration when assessing human tissue micro-trauma (via CK) *in vivo* with different loading and rest regimens, when total work volume, work time and rest time were predetermined. Although load and rest alone did not manifest as significant risk factors in this study, the interaction of load and day, as well as rest and day had significant contribution to muscle micro-trauma, and MSDs if insufficient rest was not adopted after the initial micro-trauma but forceful and repetitive exertions prolong.

Longer though less frequent rest breaks are more trauma suppressive than shorter but more frequent rest breaks, when total rest time is limited. The authors recommend to break a large workload to smaller ones, even though it may require more repetitions to complete if possible, as lower load exerted with more repetitions that sum up to the same total work volume as higher load exerted over fewer repetitions help keep the mean muscle micro-trauma response low throughout the post exertion days, although more extensive future studies on trauma development and its recovery course of different exercise and rest period and/or of different parts of the body is required.

The findings of this study add to the growing body of knowledge of human body's bio-chemical responses to eccentric muscle exertions at different load-repetition levels, and when given different rest breaks. Relevance of these research findings are applicable, but are not limited to, understanding of early stages of musculoskeletal injury development and recovery,

design and scheduling of manufacturing jobs if future studies expand the research scope to various tissue types and different work-rest regimens and confirm the findings of this study, as well as development of ergonomic assessment tools that incorporate rest intervals to better diagnose musculoskeletal injury risk in the occupational setting.

Chapter 4

Impact of Rest on Systemic Inflammation and Tissue Trauma in A Rat Model

4.1 Introduction

Musculoskeletal disorders (MSD), also referred to as repetitive or overuse injuries in soft tissues of the neck, shoulders, knees and hands, are painful and costly among working populations in the U.S. and worldwide (WHO, 2003; Hassard et al., 2014). Over 600,000 work-related MSD (WMSD) injuries and illnesses occur annually, accounting for 34% of all lost workdays and 32% of all work-related injuries and illnesses in the United States in 2014 (USBLS, 2014). MSD's course of recovery is long compared to all other types of work related injuries (Waters, 2004). Employers are paying as much as $20 billion per year for direct costs of worker's compensation, and up to $100 billion on indirect costs. There remains a need for effective measures to prevent and treat these injuries (OSHA, 2014).

Insufficient rest is often listed as a psychosocial MSD risk factor (Punnett & Wegman, 2004). However, rest and its healing value also carry characteristics of physical and physiological risk factors. A definitive frequency and duration of rest breaks required for specific load levels remain unclear (Trinkoff, Geiger-Brown, Brady, Lipscomb, & Muntaner, 2006; Lee, Ahn, Park, Kim, & Moon, 2011). The rate and degree of injury to different types of soft tissues (ex. muscle vs tendon) under certain loading conditions are also unclear (Barbe & Barr, 2006). Forced treadmill running for rats is a common method for researcher to investigate exercise-induced positive and negative physiological adaptations (Moraska, Deak, Spencer, Roth, & Fleshner, 2000). A rat model was designed to mimic a repetitive-motion work scenario which

allowed observation and comparison of consequent chronic systemic inflammation and soft tissue trauma levels between groups that were assigned with longer yet infrequent (Infrequent Long Rest) versus shorter yet frequent (Frequent Short Rest) rest breaks. Using breed, age, gender, weight and housing condition-controlled rat subjects, many confounding variables that the human subjects might demonstrate were screened out. This model also made histological and morphological observations of the soft tissue change and damage possible (Al-Shatti, Barr, Safadi, Amin, & Barbe, 2005; Barbe et al., 2008). The results were reflective of human responses to exercise in blood biochemical and histological profiles (Fedorczyk et al., 2010; Goutianos et al., 2015).

This study hypothesized that the prescribed treadmill regimen would induce systemic inflammation, tissue trauma, negative physiological adaptations (ex. decrease in body weight, individual muscle and tendon net weight, and levels of stress biomarker presence) in Sprague-Dawley rats. It was also hypothesized that rats that follow a shorter but more frequent rest schedule would exhibit higher levels of elevation in systemic inflammation, in muscle, tendon morphology, metabolic demands such as greater heart weight and muscle hypertrophy, as well as adrenal hypertrophy, a stress-sensitive measure (Moraska et al., 2000; T. G. Smith & Gallagher, 2015). Heart weight is positively related to maximum cardiac output. Increase in heart weight versus body weight is associated with increased maximum capacity to deliver blood to working muscles if muscle weight to body weight ratio remains unchanged (Oscai, Mole, Brei, & Holloszy, 1971). Therefore, in this study, outcome measures including terminal pro-inflammatory cytokine TNF-alpha and muscle damage biomarker Creatine Kinase (CK) expressions, body weights throughout the experiment period, individual tissue weight (Achilles tendon, gastrocnemius, soleus, plantaris, tibialis anterior and tricep muscles), Achilles tendon morphology, and oxidative stress biomarker Malondialdehyde (MDA), as well as terminal adrenal gland weights were examined (Moraska et al., 2000; Fedorczyk et al., 2010).

4.2 Methods

4.2.1 Animals

The experiment was approved by Auburn University Institutional Animal Care and Use Committee. A total of 24 female Sprague-Dawley rats were procured at 90 days of age, and housed in an Institutional Animal Care and Use Committee (IACUC) approved facility with 12: 12 hour light: dark cycles and free access to food and water. Female rats were used because gender is suggested an MSD risk factor, and females are more prone to WMSDs (Gerr et al., 2002; Strazdins & Bammer, 2004) A similar rat model been used in prior studies. (Al-Shatti et al., 2005; Barbe et al., 2008; Fedorczyk et al., 2010) .

4.2.2 Apparatus

A commercially available 4-lane animal treadmill (Columbus Instruments, OH) with a single belt and dividing (clear plastic walls between lanes and opaque PVC at the ends) walls suspended over the belt surface was used as the exerciser to induce musculoskeletal injuries. Each lane was 17.25"L x 2.37"W x 5"H (43.8 cm x 6 cm x 12.7 cm) in dimension and was supplied with an individually-lane-controlled electrical stimulus assembly at the beginning of the lane. The electrical stimulus could be adjusted by a dial to a range of currents from 0 to 1.5 mA at 163V. Speed could be adjusted in the range of 3-100 m/min and was set at 12 m/min for all rats. When rats were running, all lanes were covered with one piece of clear plastic board with breathing holes. A plastic block was placed under the initiating end of treadmill to achieve a 15° decline.

4.2.3 Treatment Groups

Upon procurement, rats were randomly assigned to Control (n=8), Long Infrequent Rest Breaks (LIRB, n=8), and Short Frequent Rest Breaks (SFRB, n=8) groups respectively. All rats were acclimated for 1 week post-procurement and were handled and weighed everyday by the same person who handled them throughout the experiment. The Control group served as

age-matched baseline for the working groups. During the week of acclimation, working rats were trained to run on the treadmill, which was set at the designed speed and decline for the chronic task in the following 3 weeks, starting from 1 min on the first day and ramping up to 7 min on the last day of the week. The Long Infrequent Rest group performed a downhill running task on the 4-lane rat treadmill for 2 h/day, in 40 min sessions, separated by 2 h breaks, 3 days/week for 3 weeks. The Short Frequent Rest group performed the running task on treadmill with same degrees of decline for 2 h/day, in 20 min sessions, separated by 1 h breaks, 3 days/week for 3 weeks. The treadmill was set to have a 15° decline and to run at 12 m/min (Schwane & Armstrong, 1983) for both Long Infrequent Rest and Short Frequent Rest groups (Fig. 4.1).

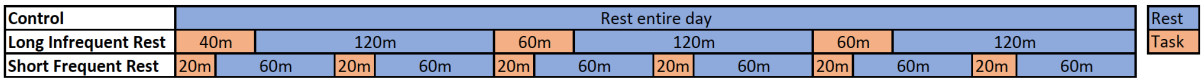

Figure 4.1: Daily schedules that the 3 treatment groups followed on Mondays, Wednesday and Fridays for 3 weeks.

4.2.4 Experimental Timeline

After 1 week of acclimation and training post-procurement, task rats followed their daily and weekly work-rest schedule for 3 weeks (Fig. 4.1, 4.2). Control rats acclimated 1 week post-procurement but did not perform training or the task throughout the 4-week experiment period. A 3-week work duration was selected as tissue injury goes through acute inflammation phase first, which involves different biochemical reaction in the body than chronic inflammation. Chronic inflammation typically begins 2-4 days after the onset of the acute response and can last for weeks even years due to repeated initiating stimulus or interference of normal healing process. However, when the stimulus level is low enough and healing rate is greater than injury rate, tissue can regenerate and recover, or even become stronger than pre-inflammation due to a training effect (Gallagher & Heberger, 2013). We designed a working scenario where chronic inflammation would start and could persist to examine whether different rest schedules lead to different tissue injury and recovery responses (Barr & Barbe, 2004).

Group	1 week	3 weeks
Control	Acclimate	Rest entire experiment
Long Infrequent Rest	Acclimate	Treadmill
Short Frequent Rest	Acclimate	Treadmill

Figure 4.2: Experiment plan for the 3 treatment groups.

4.2.5 Body Weight and Tissue Weight

Body weight over time was determined at the nearest tenth of a gram on a digital-display balance (Ohaus NV1101 Precision, New Jersey, United States) on all days (n=15) that the rats were scheduled to be handled (acclimation days 1-5, Mondays, Wednesdays and Fridays of week 1-3, and the Tuesday after week 3, which was the day of euthanasia). Weights of the heart, Achilles tendon, left gastrocnemius, soleus, plantaris, tibialis anterior, tricep muscles, and the left and right adrenals were collected post euthanasia and were determined at the nearest ten-thousandth of a gram on an analytical balance (Fisher Scientific XA, New Hampshire, United States) on the day of euthanasia.

4.2.6 Serum ELISA Analysis of Inflammatory Biomarkers

At 36 h after completion of the final task (to avoid possible acute-exercise- and stress- induced cytokine fluctuations), task rats and Control rats were deeply anesthetized using 5% isoflurane in oxygen. 3 ml of blood was collected via cardiac puncture using 21-gauge needles and was centrifuged at 2000g at room temperature for 30 min. Serum was separated and stored at -80 °C until use. Serum was assayed for 3 biomarkers using commercially available ELISA kits (Barbe et al., 2013; H. G. Gao et al., 2013) for levels of: (1) tumor necrosis factor-alpha (TNF-alpha), a pro-inflammatory cytokine (R& D Systems, Minnesota, United States) (Carp, Barbe, Winter, Amin, & Barr, 2007; D. L. Xin et al., 2011), (2) creatine kinase (CK), a muscle damage biomarker (Abcam, Massachusetts, United States) (Collinson et al., 1995; Goicoechea et al., 2008) and (3) malondialdehyde (MDA), an oxidative stress biomarker (MyBioSource, California, United States) (Carp, Barr, & Barbe, 2008).

4.2.7 Histopathological Assessment of Tendon

While anesthetized and post blood collection, the rats' Achilles tendons were harvested post-mortem from the left hind limb *en bloc*, submerged in formalin and labeled. After embedding in paraffin, the tendons were longitudinally sliced, mounted on histological slides, and stained with hematoxylin and eosin (H& E). The histological slides were scanned by a high-definition slides scanner Aperio CS2 (Leica Biosystems, Germany) and saved as .svs files. The images were analyzed in a blinded fashion using an open source digital pathology image analysis software QuPath (v0.1.2, Queen's University Belfast, UK) (Bankhead et al., 2017).

Tendon region (Fig. 4.3) was scored using a modified semiquantitative scoring method, the Bonar scale, to quantify tendon' s morphological changes. Three factors, cell shape, collagen organization, and cellularity were scored in endotendon. Cell shape was evaluated on a 0-3 scale. Cell shape scored "0" when all tenocytes were organized and slender and therefore deemed normal morphology; "1" when tendons contained mostly elongated tenocytes but a small number of oval cells; "2" if tendons contained equal number of elongated tenocytes and oval cells; "3" when tendon contained mostly oval cells. Collagen organization scored "0" if tendon fibers were closely packed together; "1" if fibers were slightly wavy but closely packed; "2" slightly wavy but separated; "3" wavy, non-parallel and separated (L. J. Soslowsky et al., 2002; Cook, Feller, Bonar, & Khan, 2004; Fedorczyk et al., 2010). Cellularity was quantified by analyzing three distinct fields of view (FOV) from the longitudinal section of a tendon. QuPath automatically assigned gridlines of 250μm x 250μm grids across the image. Four grids (2 x 2) makes a rough field of vision at 20x magnification. Therefore, the positions of the FOV were determined in a consistent fashion by first positioning the center of the FOV2 at the center of tendon section and outlining the 4 grids that fell in this FOV. The outlined area was referred to as an annotation by QuPath. FOV 1 and 3 were in captured in non-adjacent proximal and distal central portions of the tendon along its longitudinal axis. Annotations of 4-grid areas were also defined for FOV1 and 3 respectively (Fig. 4.3). A combination method of automated cell nuclei count by QuPath and manual count by the experimenter was used to measure the number of nuclei per FOV. The mean number of nuclei per grid was calculated for each tendon

by calculating the mean number of nuclei per grip among the 3 annotations (Backman, Boquist, Fridén, Lorentzon, & Toolanen, 1990; L. Soslowsky et al., 2000; Glazebrook, Wright Jr, Langman, Stanish, & Lee, 2008).

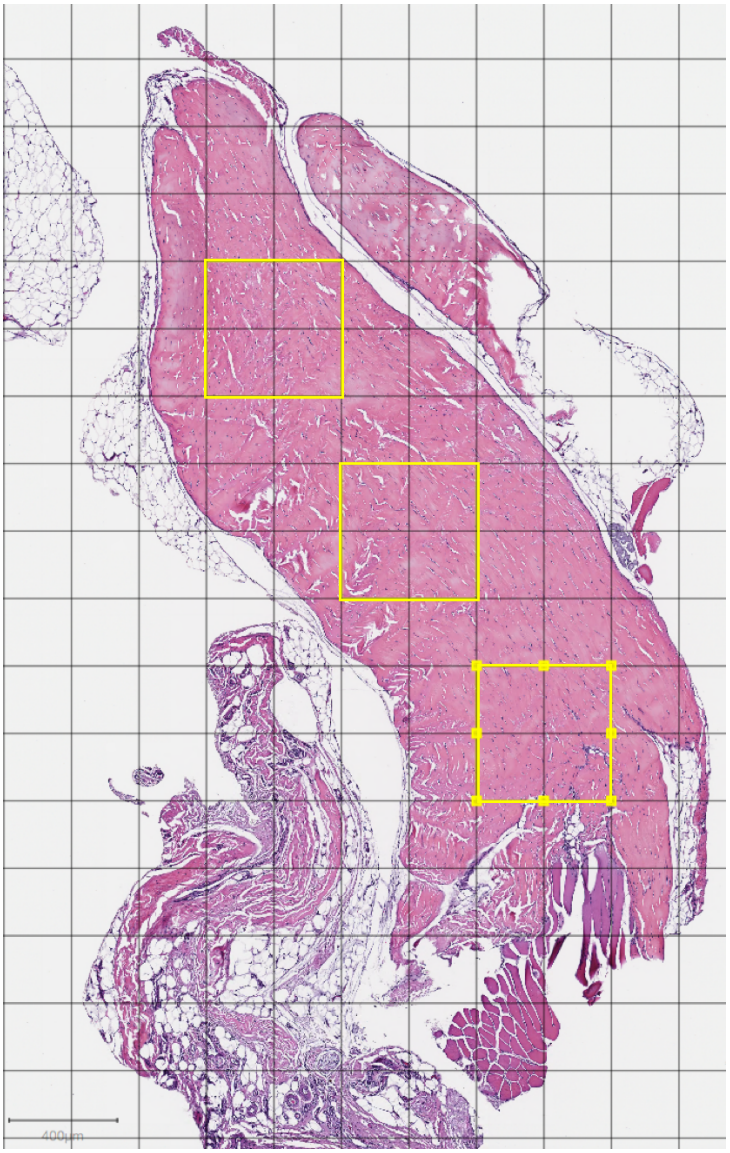

Figure 4.3: Three non-adjacent 500μm x 500μm, made up of four 250μm x 250μm QuPath grids, fields of visions were selected.

