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Association of a Repetitive Manual Task on Inflammatory Biomarker Expression, Heart Rate Variability and Rating of Perceived Exertion

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ASSOCIATION OF A REPETITITVE MANUAL TASK ON INFLAMMATORY BIOMARKER EXPRESSION, HEART RATE VARIABILITY AND RATING OF PERCEIVED EXERTION

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Dedication

I would like to dedicate this dissertation work to my family and mentors, along with a special feeling of gratitude to my loving wife Maiko Nava, whose words of encouragement, patience and unconditional love helped me throughout this journey. A special thanks to my mother Marcela Iwashige and sister Luz Marcela Conde for believing this was possible. I will always be thankful to my aunts and uncle Paty, Bertha, and Carlos Iwashige for always being there for me.

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ASSOCIATION OF A REPETITITVE MANUAL TASK ON INFLAMMATORY BIOMARKER EXPRESSION, HEART RATE VARIBILITY AND RATING OF PERCEIVED EXERTION

by

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DISSERTATION

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Table of Contents

Chapter 1: Introduction

Work-Related Musculoskeletal Disorders (WMSDs)

Musculoskeletal disorders (MSDs) are comprised of an extensive collection of degenerative and inflammatory conditions affecting tendons, muscles, joints, ligaments peripheral nerves and blood vessels. These conditions frequently produce functional impairment and pain, commonly affecting the hands, wrists, forearms, lower back, elbows, shoulders, and the neck (Buckle & Devereux, 2002; Punnett & Wegman, 2004). Classification of MSDs depends on the tissue and/or the systems affected. Some of the most common conditions include tendon-related disorders such as tendonitis and tendinosis, nerve-related disorders such as carpal tunnel syndrome, and joint-related disorders such as osteoarthritis (Buckle & Devereux, 2002). The most common MSDs are listed in Table 1. The MSDs can be classified as idiopathic, resulting from continuous or repetitive stress, or traumatic, resulting from a single incident (Kumar, 2001).

Idiopathic MSDs are generally regarded as WMSDs due to the potential association of the multifactorial causes including extreme force, repetitive motion, extended sitting and standing and awkward or sustained postures (da Costa & Vieira, 2010). The World Health Organization (WHO) and the International Labor Organization (ILO) who have defined WMSDs as acute or chronic conditions involving the function of nerves, tendons, muscles, or supporting structures of the body that occur when working conditions and activities are significant contributors, but are not the only causal or determinant, to the development or worsening of symptoms (Anil, 2013; Organization, 1985). Furthermore, the United States Bureau of Labor Statistics (BLS) defines WMSDs as

Table 1. Classification of common neck and upper limb musculoskeletal disorders. Adapted from Buckle and Devereux (2002).

Classification of Neck and Upper Limb Musculoskeletal Disorders				
Muscle Related Disorders	Tendinopathies	Nerve-related Disorders	Osteopathy	Circulatory Disorders
Tension neck Syndrome Muscle sprain and strain Myalgia and Myositis	Tendinitis Peritendinitis Tenosynovitis Epicondilitis De Quervain's desease Dupuytren's Contracture Trigger finger Ganglion cyst	Carpal tunnel syndrome Cubital tunnel syndrome Guyon canal syndrome Pronator teres syndrome Radial tunnel syndrome Neurogenic Thoracic outlet syndrome Cervical syndrome Digital neuritis	Osteoarthritis Synovitis Instability	Hypothenar hammer syndrome Raynaud's syndrome Vascular Thoracic Outlet Syndrome

cases where the type of injury or illness is strain, sprain, tear, soreness, pain, or musculoskeletal and connective tissue disorders and diseases where the event leading to the injury includes bending, reaching, climbing, twisting, overreaching, repetition or overexertion (Rosemont, 2008). Although development of WMSDs has not been directly linked to a single occupation, a high incidence and prevalence of WMSDs have been reported in positions requiring repetitive or intensive use of the upper limbs (Armstrong et al., 1993). WMSDs are the primary non-fatal occupation injuries and illnesses requiring days away from work in the USA (Statistics, 2018), therefore, their prompt diagnosis and treatment is critical to reduce the days away from work and the financial costs to the workers and their employers.

WMSDs Diagnosis and Treatment

The diagnosis of WMSDs include the identification of the workplace risks. The evaluation begins by discussing the patient's work requirements, including a detailed description of all the procedures involved in a typical day, focusing on the intensity, frequency, and duration of each of the tasks. Confirmation of the diagnosis requires laboratory and diagnostic tests to determine the damage to the muscles, tendons and nerves. One of the tests performed is a magnetic resonance imaging (MRI) which provides images of muscles, ligaments, and tendons, facilitating the diagnostic. Another test performed is the electroneuromyography (ENG) which provides information regarding muscular activity and conduction velocity of the nerves (Safety, 2020).

Besides clinical diagnosis using MRI and ENG, numerous investigators have developed different methods to diagnose and predict the development of WMSDs without the use of

specialized testing and equipment. Abdelhameed, Sato, and Morita (2015) developed a diagnosis model for shoulder instability using recorded maximal isometric contractions, resulting in a high correlation ($R = 0.99$) between the clinically diagnosed instable shoulder and the artificial neural network (ANN) model. Lugay and Matias (2015) established a regression model to evaluate the level of pain caused by WMSDs in the upper extremities of sewing machine operators. Sánchez, Iglesias-Rodríguez, Fernández, and de Cos Juez (2016) developed an algorithm to predict WMSDs based on reported