4.2.8 Sample Size Selection and Data Analysis

A sample size of n=8 per group was selected (statistical power 0.70) using effect size 3.5 pg/microgram and standard deviation 2.2 pg/microgram from a previous repetitive tendon injury study quantifying TNFalpha levels pre- and post- loading. This study recruited Sprague Dawley rats of same gender and similar age (Kirk, 1995; Fedorczyk et al., 2010).

Applicable past data of the other proposed response variable measurements (ex. effect sizes and standard deviations), including MDA, CK, and Achilles tendon Bonar scores were scarce. A sample size of 6 per group was found to be necessary to yield statistically significant results according to the most relevant studies we were able to find (Al-Shatti et al., 2005; Barbe et al., 2008). We therefore decide n=8 per group was an adequate sample size, which also allows room for unexpected subject dropout due to illness or inability to perform required tasks.

Rats' body weight over time was analyzed by two-way ANOVA with repeated measures. The main factor examined was "Rest", and the repeated measure was "Day". The effects of Rest, Day and their interaction were evaluated, as well as post-hoc multiple comparisons of mean body weights of different treatment groups of a day and body weights of each treatment group across the experiment span. Tissue and organ's terminal weight (adjusted by body weight at euthanasia), TNF-alpha, MDA and CK levels, tendon scores, and cell nuclei count were analyzed by one-way ANOVA respectively with factor Rest among the Control, Long Infrequent Rest or Low Rest groups, followed by Tukey pairwise comparisons.

4.3 Results

4.3.1 Body Weight over Time and Terminal Tissue Weight

Day and the interaction of Rest and Day had a significant impact on rats' body weight over time ($p<0.0001$). Overall, Control rats' body weights were significantly greater than the Long Infrequent Rest ones (p=0.0072, 95% CI for difference [1.123, 8.806]). Comparing different treatment groups within individual days, Control rats' body weight on Wk1 Day3 was significantly greater than the Long Infrequent Rest ones (p=0.0177, 95% CI for difference [3.209

to 31.61]). Comparing body weight across different days for each treatment group, all groups experienced significant weight loss during the week of acclimation. After some decrease on Acclimation Days 2 and 3, the Control group's body weight overall increased throughout the experiment period (except on Week2 Days 1 and 2), matching the trend of normal growth chart for Sprague-Dawley rats of the same age (Han et al., 2010). However, the body weight of the Short Frequent Rest rats was stagnant post decline from the acclimation week until the end of the experiment, while the Long Infrequent Rest rats' body weight had a statistically significant increase at the end of the experiment period (Fig. 4.4, 4.5, 4.6) (Han et al., 2010; Envigo, 2019).

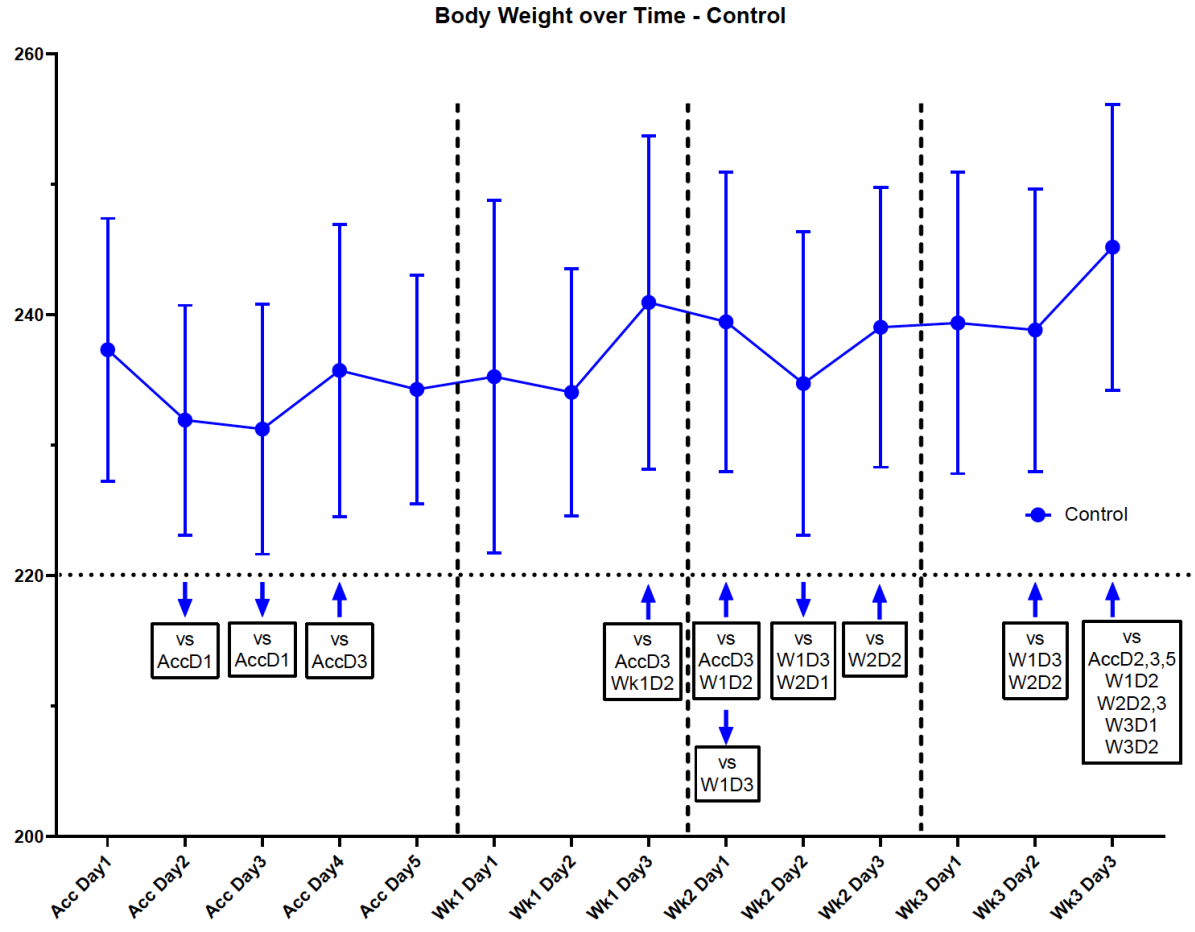

Figure 4.4: Body Weight of Control Rats over Time in Line Plot. Line plot of Control rats' mean body weight over time in grams (g) with standard deviation. Body weight increased overtime overall. Upward and downward arrows indicated a significant increase and decrease repectively, compared to one or more of prior days. Labels underneath the arrow specified the day(s) compared to that significant change was observed.

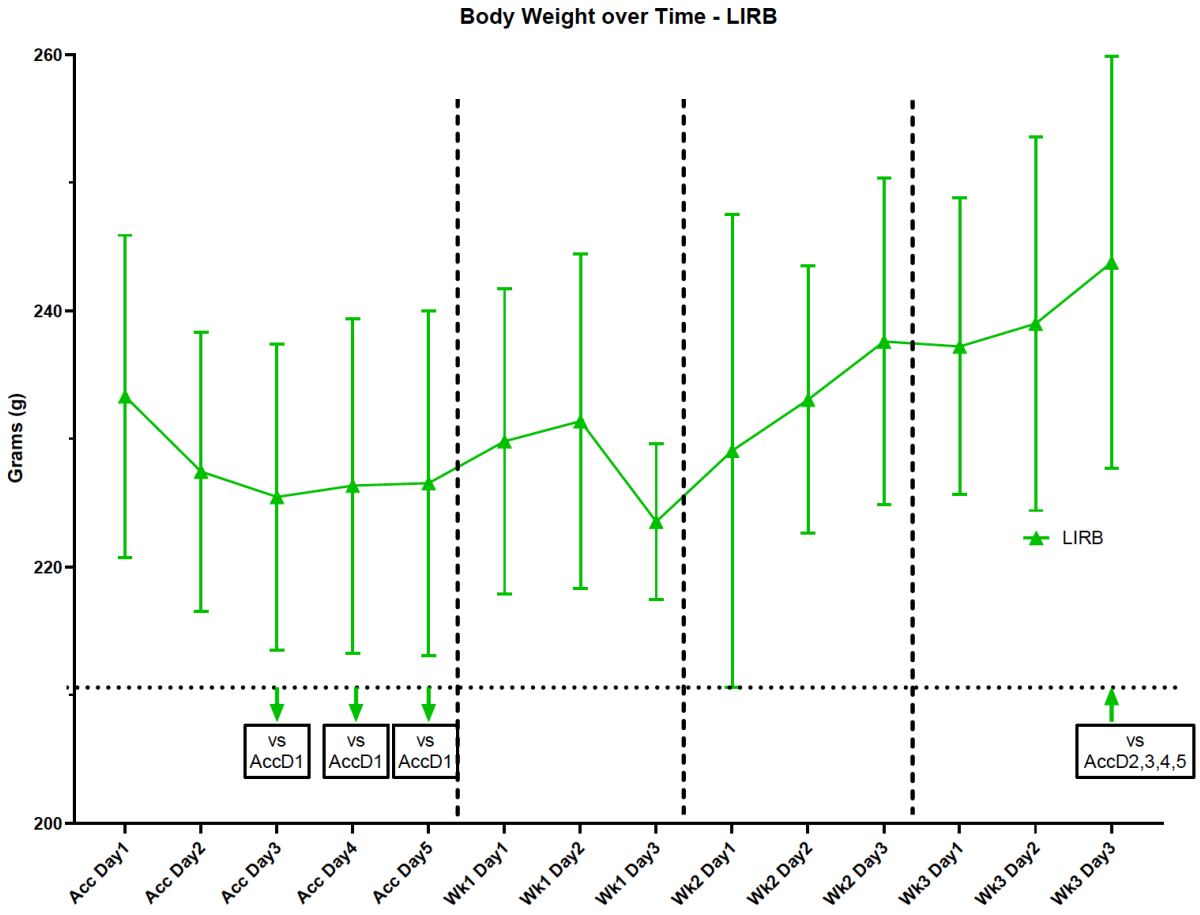

Figure 4.5: Body Weight of Long Infrequent Rest Rats over Time in Line Plot. Body weight significantly increased toward end of experiment but stayed stagnant after the initial decline for most part of the experiment.

Weights of the heart, Achilles tendon, left gastrocnemius, soleus, plantaris, tibialis anterior, tricep muscles, and the left and right adrenals were adjusted by dividing body weight at euthanasia and multiplying 10^n so the numbers compared were in the hundreds for fair comparison and ease of interpretation. Average adjusted weight of left and right adrenals was used for comparison. None of the above comparisons was statistically significant (Fig. 4.7).

4.3.2 Achilles Tendon Histopathological Changes

We observed histopathological changes in Long Infrequent Rest and Short Frequent Rest groups' Achilles tendons respectively (Fig. 4.8). Rest regimen contributed to the statistically significant increase in cellularity in the working groups' Achilles tendons (p=0.0002). Compared to the Achilles tendon cellularity in Control rats, Long Infrequent Rest and Short Frequent Rest

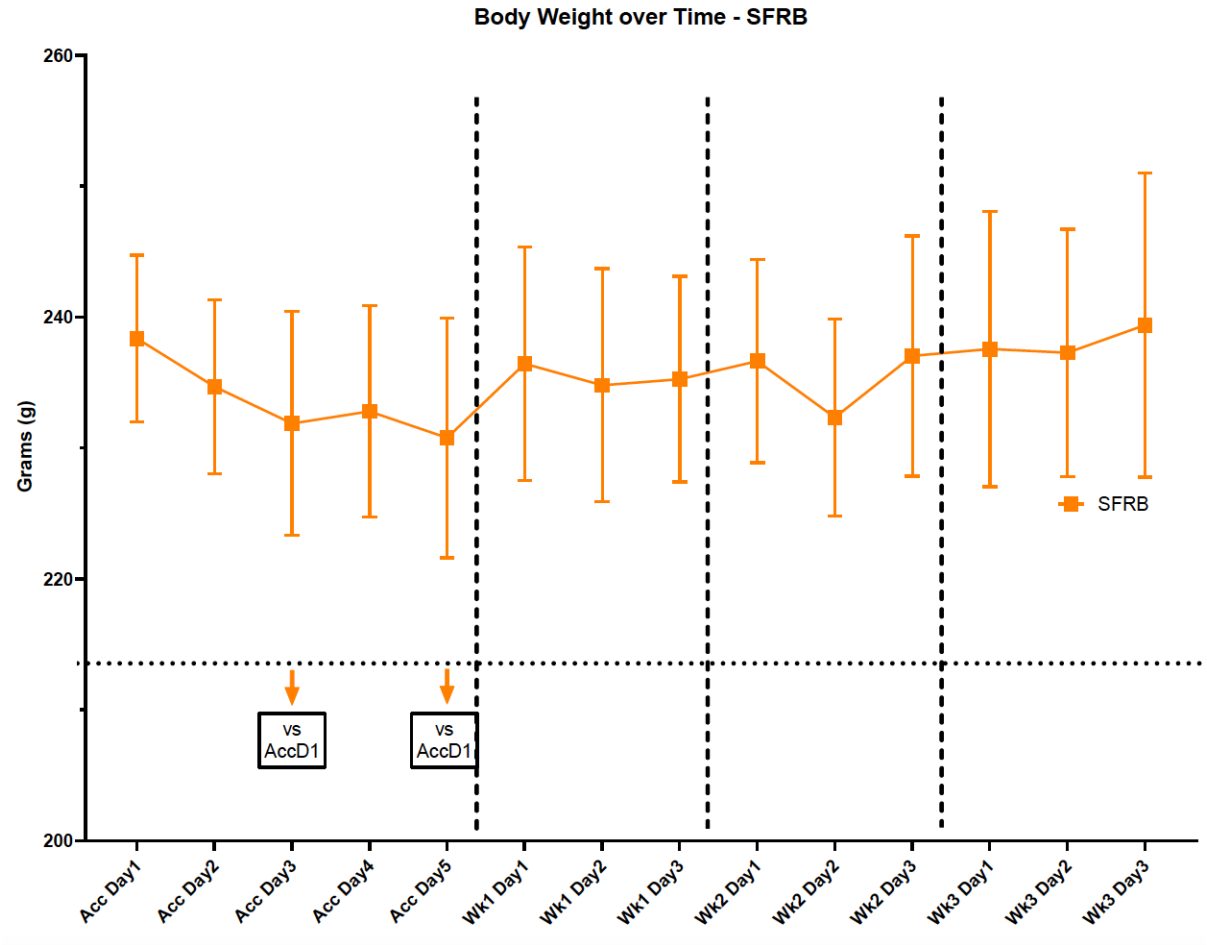

Figure 4.6: Body Weight of Short Frequent Rest Rats over Time in Line Plot. Body weight did not increase after the initial decline.

groups' Achilles tendon cellularity were significantly greater (p=0.0203, p=0.0001, Fig. 5.6A). Collagen organization and cell shape did not differ significantly across the 3 treatment groups (Fig. 5.6B, C) (Table 4.2).

4.3.3 ELISA Analysis of Inflammatory Cytokines, Enzyme and Oxidative Stress Biomarker in Serum

Rest significantly affected serum MDA levels at week 3 (p=0.0268). Long Infrequent Rest rats expressed significantly higher MDA level in comparison with the Control rats (p=0.0208), although no significance was discovered between Control and Short Frequent Rest groups (p=0.2801) or Long Infrequent Rest and Short Frequent Rest (p=0.4030) groups (Fig. 4.10A).

Table 4.1: Adjusted Tissue and Organ Weights at Euthanasia Descriptive Statistics

Heart	Mean	Stdev	n
Control	308.85	17.42	9
Long Infrequent Rest	310.00	31.97	8
Short Frequent Rest	310.51	38.48	8

Achilles	Mean	Stdev	n
Control	623.02	197.91	9
Long Infrequent Rest	603.57	180.51	8
Short Frequent Rest	670.26	243.74	8

Adrenal (Average)	Mean	Stdev	n
Control	105.22	14.62	9
Long Infrequent Rest	106.58	14.38	8
Short Frequent Rest	111.11	13.94	8

Tricep	Mean	Stdev	n
Control	368.28	41.56	9
Long Infrequent Rest	383.68	62.10	8
Short Frequent Rest	402.58	31.49	8

Gastrocnemius	Mean	Stdev	n
Control	580.18	23.81	9
Long Infrequent Rest	556.21	33.27	8
Short Frequent Rest	577.68	59.64	8

Soleus	Mean	Stdev	n
Control	359.47	34.31	9
Long Infrequent Rest	346.61	36.17	8
Short Frequent Rest	373.68	32.30	8

Plataris	Mean	Stdev	n
Control	106.41	5.13	9
Long Infrequent Rest	105.44	8.40	8
Short Frequent Rest	112.54	13.03	8

Tibialis Anterior	Mean	Stdev	n
Control	223.96	62.69	9
Long Infrequent Rest	186.80	10.82	8
Short Frequent Rest	224.22	43.03	8

Table 4.2: Tendon Score Descriptive Statistics

Cellularity: Achilles Tendon	Mean	Stdev	n
Control	76.00	22.00	7
Long Infrequent Rest	113.00	13.00	6
Short Frequent Rest	140.00	27.00	7

Collagen Organization: Achilles Tendon	Mean	Stdev	n
Control	2.00	1.00	7
Long Infrequent Rest	2.10	0.69	7
Short Frequent Rest	2.20	0.97	9

Cell Shape: Achilles Tendon	Mean	Stdev	n
Control	2.00	1.00	7
Long Infrequent Rest	1.70	0.76	7
Short Frequent Rest	2.30	0.87	9

Rest did not affect serum CK or TNF-alpha levels at week 3 among the 3 treatment groups (p=0.2442, p=0.2042). None of the pair-wise comparisons for CK and TNF-alpha among the 3 treatment groups was statistically significant (Fig. 4.10B, C) (Table 4.3).

4.3.4 Discussion

We show here for the first time in a chronic forced treadmill running model of WMSDs that work-rest scheduling had quantifiable effects on subjects' physiological adaptations, systemic

Table 4.3: Serum Cytokine Levels at Euthanasia Descriptive Statistics

MDA	Mean	Stdev	n
Control	48.00	7.50	9
Long Infrequent Rest	66.00	16.00	8
Short Frequent Rest	58.00	13.00	8

CK	Mean	Stdev	n
Control	1230.00	107.00	9
Long Infrequent Rest	1082.00	235.00	8
Short Frequent Rest	1166.00	169.00	8

TNF-alpha	Mean	Stdev	n
Control	6.40	4.20	9
Long Infrequent Rest	5.40	4.70	8
Short Frequent Rest	2.60	4.20	8

stress levels and histomorphological changes. The equivalent total workload and cumulative rest time provided ground for quantifications of comparisons of the above-mentioned measures. The duration of the experiment allowed observation and measurement of effects of chronic injuries to musculoskeletal tissues.

The findings of this study and its future follow-up studies contribute to further understandings of tissue damage per work cycle and recovery rate, and contribute to a more accurate interpretation and prediction of how and when (ex. under what loading levels and after how many repetitions) tissue trauma would occur. This study is meant to contribute to NORA strategic goals across manufacturing, healthcare and social assistance, agriculture, forestry and fishing, construction, mining and public safety in the realm of reducing of incidence and prevalence of MSD, developing interventions, and controlling MSD risk factors (*National Occupational Research Agenda (NORA): Agriculture, Forestry, and Fishing Agenda*, 2008; *National Occupational Research Agenda (NORA): Construction Agenda*, 2008; Marras, Cutlip, Burt, & Waters, 2009; *National Occupational Research Agenda (NORA): Manufacturing Sector Agenda*, 2010; *National Occupational Research Agenda (NORA): Healthcare and Social Assistance Agenda*, 2013; *National Occupational Research Agenda (NORA): Public Safety Agenda*, 2013; *National Occupational Research Agenda (NORA): Mining Agenda*, 2015).

We observed negative physiological adaptations, such as decrease and stagnancy in body weight among the working groups in comparison with the Control group and against the normal growth chart despite food provision being *ad libitum*. In the past, reduction of body weight had been used as a gross measure of increased metabolic rate or energy expenditure (Rodnick, Reaven, Haskell, Sims, & Mondon, 1989; Moraska et al., 2000). Long Infrequent Rest groups' body weight increased significantly only on the last day and was significantly lower than the Control groups' on Wk1 Day3, while Short Frequent Rest groups' were stagnant after the decrease during acclimation and did not differ significantly from the other groups on any particular day. Therefore, the task did appear to have caused an energy expenditure imbalance among the working groups. The author speculates that the Long Infrequent Rest group was more adversely affected physiologically by the task near the beginning of the task but was starting to overcome the energy expenditure imbalance, while the energy expenditure deficit that the Short Frequent Rest group was not as expressive during the experiment duration but did not show signs of recovery either.

We hypothesized that the forced downhill treadmill running task would stimulate stress responses in the task groups and would possibly result in different levels of such stress responses in Long Infrequent Rest and Short Frequent Rest groups. In a few previous studies, it was found that forced long-term treadmill running produced chronic mental stress because animals were adversely motivated to run (Moraska et al., 2000; Tekin, Dursun, & Ficicilar, 2008). In this study, evidence was present for task-induced stress. Long Infrequent Rest and Short Frequent Rest rats displayed significant tendon morphology respectively in comparison with the Control ones. Long Infrequent Rest groups' MDA level was significantly higher than the Control group, although no significant difference was found between Short Frequent Rest and Control groups, or Short Frequent Rest and Long Infrequent Rest groups. Neither the Long Infrequent Rest or the Short Frequent Rest group experienced adrenal hypertrophy. A previous study presented supportive evidence that inflammation began earlier than histomorphological tendon changes and these responses were exposure dependent with greater tissue responses over time and with higher task demands (Fedorczyk et al., 2010). MDA is a marker for oxidative stress, a result

of expressive reactive oxygen species (ROS), which is closely related with severity of inflammation (Szczurek & Szygula-Jurkiewicz, 2015). The author interprets the findings as that the Long Infrequent Rest group might have gone under greater oxidative stress than the Short Frequent Rest group although the task adversely affected musculoskeletal health in both groups due to the significant increase of MDA in the Long Infrequent Rest group in comparison with the Control group but not in the Short Frequent Rest group. It is possible that the greater fluctuation in physiological adaptations (body weight over time) that the Long Infrequent Rest group experienced, despite the weight increase towards end of the experiment indicative of adapting to the prescribed regimen and catching up to the normal trend, contributed to the significant oxidative stress.

There are several limitations associated with this study. First, only female subjects and one age group were studied, which eliminated gender and age as confounders, but could be expanded to be more representative to the working population. Second, the HRHF repetitive task rat model has been successfully adopted in the past to demonstrate MSD risks and control in humans (Kietrys, Barr, & Barbe, 2011; Kietrys et al., 2012; H. G. Gao et al., 2012; Massicotte et al., 2015; D. Xin et al., 2017; Bove et al., 2019). However, clinical validation is required. Third, only one task intensity level and duration was explored in this study. Future study could consider sampling a spectrum of task regimens that vary in intensity and duration to establish a dose-response model between regimen and trauma to contribute to musculoskeletal injury prediction.

In conclusion, a novel rat model was built to simulate 2 different work-rest shifts to study long-term eccentric exertion task's physiological, inflammatory, stress and histomorphological effect on musculoskeletal disorder development. Chronic forced downhill treadmill task suppressed normal weight gain in young adult female Sprague-Dawley rats. Task rats experienced weight loss upon arrival and never regained the lost weight to match the normal growth curve. Tendon morphology was observed in both task groups. Increased oxidative stress and significant contrast of weight at the beginning of the task period in comparison with the Control rats before its eventual weight gain indicated greater magnitude of response to the task than the

Short Frequent Rest rats, whose weight stagnated after the initial decrease and whose oxidative stress level did not see a significant increase.

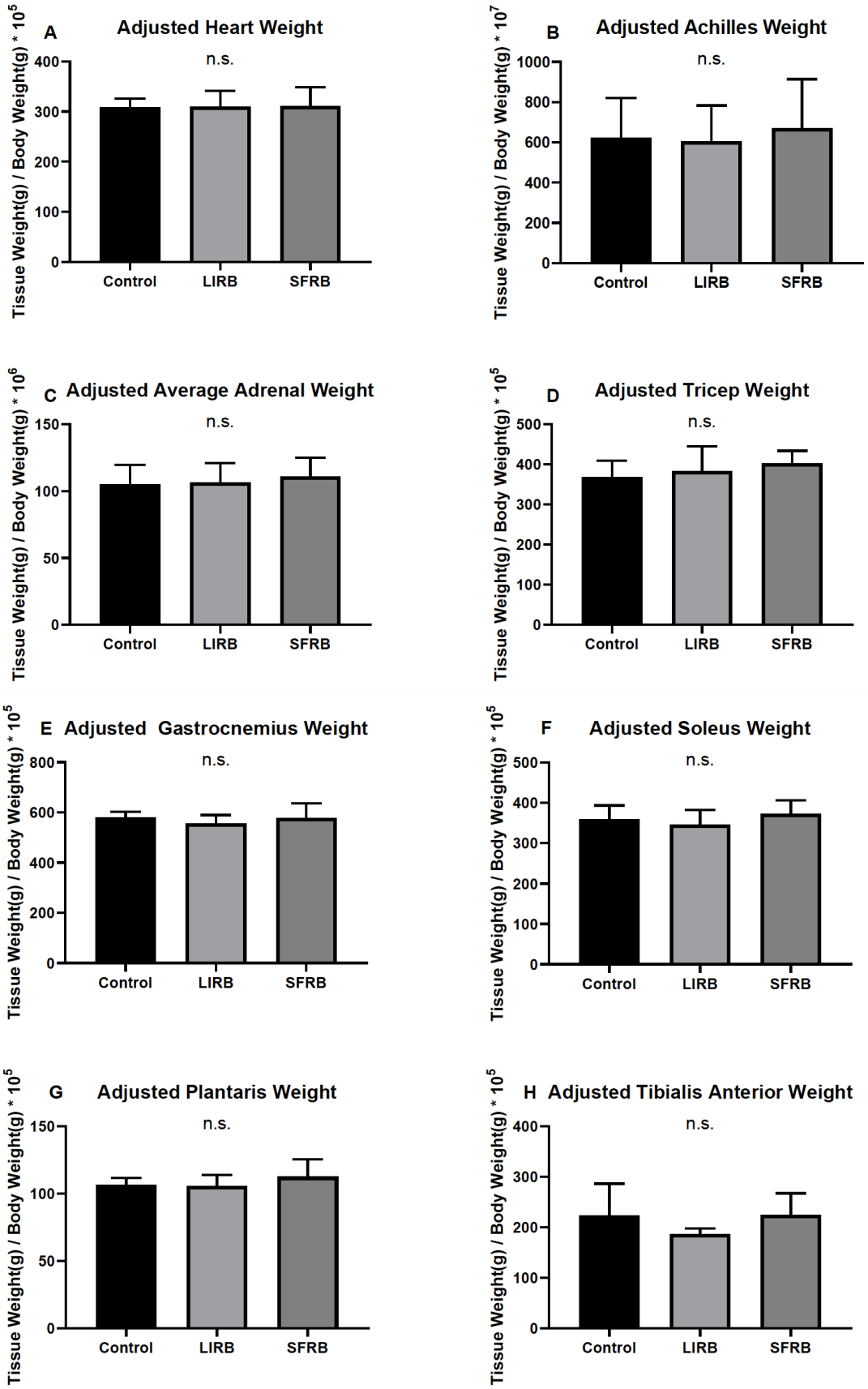

Figure 4.7: Adjusted Tissue and Organ Weights at Euthanasia. Adjusted Tissue Weight = Net Tissue Weight (g) / Body Weight at Euthanasia (g) * 10n. N was decided so that the adjusted weights were in the hundreds. N.s. denotes $p<0.05$ in post-hoc pairwise comparisons.

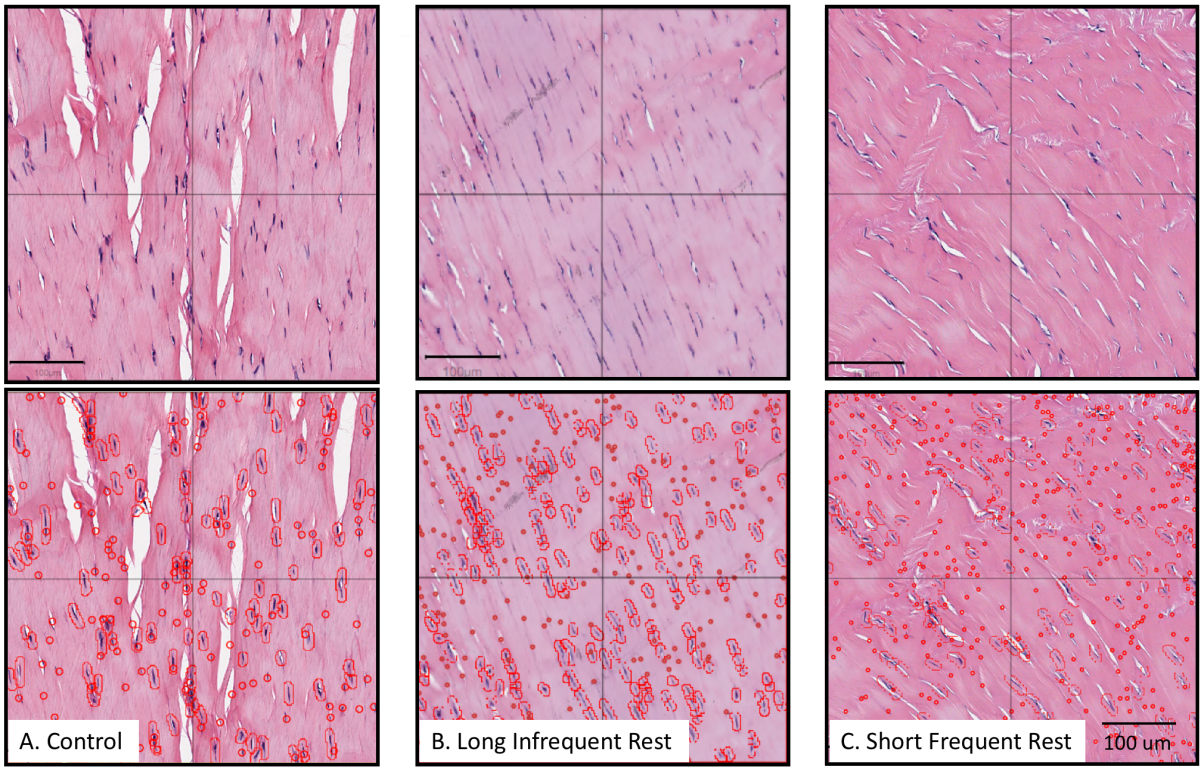

Figure 4.8: Fields of Visions (FOVs) of the longitudinal section of an Achilles Tendon. Achilles tendon logitudinal section images were scanned by a high definition scanner and viewed though image analysis software QuPath. (A) Top image shows 1 of the 3 FOVs chosen for one of the Control Achilles Tendons, comprised of 2 x 2 250μm x 250μm grids. Bottom Image shows auto-detected tenocyte nuclei, marked by fuzzy-bordered circles, and manually detected tenocyte nuclei, marked by circles.

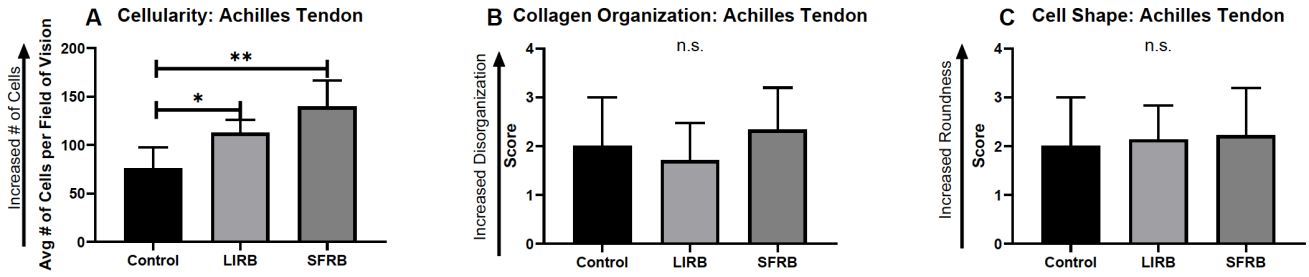

Figure 4.9: Degenerative Change in Longitudinal Sections of The Achilles Tendon. (A) Average number of tenocytes per 250μm x 250μm area across 3 fields of vision (FOV) examined in the top, middle and bottom along the longitudinal axis of the tendon's longitudinal section. (B) Tendon pathology scores for collagen organization. (C) Tendon pathology scores for cellularity. (D-F) H& E stained tendons from the Control, Long Infrequent Rest and Short Frequent Rest groups respectively. Scale bars are 100μm.

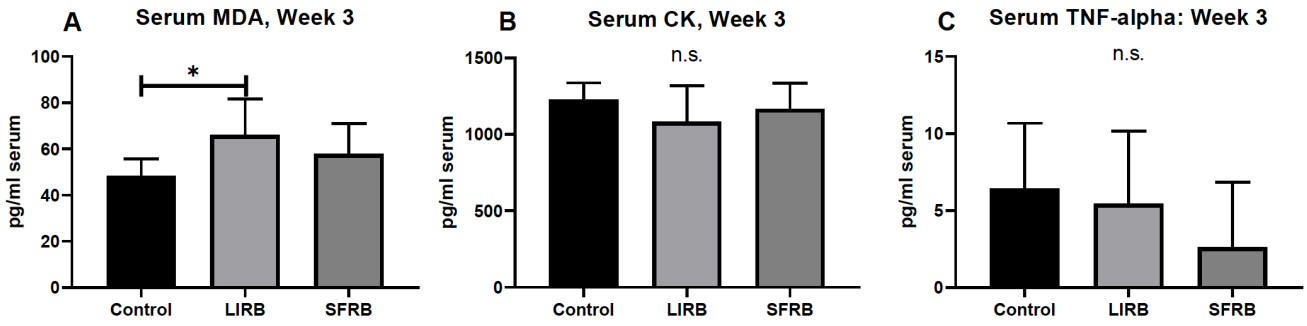

Figure 4.10: Serum Cytokine Levels at Euthanasia. Serum levels of oxidative stress biomarker Malondialdehyde (MDA), skeletal-muscle-injury expressing enzyme Creatine Kinase (CK), and pro-inflammatory cytokine Tumor Necrosis Factor-alpha (TNF-alpha) assayed using multiplex ELISA. Results of one-way ANOVAs for parameter are shown in (A) (B) and (C) respectively, using factor Rest. *: $p<0.05$, compared to age-matched Control rats.

Chapter 5

Impact of Secondary Intervention on Tissue Inflammation and Sensorimotor Behavioral Declines in A Rat Model

5.1 Introduction

Musculoskeletal Disorders (MSDs), also commonly referred to as Cumulative Trauma Disorders (CTDs) and Repetitive Strain Injuries (RSIs) are prevalent at workplaces worldwide. According to the latest report from Bureau of Labor Statistics, musculoskeletal disorders (MSDs) accounted for 31% of all nonfatal occupational injuries and illnesses involving days away from work in 2015 (BLS, 2015). MSDs are a result of one or a combination of risk factors, including physical risk factors such as forceful exertions, repetitive tasks, awkward posture, vibration, heat or chemical exposure, temporal aspects such as work-rest scheduling and work pace, psychosocial risk factors such as low job control, insufficient rest, time pressure, monotonous work, low support from management and coworkers, and individual factors such as age, gender, BMI, smoking and more (Sauter & Moon, 1996). Musculoskeletal Heath has recently become a cross-sector council in the NIOSH National Occupational Research Agenda (NORA); and musculoskeletal disorder prevention has remained a prioritized goal in the Manufacturing Sector.

MSDs' impact are tangible and significant, and may include workers' negative health outcomes including surgery, chronic pain, depression and employers' financial loss, (Yelin, Callahan, & Group, 1995). The current widely accepted biopsychosocial approach in treating MSDs' pain and disability aims at rehabilitation rather than cure, taking into consideration of the complex and dynamic interaction among physiologic, psychologic and social factors of pain

and disability, which is very different than the traditional biomedical reductionistic approach which promised a cure to MSDs by cutting or blocking pain pathways pharmacologically or surgically (Gatchel, 2004). The treatment, prevention and intervention are classified into three levels: primary, secondary and tertiary. Primary care is mostly applicable to acute cases and targets pain relief. Primary prevention represents intervention methods, such as the static or dynamic stretching programs at manufacturing sites, applied to the worker population that is uninjured in order to avoid injury. Secondary care typically takes place within six months after the injury and before the condition turns chronic and is therapy-driven. Secondary intervention, such as job-modification programs, aids patients to return to work as soon as possible. Tertiary care treats the patients with chronic pain and/or disability. Tertiary intervention, such as vocational rehabilitation, include intensive and sometimes individual treatments for a small percentage of patients in order to prevent permanent loss of productivity and ongoing disability (Gatchel, 2004; Tullar et al., 2010; Gatchel & Schultz, 2014; Goldenhar & Stafford, 2015).

There remains a need for definitive understanding of MSD injury and recovery mechanisms at different stages, which are crucial in effective MSD prevention and intervention. The current general understanding of MSD development and progression is that when micro-trauma form in the muscle, connective tissue, and/or bones and joints due to one or more than one risk factors, it either heals and returns to tissue homeostasis through removal of persistent injury stimuli and sufficient rest or progresses to a sub-acute phase in which local inflammatory factors (i.e., cytokines) are released. The sub-acute phase may eventually heal with scar tissue and chronic fibrosis formations or may be exacerbated to a chronic inflammation and/or fibrosis phase and eventually stimulate systemic chronic immune response (L. L. Smith, 2000; H. G. Gao et al., 2013; Fisher et al., 2015).

Cytokines are protein molecules that are released by a wide range of cells from the immune system. They communicate with cells that function in immune response and signal cell movement to inflammation, infection and trauma sites (Zhang & An, 2007). Many cytokines released from skeletal muscles are referred to as myokines, which demonstrate an association between exertion and inflammation (Handschin & Spiegelman, 2008). Cytokines have been used in

both human and animal studies of upper extremity work-related MSDs as serum biomarkers, which aid quantification of pathophysiological responses of systemic inflammation, fibrosis and degeneration in serum, nerves and musculotendinous tissues and identification of different pathological stages (H. G. Gao et al., 2013). Macrophages release cytokines through a series of orchestrated pathways (Arango Duque & Descoteaux, 2014). Neural macrophage infiltration in tissues has been documented to link to imflammation-induced mechanical sensitivity of axons (Bove et al., 2019).

We adapted a previously developed unique rat model of prolonged voluntary repetitive task of high-force-high-repetition lever reaching, grasping and pulling in order to induce exposure-dependent tissue injury and inflammation. It has been hypothesized that systemic and tissue specific inflammation decreases tissue tolerance, and in turn leads to increased tissue damage (Barr & Barbe, 2004; Barbe et al., 2013). Studies have shown that aerobic exercises were effective in recovering motor performance reducing systemic inflammation and pain associated with MSDs. As a potential secondary intervention to the tissue damage incurred by the high-force-high-repetition grasping task, flat treadmill running was introduced to certain treatment groups in order to examine its effect in treating decreases in motor performance due to exposure to several physical MSD risk factors, such as reducing systemic inflammation and pain-related behaviors (Gamble, Boreham, & Stevens, 1993; Petersen & Pedersen, 2005; S. K. Kim, Jung, & Kim, 2008).

We hypothesized that the high repetition high force reaching task had adverse effects in voluntary task performance, pain and dicomfort behavior, systemic inflammation, and tissue histopathology, while rest and treadmill intervention would attenuate such effects. We were interested in comparing the above outcome parameters between the FRC and 10wk HRHF groups, and TRHF and 10wk HRHF groups to evaluate effect of the HRHF task; between the 10wk HRHF and 10wk HRHF+TM groups, and TRHF+Rest and TRHF+Rest+TM groups to evaluate the effect of the treadmill; between TRHF and TRHF+Rest groups to evaluate the effect of rest; and between the FRC and TRHF groups to evaluate the effect of training.

5.2 Methods

Subjects

This experiment was approved by the Institutional Animal Care and Use Committee and was compliant with NIH guidelines for the human care and use of laboratory animals. All animals recruited by the experiment were housed in an AAALAC-accredited animal facility with a 12-h light: dark cycle. Rats had free access to water, were assigned to be group-housed in cages (2 rats per cage) and were provided with environmental enrichment (chew toys and tunnels). All rats were handled at least 3 times per week by the same person to reduce investigator-induced stressors. Female rats were procured to eliminate gender as a potential confounder, also because females are reported to be more prone to work-related MSDs (Finsterer, 2012). All rats were procured at 4-7 weeks of age, housed and handled until they reached young adulthood (2.5 months).

All rats were food restricted to body weights of no less than 10% lower than age-matched normal controls so as to be motivated for the reinforcement provided upon a successful completion of the prescribed lever pulling task. All rats received grain-based Purina rat chow daily, in addition to food pellet rewards provided during the food reward task. A 1: 1 mix of purified grain and banana 34 mg flavored pellets was used (Bioserve, NJ, USA). Control rats that did not perform the task were provided similar amounts of food reward pellets as task rats. Rats were inspected weekly and postmortem for illnesses and tumors that could contribute to elevation of serum cytokine; none was observed.

A total of 75 young adult female Sprague-Dawley rats were used. Rats were randomly assigned to the following six treatment groups (Table 5.1).

(1) FRC (n=9; food restricted control) rats that went through no training or task performance. They were euthanized at week 10, at matched time points as the 10wk HRHF and 10wk HRHF+TM rats.

(2) TRHF (n=10; trained to High Force) rats that trained only for the first 4 weeks to a high force task with no specific reach rate (TRHF, n=10) and euthanized post training within 36

hours of the last training session.

(3) TRHF+Rest (n=11; trained to High Force then Rested) rats that rested for 10 weeks after training (TRHF+Rest, n=11) and then euthanized at matched time points as the 10wk HRHF and 10wk HRHF+TM rats.

(4) TRHF+Rest+TM (n=10; trained to High Force, Rested, then ran on Treadmill) rats that rested for 4 weeks post training, and that were then treadmill-exercised for 6 weeks. They were euthanized at matched time points as the 10wk HRHF and 10wk HRHF+TM rats.

(5) 10wk HRHF (n=25; trained then performed 10 weeks of High Repetition High Force task) rats that only performed a high repetition, high force task for 10 weeks post training and were euthanized post task.

(6) 10wk HRHF+TM (n=10; trained, performed 10 weeks of High Repetition High Force task, and ran on Treadmill in the last 6 weeks of the 10-week task period) rats that performed the high repetition, high force task for 10 weeks, in parallel with treadmill-exercise in the last 6 weeks of the 10-week task. They were euthanized post task and treadmill-exercise.

Group	Group Name	4 weeks	4 weeks	6 weeks
Food Restricted Control	**FRC (n=9)**	Rest entire experiment		
Trained to HF	**TRHF (n=10)**	Train		
Trained to HR + Rest, but no task	**TRHF+Rest (n=11)**	Train	10wk Rest	
Trained to HF + Treadmill + Rest, but no task	**TRHF+Rest+TM (n=10)**	Train	4wk Rest	6wk Treadmill
Trained to HF + HRHF task	**10wk HRHF (n=25)**	Train	10wk HRHF	
Trained to HF + HRHF task + Treadmill	**10wk HRHF+TM (n=10)**	Train	4wk HRHF	6wk HRHF + Treadmill

Table 5.1: Experiment Plan

Behavioral Apparatus

A total of 16 operant chambers were utilized for these experiments. Larger sound dampening boxes (Med Associates, St. Albans, VT) were integrated with standard open field boxes placed inside with custom-designed force apparati to accommodate the rat's lever pulling task with reinforcement of food pellets. The force lever bar, which task rats were trained to reach and pull on, was a metal bar of 1.5mm in diameter, placed 2.5 cm outside of each operant chamber wall, and at the rat's shoulder height. The lever bar was attached to a miniature tension-compression load cell (Model LSB200, Futek Advanced Sensor Technology, Irvine, CA) connected with a

strain-gauge amplifier (Model CSG110, Futek). The load cell signal low pass filtered at 50 Hz and was sampled digitally at 100 Hz by the Force Lever activity software (ENV-118 M, Product Number SOF-808, Med Associates) that allowed the investigator to select the force level at which the rat exerted in order to receive the food reward. A successful lever-pull by the rat was when the rat recognized the cue provided by the auditory indicator (Med Associates), prompted a reaching attempt and pulled on the lever bar within the desired time frame) and pulled at the target percentage of maximum isometric force within a 500 ms cueing period. If the lever bar was pulled in the correct time frame to the correct grasp force requirement, a reward light would turn on indicating disposal of a 45 mg food pellet (Bioserve, NJ) into a trough at floor height (Barbe et al., 2013; Carp et al., 2007; Massicotte et al., 2015; D. Xin et al., 2017).

Task and Training Regimen

All rats were handled and acclimated every day for 1 week upon arrival. A subset of rats was randomly selected as the food restricted control (FRC, n=9) rats. These FRC rats remained sedentary for 14 weeks post procurement. All other rats were trained to be able to reach and pull a handle at 60% maximum pulling force (60%MPF, 120 gf or 1.176 Newtons) for 10 min/day for 5 days/week for 4 weeks post-procurement (Fig. 5.1). The week immediately post training was denoted as HRHF week 0. A randomly assigned subset of rats, TRHF (n=10), were euthanized immediately post training.

The rest of the rats were randomly assigned to TRHF+Rest, TRHF+Rest+TM, 10wk HRHF, and 10wk HRHF+TM groups (n=11, 10, 25, 10, respectively). The TRHF+Rest and TRHF+Rest+TM groups did not perform the HRHF task in the 10 weeks following the 4-week training period, while the 10wk HRHF and 10wk HRHF+TM groups did. The 2 task groups performed the HRHF task for 2 h/day and 3 days/week for 10 weeks. The daily task was scheduled as four 30-min sessions, separated by 1.5 h in order to prevent satiation. If the rats could meet the HRHF 60% MPF pulling force target for no longer than 0.5 sec and no shorter than

0.1 sec during the required timeframe after the auditory cue was delivered, a food reward would be delivered and the pull was considered a successful one.

The groups that were assigned with treadmill intervention regimen performed flat treadmill running exercises in the last 4 weeks. These rats ran on the treadmill for 1 hour/day, 5 days/week, ramping up to 23 m/min in the last 20 min on each day. The 10wk HRHF+TM group performed the HRHF task for 10 weeks, in addition to performing the forced treadmill exercise program in the last 4 weeks of the task. The TRHF+TM rats did not perform the lever-pulling task; instead, they rested post training for six weeks before then performing the forced treadmill exercise program for four weeks.

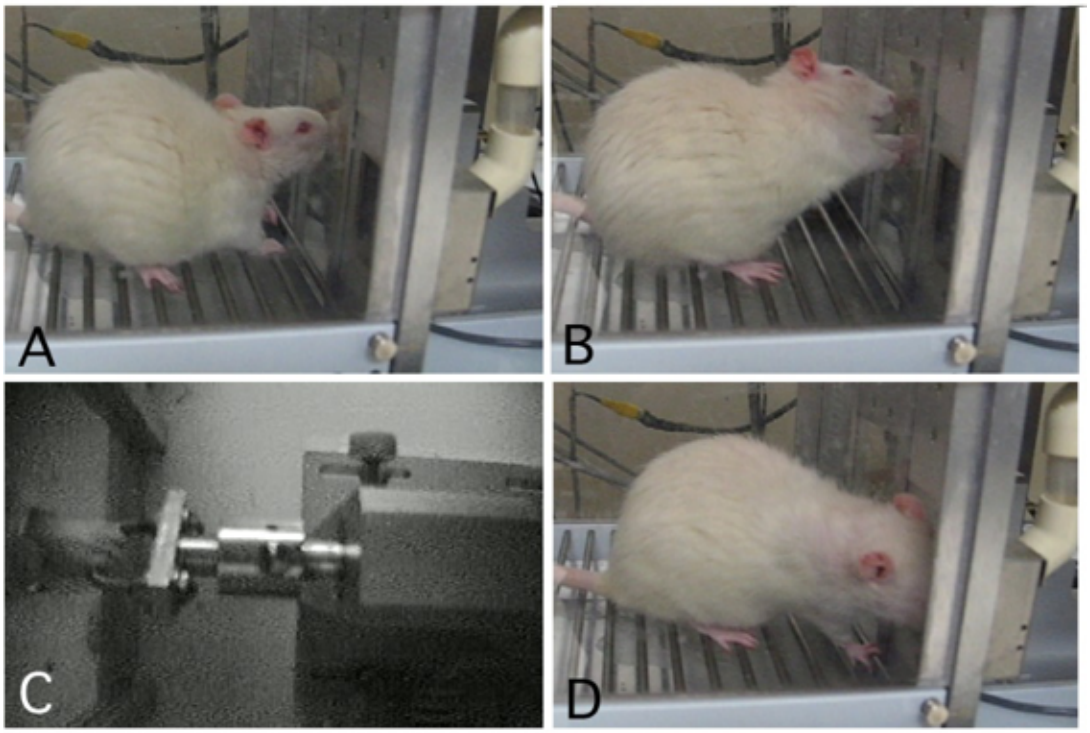

Figure 5.1: Rat Performing The HRHF Repetitive Lever Bar Pulling Task. (A) Rat awaits auditory stimulus with snout in portal. (B) Rat reaches for force lever bar with left forepaw. (C) Rat grasps and isometrically pulls the force lever bar, which is attached to a force transducer, until a force threshold of +/- 60% MPF is reached and held for at least 50 ms. (D) Upon successful achievement of reach force and time criteria, the rat releases bar and retrieves food pellet reward by mouth from a food trough located within the operant chamber.

Determination of Task Performance in Task Rats

Voluntary task performance outcomes, including grasp force, grasp time, reach rate and success rate, were assessed in 10wk HRHF and 10wk HRHF+TM groups at week 10. Grasp force and grasp time were generated from data collected by the force lever computer program during lever pulling activity. This data was collected and recorded continuously through an automated script (MatLab; Mathworks, Natick, MA). Grasp force (in Newtons) was the mean recordable force of all reaches in a given day. Grasp time (in seconds) was the average time the rat spent exerting force on the lever bar over the total number of pulls she performed in that day. Grasp time and grasp force were calculated using the interval which started when a reach was detected on the lever bar and ended when the force fell below 2.5% of the minimum required force, 110 gf (Fig. 5.2) (Barbe et al., 2013, 2018). Success rate was the total number of successful reaches that resulted in a food reward in a day out of all recordable reaches. Reach rate was the average number of total reaches per minute, including partial and full pulls on the lever bar, on a given day.

Reflexive Grip Strength

Reflexive grip strength was measured on both forelimbs of the rats in all groups by holding the rat by its tail, allowing it to grip a rigid horizontal bar, then slowly pulling the tail upward until the rat releases the bar. The bar was connected to a force transducer with digital display and recording unit (Stoelting, Wood Dale, IL). The test was repeated 3 to 5 times on each forelimb. Maximum grip strength was determined by the peak force recorded from the force transducer among these tests. (Massicotte et al., 2015) Reflexive grip strength was measured every week for the FRC rats, at the week immediately post training, TRHF week 0, before euthanasia for the TRHF rats, at week 10 post-training and before euthanasia for the TRHF+Rest, TRHF+Rest+TM, 10wk HRHF and 10wk HRHF+TM rats.

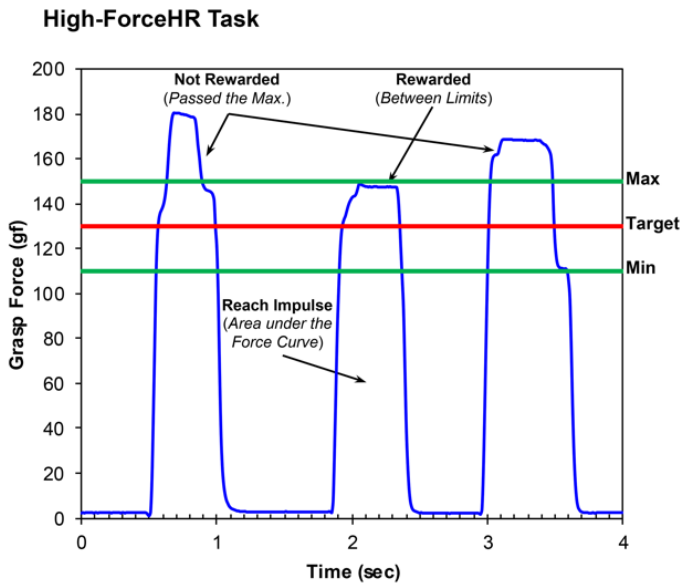

Figure 5.2: Reach Impulse Diagram. Reach impulse is represented as the area under the force curve. Reaches receiving a food reward had to occur between the maximum and minimum force thresholds (upper and lower green lines). The target force is indicated by the red line. Reaches occurring over the maximum force threshold were not rewarded. Used by permission from Massicotte et al., 2018, in which it was Supplemental Figure 1.

Forepaw Mechanical Sensitivity

The "up-down" von Frey testing method was used for forepaw sensory testing. (Deuis, Dvorakova, & Vetter, 2017) Von Frey filaments (North Coast Medical, Morgan Hill, CA) of different diameters were used to elicit a withdraw response to the rats' preferred front paws. The force (in grams) of the smallest-sized filament was recorded as the withdrawal threshold. The same person carried out the von Frey tests for all rats and was blinded to the rats' group assignments.

Median Nerve and Flexor Digitorum Tendon Analyses

Animals were deeply anesthetized with a terminal dose of sodium pentobarbital (120 mg/kg of body weight). Then, serum was collected and centrifuged as previously described (Barbe et al., 2008), and stored at -80 ° C until assayed as described below. Animals were perfused intracardially with 4% paraformaldehyde on phosphate buffer using a perfusion pump, before

collection of forearm tissues for later analyses described below. Flexor digitorum tendons from both forelimbs were removed from the bones, fixed in formalin for a few days, equilibrated in sucrose buffer for 2 days, and cryosectioned into 12-micrometer longitudinal sections.

Subsets of sections were immunostained for CD68, which is a marker of phagocytic tissue macrophages, with antibody anti-CD68 (1:500 dilution in phosphate buffered saline (PBS), Abcam, Massachusetts, United States). After 15 minutes of 0.5% pepsin antigen retrieval at room temperature, sections were incubated for 20 minutes in 4% goat serum in PBS, then incubated with primary antibody at the listed dilution in PBS at 4 ° C overnight, incubated with secondary antibodies, AffiniPure F(ab')2 fragments, conjugated to green or red fluorescent cyanine dyes (Cy2 or Cy3; Jackson ImmunoResearch, West Grove, PA) at a dilution of 1:100 each at room temperature for 2 hours. Then DAPI was used as a nuclear counterstain. Individuals blinded to group assignment quantified the immunostained macrophages with an epifluorescent microscope (E800 Nikon, New York, United States). Images were captured and analyzed using a digital camera (Retiga 4000R QImaging Firewire Camera, British Columbia, Canada) and an image analysis software (Bioquant Image Analysis Corporation, Tennessee, United States). Median nerve was observed at 20X magnification under the microscope. The number of CD68 immunopositive cells were counted inside the epineurium in one field of vision per section of three non-adjacent sections (Bove et al., 2016).

Subsets of the cryosections were mounted onto positively charged slides, stained with hematoxylin and eosin (H& E). The slides were observed under bright-field microscope using 40x magnification. Tendons were scored using a semiquantitative method, the modified Bonar scale (Fedorczyk et al., 2010; L. J. Soslowsky et al., 2002; Cook et al., 2004). Intramuscular and distal tendon cell shape and cellularity were examined and scored respectively using a scale from 0 to 3, with 0 representing normal histological appearance (ex. elongated cell shape and aligned fibers for cell shape, and even distribution of the cells for cellularity) and 3 representing advanced pathological changes (ex. rounded cell shape and wavy fibers for cell shape, and dense, clustered distribution of the cells for cellularity). The person who performed the scoring was blinded to group assignment (Kietrys et al., 2012).

ELISA Analysis of Cytokines, Chemokines and Myokines in Serum

Blood was collected from all rats through cardiac puncture with 23-gauge needles following anesthesia using sodium pentobarbital through intraperitoneal injection at 120 mg/kg body weight, 18 hours after final task and behavioral testing were completed to avoid possible serum cytokine or chemokine fluctuations induced by exercise or acute stress. After collection, blood was immediately centrifuged at 1000g at 4 ° C. Serum was extracted and stored at -80 ° C until use. Custom rat multiplex ELISA kits were used to assay 6 cytokine and chemokines: (1) IL10, an anti-inflammatory cytokine; (2) MIP2, a macrophage and mast cell secreted, wound-healing signaling inflammatory chemokine; (3) IL6, proteic cytokine with both pro-inflammatory and anti-inflammatory properties; (4) TNF-alpha, IL-1alpha, IL-1beta, all pro-inflammatory cytokines. The array sensitivity of the serum analytes was 0.8 pg/ml for IL10, 0.2 pg/ml for CXCL or MIP2, 6 pg/ml for IL6, 3.1 pg/ml for TNF-alpha, 1.5 pg/ml for IL-1alpha and 6.2 pg/ml for IL-1beta (D. L. Xin et al., 2011).

Statistical Analysis

Results are reported as mean and standard deviation (SD). Two main factors assessed were "Rest" and "Treadmill Intervention", although a total of four factors were considered: "Train", "Rest", "Treadmill Intervenion", and "Task" (Fig. 5.3). The main effects that the authors were aiming to assess are the effects of HRHF task, treadmill intervention, rest and training, as well as any interaction among the above-mentioned potential effects. The specific post-hoc pairwise comparisons carried out were: FRC vs 10wk HRHF and TRHF vs 10wk HRHF to assess the effect of the task, 10wk HRHF vs 10wk HRHF+TM and TRHF+Rest vs TRHF+Rest+TM to assess the effect of the treadmill intervention, TRHF no Rest vs TRHF+Rest to assess effect of rest, and FRC vs TRHF no Rest to assess the effect of training.

Unpaired t tests were used to compare voluntary grasp force, grasp time on lever bar, success rate (number of pulls that resulted in a food reward out of all reaches) and reach rate (number of full and partial reaches on the lever bar per minute) at week 10 between the 10wk HRHF and 10wk HRHF+TM groups. Reflexive grip strength, forepaw mechanical sensitivity,

				Treadmill			
				0		1	
				Train	Train	Train	Train
				0	1	0	1
Task	0	Rest	0	FRC	TRHF		
		Rest	1		TRHF+Rest		TRHF+Rest+TM
	1	Rest	0		10wk HRHF		10wk HRHF+TM
		Rest	1				

Figure 5.3: Partial Factorial Experiment Design. The factors considered are "Train", "Rest", "Treadmill Intervention", and "Task", represented by the 4 axes. Each factors has 2 levels, "0" and "1". The 6 treatment groups compared are colored and labeled as they are in Table 5.1.

tendon cellularity and cell shape, as well as serum cytokines levels of all treatment groups at week 10 were analyzed using two-way ANOVA, followed by Tukey post hoc pairwise comparisons. All statistical analyses and data visualization were conducted with aid of GraphPad Prism 8.0.2. For conciseness, figures are grouped into panels. Statistical significance is indicated in figures when p value is below 0.05. Alpha level of 0.05 was selected.

5.3 Results

Voluntary Reach Performance Unscreened by Treadmill Intervention

At week 10, 10wk HRHF+TM group's grasp force was statistically significantly lower than the 10wk HRHF group ($p=0.0046$; Fig. 5.4A). In contrast, grasp time did not statistically differ between the two groups ($p=0.8510$; Fig. 5.4B). The proportion of successful reaches that resulted in a food reward out of all reaches in 10wk HRHF+TM rats was statistically significantly lower than in 10wk HRHF rats ($p<0.0001$; Fig. 5.4C). In contrast, the number

of all reaches registered on the lever bar, including partial and full pulls, was not statistically significantly different between these two groups (p=0.8629; Fig. 5.4D), meaning that all HRHF rat reached over the target reach rate (Table 5.2).

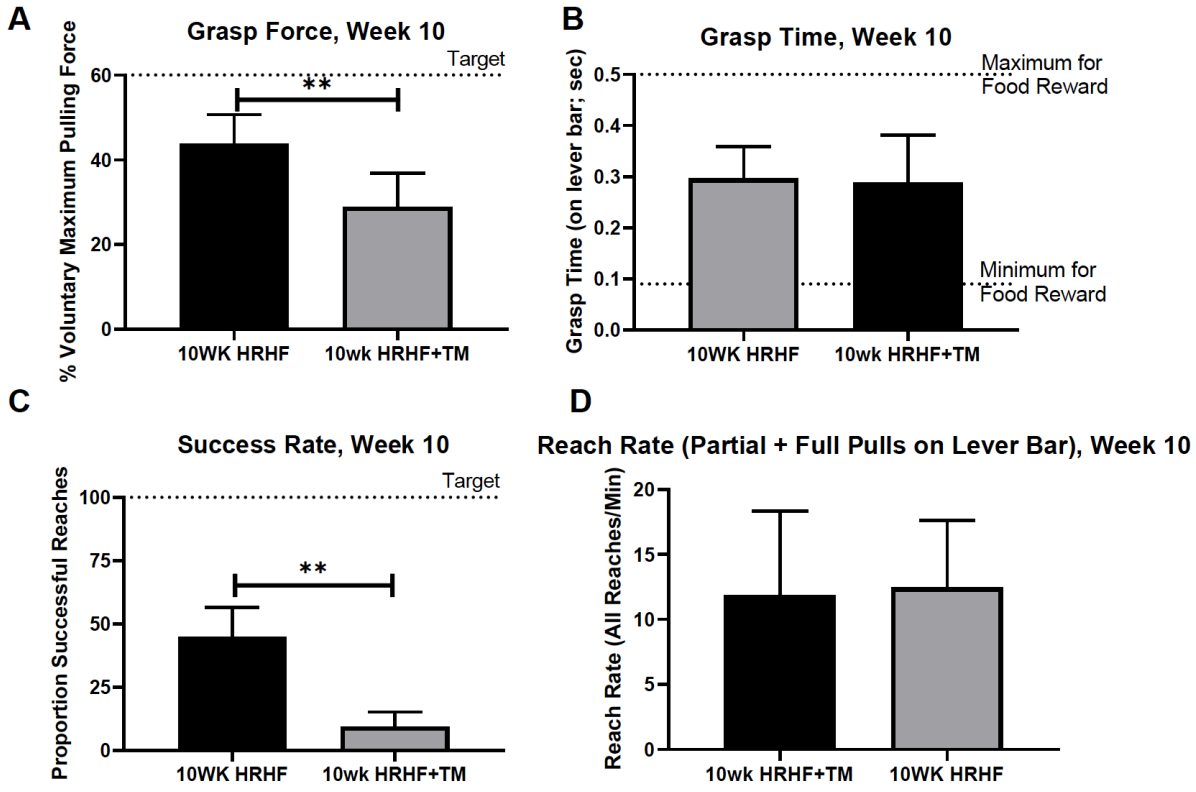

Figure 5.4: Voluntary Task Performance. (A) Grasp force: force exerted by pulling on lever bar (percentage of maximum pulling force [MPF]). (B) Grasp time: time spent grasping and exerting force on the lever bar. (C) Success rate: percentage of successful reaches of all reaches. (D) Reach rate: number of partial and full pulls on lever bar per minute. Mean + SD shown. **: $p<0.01$, between groups as depicted.

Reflexive Grip Strength and Forepaw Mechanical Sensitivity Unscreened by Treadmill Intervention; Forepaw Mechanical Sensitivity Improved with Rest in Trained-only Rats

Reflexive grip strengths were statistically significantly lower in TRHF, 10wk HRHF and 10wk HRHF+TM groups in comparison to the FRC group ($p<0.0001$, $p<0.0001$ and $p<0.0001$, respectively; Fig. 5.5A; Table 5.3). Compared to the TRHF group, grip strength decreased in the TRHF+Rest rats ($p<0.0001$; Fig. 5.5A). Also, grip strength was lower in the 10wk HRHF+TM than in 10wk HRHF rats ($p<0.0001$; Fig. 5.5A).

Table 5.2: Voluntary Task Performance Descriptive Statistics

Grasp Force (g)					
		Treadmill			
		0		1	
		Train	Train	Train	Train
		0	1	0	1
Task 0	Rest 0				
	Rest 1				
Task 1	Rest 0		N=8 Mean=0.30 Stdev=0.06		N=5 Mean=0.29 Stdev=0.09
	Rest 1				

Grasp Time (sec)					
		Treadmill			
		0		1	
		Train	Train	Train	Train
		0	1	0	1
Task 0	Rest 0				
	Rest 1				
Task 1	Rest 0		N=8 Mean=25.00 Stdev=6.50		N=5 Mean=12.00 Stdev=2.70
	Rest 1				

Success Rate (%)					
		Treadmill			
		0		1	
		Train	Train	Train	Train
		0	1	0	1
Task 0	Rest 0				
	Rest 1				
Task 1	Rest 0		N=8 Mean=45.00 Stdev=9.40		N=5 Mean=12.00 Stdev=5.80
	Rest 1				

Reach Rate (%)					
		Treadmill			
		0		1	
		Train	Train	Train	Train
		0	1	0	1
Task 0	Rest 0				
	Rest 1				
Task 1	Rest 0		N=8 Mean=12.00 Stdev=5.20		N=5 Mean=12.00 Stdev=6.40
	Rest 1				

The size of von Frey filament needed to register a forepaw withdrawal response, also called the withdrawal threshold, were significantly smaller in TRHF, 10wk HRHF, and 10wk HRHF+TM groups compared to the FRC group ($p=0.0002$, $p=0.0091$ and $p<0.0001$, respectively; Fig. 5.5B), indicative of a lower withdrawal threshold and thus, increased forepaw mechanical sensitivity. The forepaw withdrawal thresholds were significantly lower in the TRHF and TRHF+Rest+TM groups than in the TRHF+Rest group ($p=0.0001$ and $p=0.0075$, respectively; Fig. 5.5B) (Table 5.3).

Histomorphological Changes

When quantified with the Bonar scale, distal flexor digitorum tendon (FDT)' s cellularity was statistically significantly higher in TRHF and 10wk HRHF groups than in the FRC group ($p=0.0024$, $p=0.0079$, respectively; Fig. 5.6A). The post hoc comparisons conducted among treatment groups in intramuscular FDT's cellularity and cell shape, and distal FDT' s cell shape

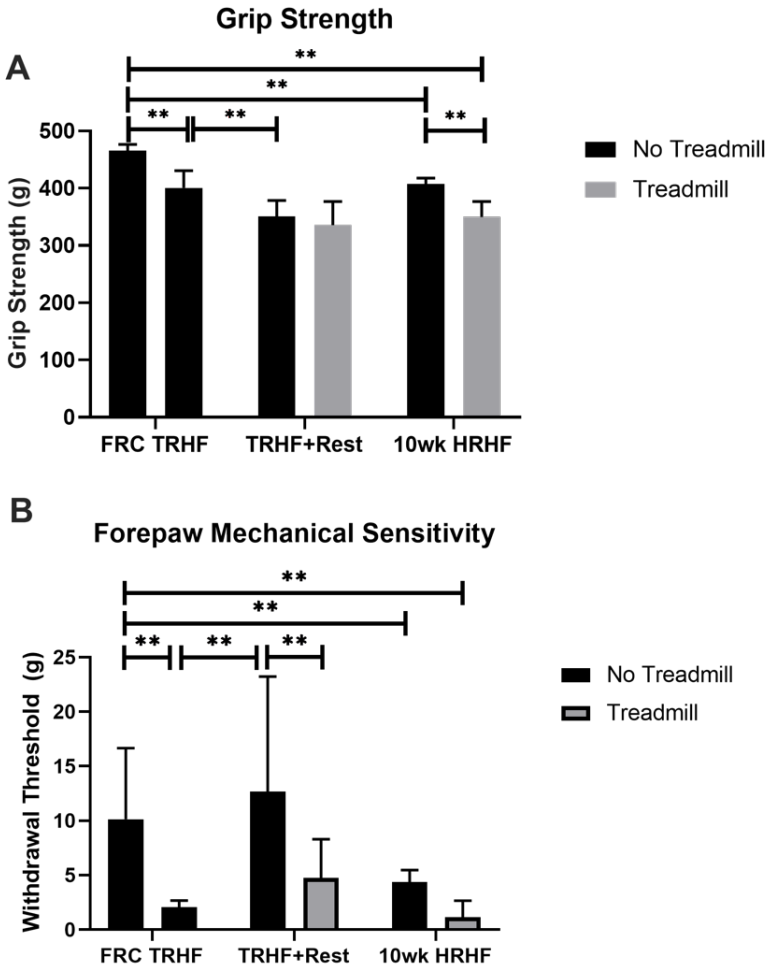

Figure 5.5: (A) Reflexive Grip Strength; (B) Forepaw Mechanical Sensitivity. Mean + SD shown. **: $p<0.01$, between groups as depicted.

did not express statistical significance (Fig. 5.6B, C, D). Microscope images of intramuscular and distal FDT from each treatment group are displayed in Fig. 5.7. The HRHF task had a negative effect on distal tendon cellularity. In contrast, TRHF+Rest, with or without treadmill exercise, showed no increase in tendon cellularity, indicating that rest was an effective treatment. Also, the 10wk HRHF+TM rats did not show increased cellularity, compared to FRC rats, indicating that the treadmill intervention was effective in remedying tendon pathology (Table 5.4).

Table 5.3: Reflexive Grip Strength and Forepaw Mechanical Sensitivity Descriptive Statistics

				Grip Strength (g)			
				Treadmill			
				0		1	
				Train	Train	Train	Train
				0	1	0	1
Task	0	Rest	0	N=87 Mean=10.57 Stdev=465.70	N=14 Mean=400.60 Stdev=30.17		
		Rest	1		N=20 Mean=250.50 Stdev=27.97		N=16 Mean=335.50 Stdev=41.35
	1	Rest	0		N=9 Mean=407.70 Stdev=10.20		N=10 Mean=349.90 Stdev=26.81
		Rest	1				

				Forepaw Withdraw Threshold (g)			
				Treadmill			
				0		1	
				Train	Train	Train	Train
				0	1	0	1
Task	0	Rest	0	N=14 Mean=10.14 Stdev=6.51	N=13 Mean=2.06 Stdev=0.62		
		Rest	1		N=6 Mean=12.67 Stdev=4.75		N=13 Mean=10.56 Stdev=3.55
	1	Rest	0		N=16 Mean=4.38 Stdev=1.09		N=10 Mean=1.14 Stdev=1.51
		Rest	1				

Inflammatory Cytokines in Flexor Digitorum Tendons

Anti-inflammatory cytokine IL10 levels were significantly impacted by treatment group assignment, treadmill, and the interaction of group assignment and treadmill (p¡0.0001, p¡0.0001, p¡0.000). IL10 level was significantly higher in TRHF than TRHF+Rest (p=0.0312), and 10wk HRHF+TM rats, than in FRC and 10wk HRHF rats ($p<0.0001$ and $p<0.0001$, respectively; Fig. 5.8A). Levels of wound healing indicator chemokine MIP2 were significantly impacted by the interaction of group assignment and treadmill (p¡0.0001). MIP2 level was higher in TRHF rats than in TRHF+Rest rats (p=0.0400), and in TRHF+Rest+TM rats, than in TRHF+Rest rats (p=0.0038), and in 10wk HRHF rats than in 10wk HRHF+TM rats (p=0.0476; Fig. 5.8B). Levels of the proteic cytokine IL-6 (has both pro-inflammatory and anti-inflammatory properties) were significantly impacted by treatment group assignment, treadmill, and the interaction of group assignment and treadmill (p=0.0001, p=0.02, p=0.0008). IL-6 level was higher in the TRHF+Rest+TM group than in the TRHF+Rest group (p=0.0004; Fig. 5.8C). Level of

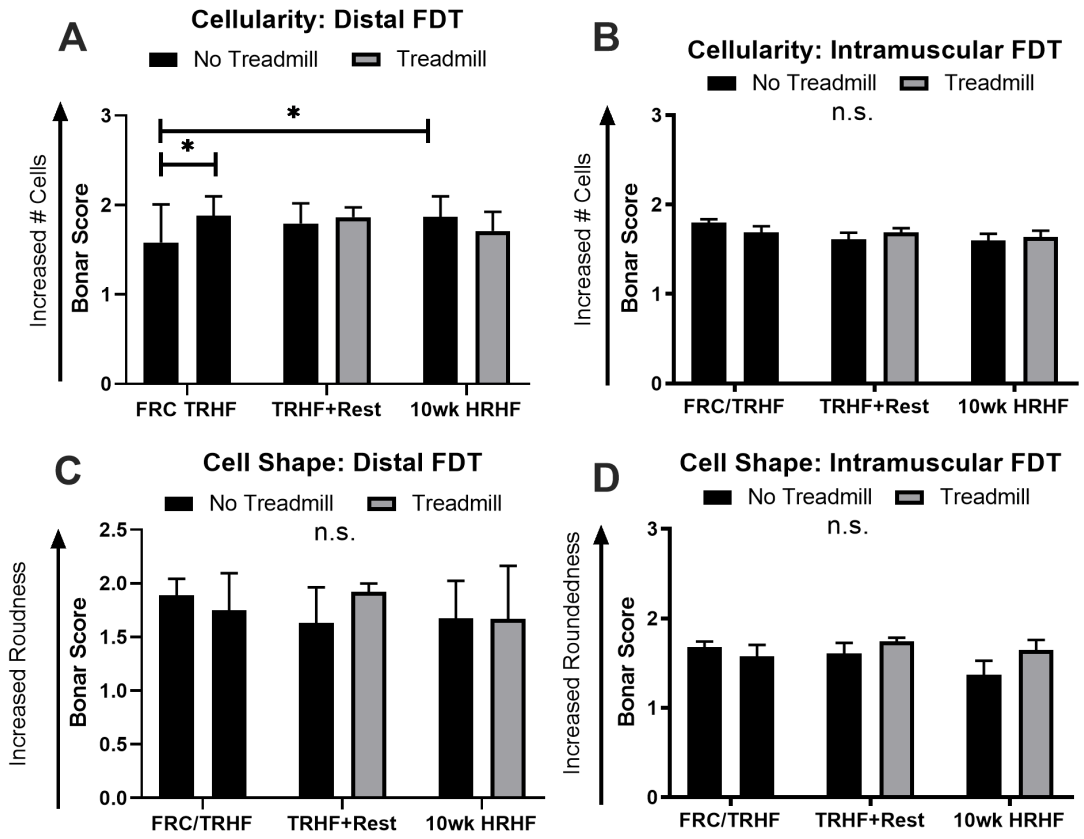

Figure 5.6: Tendon scores. Mean + SD shown. *: p <0.05, between groups as depicted. N.s. = not significant.

pro-inflammatory cytokines, IL-1alpha, were significantly impacted by treatment group assignment, treadmill, and the interaction of group assignment and treadmill (p=0.0241, p=0.0237, p=0.0116). IL-1alpha level was higher in the TRHF group than in the TRHF+Rest group (p=0.0143; Fig. 5.8D). No significant pair-wise difference was observed in IL-1beta and TNF-alpha, alhtough TNF-level was significantly impacted by the interaction of group assignment and treadmill (Fig. 5.8E, F) (Table 5.5).

The median nerve at the level of the wrist was examined for presence of activated macrophages, which are CD68-immunopositive. Significantly increased numbers were observed in TRHF rats ($p<0.05$), as well as in 10wk HRHF and 10wk HRHF+TM rats ($p<0.01$, $p<0.01$), compared to FRC rats only. An increase was also observed in TRHF+Rest+TM rats, although this increase was not significantly different (Fig. 5.9) (Table 5.6).

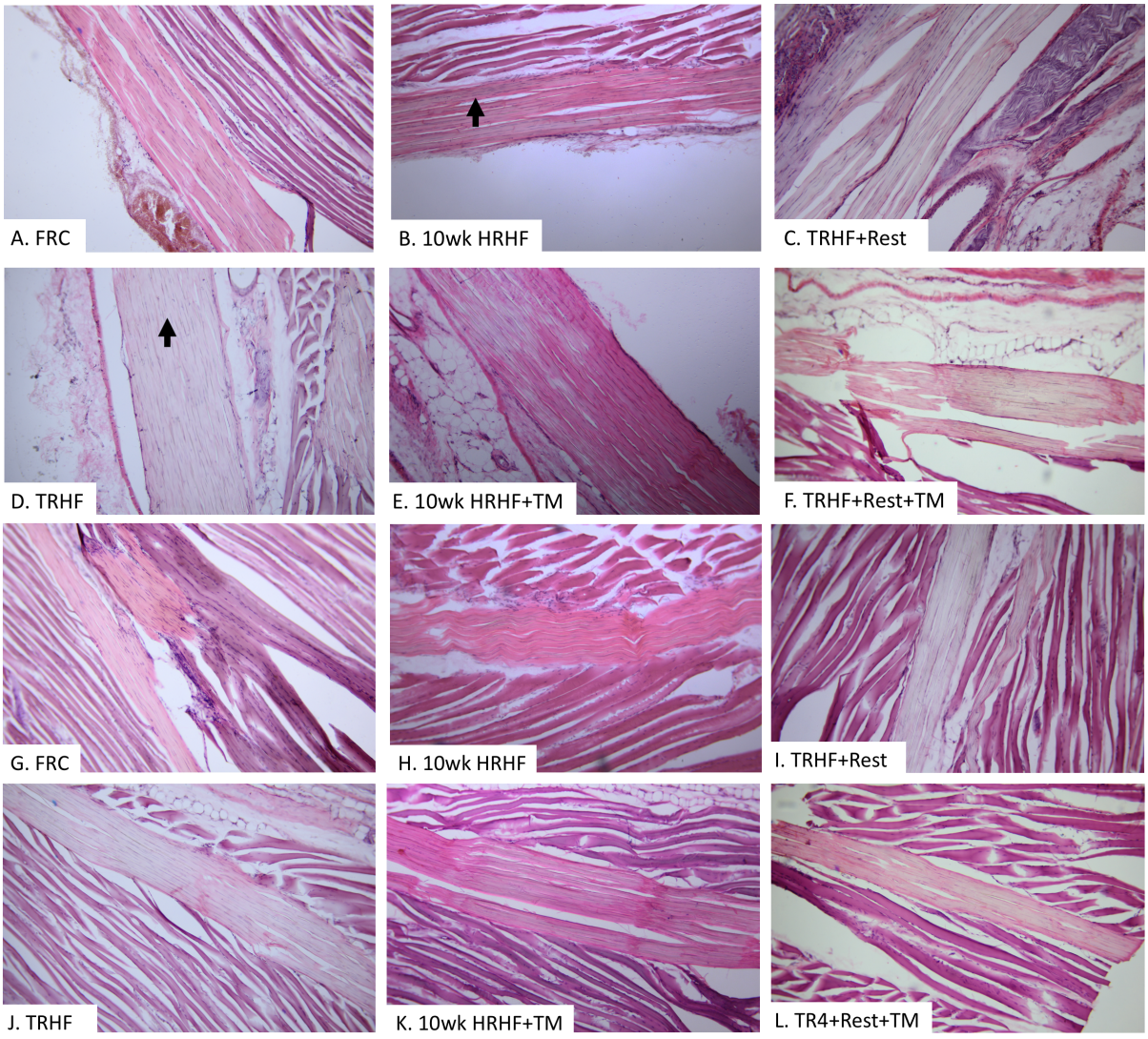

Figure 5.7: Microscope images of representative distal (A-F) and intramuscular (G-L) flexor digitorum tendons from each treatment group. Tendons are stained with hematoxylin and eosin.

Table 5.4: Tendon Scores Descriptive Statistics

				Tendon Cellularity Distal			
				Treadmill			
				0		1	
				Train	Train	Train	Train
				0	1	0	1
Task	0	Rest	0	N=13 Mean=1.58 Stdev=0.42	N=18 Mean=1.88 Stdev=0.22		
		Rest	1		N=11 Mean=1.79 Stdev=0.23		N=11 Mean=1.86 Stdev=0.11
	1	Rest	0		N=12 Mean=1.87 Stdev=0.23		N=7 Mean=1.71 Stdev=0.21
		Rest	1				

				Tendon Cellularity Intramuscular			
				Treadmill			
				0		1	
				Train	Train	Train	Train
				0	1	0	1
Task	0	Rest	0	N=14 Mean=1.80 Stdev=0.14	N=18 Mean=1.69 Stdev=0.29		
		Rest	1		N=12 Mean=1.61 Stdev=0.26		N=11 Mean=1.69 Stdev=0.15
	1	Rest	0		N=12 Mean=1.60 Stdev=0.26		N=8 Mean=1.64 Stdev=0.20
		Rest	1				

				Tendon Cell Shape Distal			
				Treadmill			
				0		1	
				Train	Train	Train	Train
				0	1	0	1
Task	0	Rest	0	N=13 Mean=1.89 Stdev=0.15	N=18 Mean=1.75 Stdev=0.34		
		Rest	1		N=11 Mean=1.63 Stdev=0.33		N=11 Mean=1.92 Stdev=0.08
	1	Rest	0		N=12 Mean=1.68 Stdev=0.35		N=7 Mean=1.67 Stdev=0.50
		Rest	1				

				Tendon Cell Shape Intramuscular			
				Treadmill			
				0		1	
				Train	Train	Train	Train
				0	1	0	1
Task	0	Rest	0	N=14 Mean=1.68 Stdev=0.23	N=18 Mean=1.57 Stdev=0.55		
		Rest	1		N=12 Mean=1.61 Stdev=0.41		N=11 Mean=1.74 Stdev=0.15
	1	Rest	0		N=12 Mean=1.37 Stdev=0.54		N=8 Mean=1.65 Stdev=0.32
		Rest	1				

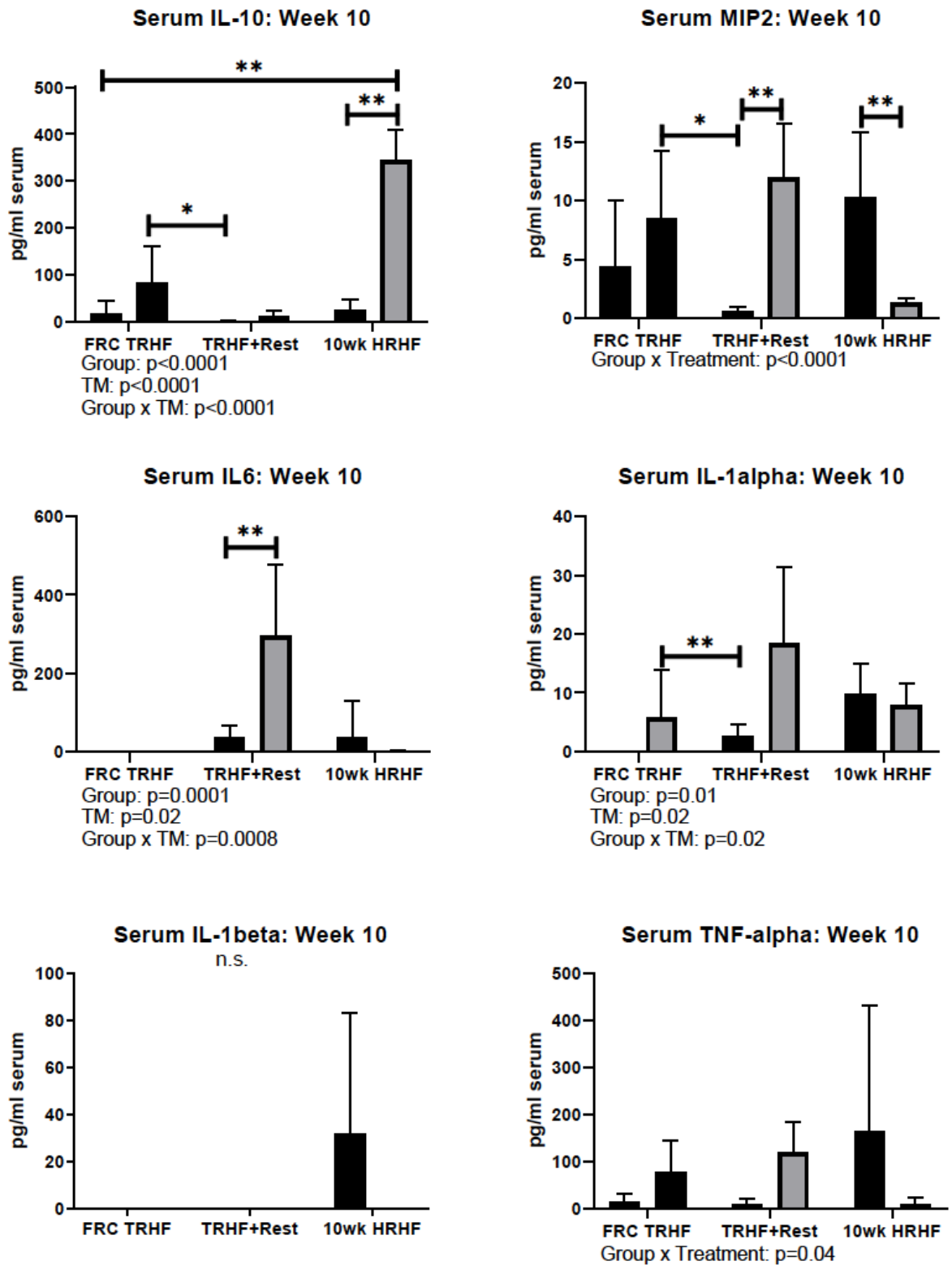

Figure 5.8: Serum Cytokine Levels at Week 10. (A) IL-10; (B) MIP2; (C) IL6; (D) TNF-alpha; (E) IL-1alpha; (F) IL-1beta. Mean + SD shown. *: $p<0.05$, and **: $p<0.01$, between groups as depicted. n.s. = not significant.

Table 5.5: Serum Cytokine Levels at Week 10 Descriptive Statistics

IL10 (pg/ml)						
			Treadmill			
			0		1	
			Train	Train	Train	Train
			0	1	0	1
Task	0	Rest 0	N=4 Mean=17.84 Stdev=26.54	N=11 Mean=83.37 Stdev=77.67		
		Rest 1		N=6 Mean=0.67 Stdev=1.63		N=7 Mean=11.54 Stdev=12.83
	1	Rest 0		N=5 Mean=23.58 Stdev=21.97		N=4 Mean=344.40 Stdev=66.34
		Rest 1				

MIP2 (pg/ml)						
			Treadmill			
			0		1	
			Train	Train	Train	Train
			0	1	0	1
Task	0	Rest 0	N=6 Mean=4.38 Stdev=5.63	N=13 Mean=8.54 Stdev=5.69		
		Rest 1		N=5 Mean=0.60 Stdev=0.37		N=7 Mean=11.98 Stdev=4.52
	1	Rest 0		N=8 Mean=10.32 Stdev=5.44		N=4 Mean=1.30 Stdev=0.42
		Rest 1				

IL6 (pg/ml)						
			Treadmill			
			0		1	
			Train	Train	Train	Train
			0	1	0	1
Task	0	Rest 0	N=7 Mean=0.00 Stdev=0.00	N=7 Mean=0.00 Stdev=0.00		
		Rest 1		N=5 Mean=36.48 Stdev=28.93		N=7 Mean=296.04 Stdev=182.69
	1	Rest 0		N=7 Mean=35.71 Stdev=94.49		N=4 Mean=1.25 Stdev=2.50
		Rest 1				

TNF-alpha (pg/ml)						
			Treadmill			
			0		1	
			Train	Train	Train	Train
			0	1	0	1
Task	0	Rest 0	N=6 Mean=13.52 Stdev=17.30	N=10 Mean=77.49 Stdev=68.08		
		Rest 1		N=6 Mean=9.17 Stdev=10.75		N=7 Mean=120.09 Stdev=65.28
	1	Rest 0		N=8 Mean=163.75 Stdev=267.89		N=4 Mean=9.65 Stdev=12.79
		Rest 1				

IL1alpha (pg/ml)						
			Treadmill			
			0		1	
			Train	Train	Train	Train
			0	1	0	1
Task	0	Rest 0	N=7 Mean=1.71 Stdev=4.52	N=11 Mean=5.73 Stdev=8.21		
		Rest 1		N=6 Mean=2.68 Stdev=2.02		N=7 Mean=18.34 Stdev=13.09
	1	Rest 0		N=8 Mean=21.36 Stdev=32.92		N=4 Mean=7.80 Stdev=3.89
		Rest 1				

IL1beta (pg/ml)						
			Treadmill			
			0		1	
			Train	Train	Train	Train
			0	1	0	1
Task	0	Rest 0	N=7 Mean=0.00 Stdev=0.00	N=12 Mean=0.00 Stdev=0.00		
		Rest 1		N=6 Mean=0.00 Stdev=0.00		N=7 Mean=0.00 Stdev=0.00
	1	Rest 0		N=8 Mean=31.85 Stdev=51.37		N=4 Mean=0.00 Stdev=0.00
		Rest 1				

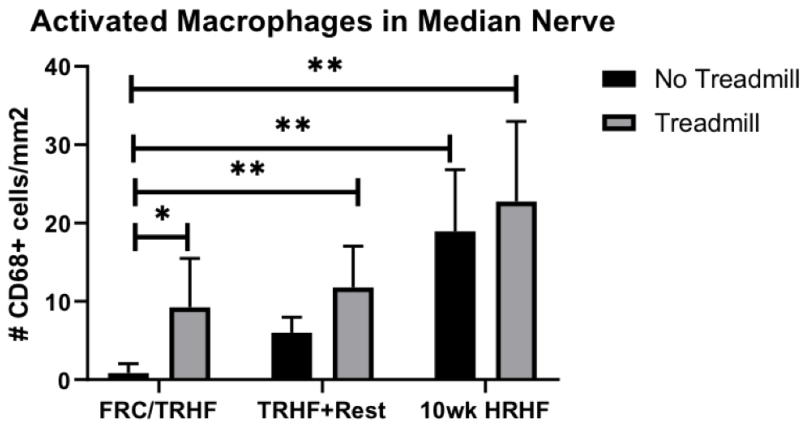

Figure 5.9: Number of Activated Macrophages (CD68-immunopositive) in Median Nerve at Level of The Wrist at Week 10. Mean + SD shown. *p<0.05 and **p<0.01, compared to groups as depicted.

Table 5.6: Number of Activated Macrophages in Median Nerve at Level of The Wrist at Week 10 Descriptive Statistics

				Activated Macrophages in Median Nerve			
				Treadmill			
				0		1	
				Train	Train	Train	Train
				0	1	0	1
Task	0	Rest	0	N=10 Mean=0.83 Stdev=1.23	N=10 Mean=9.23 Stdev=6.25		
		Rest	1		N=5 Mean=6.01 Stdev=1.98		N=5 Mean=11.77 Stdev=5.29
	1	Rest	0		N=5 Mean=18.96 Stdev=7.87		N=5 Mean=22.74 Stdev=10.25
		Rest	1				

5.4 Discussion

We hypothesized that rest and treadmill intervention might improve task performance, attenuate pain and discomfort behavior, reduce systemic inflammation and improve tissue histomorphology. We were interested in comparing the above outcome parameters between the FRC and 10wk HRHF groups, and TRHF and 10wk HRHF groups to evaluate effect of the HRHF task; between the 10wk HRHF and 10wk HRHF+TM groups, and TRHF+Rest and TRHF+Rest+TM groups to evaluate the effect of the treadmill; between TRHF and TRHF+Rest groups to evaluate the effect of rest; and between the FRC and TRHF groups to evaluate the effect of training.

Voluntary grasp force and success rate did not improve, but in contrast decreased, among task rats that were assigned with treadmill intervention in comparison with task rats that were not. Both 10wk HRHF and 10wk HRHF+TM groups' grip strength decreased significantly in comparison with the FRC rats. The 10wk HRHF rats' reflexive grip strength decreased more significantly in comparison with the treadmill intervention task rats. The forepaw mechanical sensitivity increased in both 10wk HRHF and 10wk HRHF+TM groups compared to the FRC group, although no significance was observed when comparing the 10wk HRHF and 10wk HRHF+TM groups. Therefore, the HRHF task did have an adverse effect on rats' voluntary task performance, grip strength and forepaw mechanical sensitivity, yet treadmill intervention did not seem to remedy such effect.

Training induced nerve inflammation and pain, indicated by the significantly elevated number of activated macrophages in median nerve in TRHF group in comparison with FRC. The adverse training effect appeared to be exacerbated instead of remedied by treadmill running, leading to significant increase of macrophages in median nerve in TRHF+Rest+TM rats. The nerve inflammation and pain did not improve in 10wk HRHF rats, and were worse in 10wk HRHF+TM rats, indicating that the treadmill intervention did not serve in reducing nerve inflammation and pain, which confirmed the findings from the other outcome measures, such as voluntary task performance, reflexive grip strength, forepaw mechanical sensitivity, and systemic inflammation levels (Fig. 5.9).

The significant deficit in both voluntary grasp force and in proportion of successful reaches in the 10wk HRHF+TM group were indications of pain and discomfort (Fig. 5.7). Reflexive grip strength significantly decreased post-training and did not recover with rest, perhaps because the animals are guarding the injured limb and muscle is atrophying. Although not statistically significant, 10wk HRHF rats had regained some grip strength compared to the TRHF rats due to likely muscle strengthening with the task, yet still lower than FRC rats. The 10wk HRHF+TM rats had significant grip strength deficit in comparison with the 10wk HRHF rats, indicative of treadmill intervention not able to remedy the pain and discomfort induced by task in working rats (Fig. 5.5A).

Forepaw mechanical sensitivity, indicative of mechanical allodynia (Elliott et al., 2009), significantly increased post-training, was attenuated by rest, but appeared to be exacerbated by treadmill running in the TRHF+Rest+TM group. 10wk HRHF rats' mechanical sensitivity recovered some from training (in comparison with TRHF, although not statistically significant), possibly due to muscle strengthening by performing the task, but not back to the pre-injury state. Intended treadmill intervention was not effective in reducing but contributed to an increase in the mechanical sensitivity in the working rats (Fig. 5.5B).

Levels of quite a few inflammatory cytokines (TNF-alpha, IL1alpha, IL1beta and IL6) and chemokine MIP2 decreased with treadmill running, which is in line with previous findings (Chen, Chiu, Hsieh, Hung, & Wang, 2015). Levels of MIP2 and IL6 in the TRHF+Rest+TM group were significantly elevated than the TRHF+Rest group. Without task, it is indicative that the TRHF+Rest+TM group' s inflammation level increased due to muscle activities associated with treadmill running. The decrease of MIP2, IL6 and a few other pro-inflammatory cytokines in 10wk HRHF+TM in comparison with 10wk HRHF rats might be explained by the significant increase of IL10, an anti-inflammatory cytokine that inhibits production of certain pro-inflammatory cytokines (Fig. 5.8) (Wang, Wu, Siegel, Egan, & Billah, 1995).

Increased activated macrophages in nerve were immune responses of nerve injury and abundant sources of cytokines (Richner et al., 2018). The possible reasons for treadmill intervention not effective in reducing nerve inflammation and pain are demyelination (Bove et

al., 2019), local nerve injury induced blood-nerve-barrier imbalance (Richner et al., 2018), increased ectopic nerve firing (Campbell & Meyer, 2006), and decreased compound nerve velocity (Clark, Al-Shatti, Barr, Amin, & Barbe, 2004). It was strongly indicated in previous similar rat HRHF reaching models that the HRHF task induced nerve injury and dysfunction (Elliott et al., 2009; Bove et al., 2019).

Rest appeared to reverse the pathological changes in TRHF rats' tendons (i.e., TRHF+Rest) and to improve forepaw mechanical sensitivity in TRHF+Rest rats through von Frey tests and the decrease of serum IL-1alpha and MIP2 levels in the TRHF+Rest rats in comparison with TRHF-only rats, although reflexive grip strength decreased in the TRHF+Rest group compared with TRHF.

There are several limitations associated with this study. More investigation and validation are required to relate the study' s results to human applications. Despite the similarity between rat upper and lower extremity anatomy with the human' s in comparison with many other animals, the difference in biomechanics of quadrupeds and humans in treadmill running require more clinical validation (Longo, Forriol, Campi, Maffulli, & Denaro, 2011). Front limbs of the rats were used in both the HRHF task and in treadmill running, therefore making it more difficult to distinguish the possible recovery and/exacerbation associated with treadmill in remedying the trauma induced by the HRHF task. Treadmill interventions with different levels of intensity, including considerations of speed and duration, need to be studied in order to observe whether there is a range of treadmill intervention intensity that not only reduces pain and discomfort but also is remedial to task performance. The authors also plan on examining muscle sections for possible morphological changes indicative of injury.

In conclusion, the HRHF task negatively affected rats' grip strength, caused increased mechanical sensitivity, and provoked tendon's morphological changes. Rest attenuated the mechanical sensitivity and systemic chemokine levels, remedied tendons pathological changes, yet did not improve the rats' grip strength. Treadmill running did not remedy but worsened the grip strength loss and task performance decline due to task, did not make a difference in pro-inflammatory levels or tendon morphological changes but were associated with an increase

in the anti-inflammatory cytokine, which indicated that the treadmill intervention might have decreased rats pain sensations due to mechanical allodynia.

Chapter 6

Conclusion

This dissertation had the overall aim of furthering the understanding of the impact of rest and secondary intervention on development of and recovery from musculoskeletal disorders. Efforts were made to validate epidemiological speculations of insufficient rest's negative psychosocial impact on MSD development and the remedial benefits of secondary intervention to MSD recovery. Three specific aims were established and addressed through the completion of three studies.

Specific Aim 1: Investigate long and short between-work rest breaks' impact on muscle micro-trauma development.

Specific Aim 2: Develop a forced downhill treadmill running rat model in order to measure long and short between-work rest breaks' different impacts on Achilles tendon morphology, voluntary task performance, reflexive strength and pain behavior, and systemic inflammation induced by chronic repetitive and forceful exertions.

Specific Aim 3: Examine the efficacy of commonly applauded secondary intervention methods for MSDs, treadmill running and rest, through a voluntary high repetition high force lever-reaching and pulling task for rats.

The first specific aim was addressed by the first study in Chapter Three. It was the first time that impact of rest was quantified in the context of work-rest scheduling by assessing muscle micro-trauma of different loading and rest regimens when total workload, work time and rest

time were equal. Young adult male human subjects were randomly assigned with one of the four repetitive eccentric task regimens: High Load High Rest, High Load Low Rest, Low Load High Rest and Low Load Low Rest. Serum Creatine Kinase levels, a biomarker for muscle damage, was measured before task, on days 1, 2, 4 and 8 post-task. Effect of Rest, Loading and Day were assessed by two-way ANOVA with repeated measures. Rest and Day, Load and Day, as well as Rest, Load and Day all have significant impact on muscle micro-trauma development.

The second specific aim was addressed by the second study in Chapter Four. It was the first time that a forced downhill running rat model was created to assess impact of rest scheduling in work-rest cycles of repetitive tasks by measuring physiological adaptation, stress expressions, systemic inflammations, and tendon histopathology. Effect of rest was analyzed by one-way ANOVA on the above-mentioned outcome measures. Young adult female Sprague-Dawley rats were randomly assigned to perform a moderately intense downhill daily running task over 4 weeks with Long Infrequent or Short Frequent between-task rest breaks. Total workload, work time and rest time were equivalent across groups. The task rats with different rest schedules expressed significant difference in physiological adaptations and stress responses. Rats that followed long but infrequent rest scheduling experienced greater body weight gain and loss as well as oxidative stress fluctuations in comparison with ones that followed short but frequent rest scheduling, although both working groups experienced significant deficit in body weight during the experiment period as well as significant pathological changes in the Achilles tendon.

The third specific aim was addressed by the third study in Chapter Five. Remedial effect of secondary intervention of flat treadmill running and rest on upper extremity musculoskeletal disorders were both assessed using a custom-created lever-reaching and pulling operant chamber for rats. Young adult female Sprague-Dawley rats were randomly assigned to be trained to perform a high force high repetition (HRHF) reaching and pulling task at targeted force level and rate of repetition with or without treadmill intervention or rest. Effect of Rest and Treadmill were assessed by two-way ANOVA on the following outcome measures: reflexive grip strength, pain behavior, systemic inflammation, and tendon histopathology. Effect of Treadmill

on voluntary task performance was assessed between 10wk HRHF vs 10wk HRHF+TM groups using t test. Rest attenuated the mechanical sensitivity and systemic inflammation levels, and remedied tendon's pathological changes from the significant adverse effect of the HRHF task, yet did not improve the rats' grip strength. Treadmill running did not improve voluntary task performance or reflexive grip strength, likely due to nerve inflammation and injury induced by the HRHF task. Such adverse effect was not remedied by rest and was exacerbated by the treadmill running. Mechanical allodynia was attenuated by rest but significantly increased with effect of task, and did not improve with treadmill running.

Overall, rest was found to be remedial to musculoskeletal injuries incurred by moderate to high intensity repetitive work. Specifically, long infrequent rest breaks between eccentric exertion bouts might have more suppressive effect on MSDs than short frequent rest breaks when equivalent workload, total duration of work and rest were subscribed. Flat treadmill running might have mixed effects on MSD control as a secondary intervention method. While it did appear to have attenuated tendon histopathological changes induced by high force high repetition tasks, several other outcome measures indicated that it did not serve to improve task performance, grip strength, mechanical sensitivity and nerve inflammation. These findings have practical industrial and clinical meanings and may serve in creation and improvement of future work design and scheduling guidelines, as well as MSD diagnosis and rehabilitation.

It was identified as a future musculoskeletal health research need of National Occupational Research Agenda (NORA) to quantitatively link epidemiological, biomechanical loading, soft tissue tolerance and psychosocial studies (Marras et al., 2009). The efforts made in this dissertation in improving the understanding of rest and secondary intervention were intended to contribute in this direction. There is a need for future studies to design methods in describing dynamic work (work-rest cycles)'s effects on development of localized muscle fatigue, as specific pathophysiological relationships between fatigue incurred by dynamic work with intermittent rest breaks and injury are not well understood(Nussbaum, Clark, Lanza, & Rice, 2001). Efforts need to be made in examining variation of load levels, length and frequency of rest breaks aiming at modeling potential the dose response relationship between trauma and load,

rest, and/or both. Populations of different characteristics, such as ones listed as potential MSD risk factors in the personal characteristics category (i.e. age, gender, BMI, previous injuries) in the epidemiology reviews, could be included and compared with results derived from the young and healthy populations, which were presented in this dissertation. Findings from the above extensions of research could contribute to refining existing ergonomic tools and creating new tools that reflect and assess real work-rest cycles in work shifts more closely. In addition, future research could consider using one or a combination of inertial measurement units (IMUs) and motion capturing and analysis systems to capture kinematic information generated during work-rest cycles of varying intensity, numbers of repetition and postures in lab and in field (S. Kim & Nussbaum, 2013; Schall Jr, Fethke, Chen, Oyama, & Douphrate, 2016).

In summary, the results presented in this dissertation provide an original contribution to the scientific literature concerning quantification of impact of rest and secondary intervention on MSD development and recovery. Novel and practical experiment models were developed to facilitate further exploration of different aspects of MSD injury mechanisms. These efforts are promising initiations to be developed into scheduling guidelines in real manufacturing and other work settings. We believe they hold great promise for future integrative MSD prevention research.

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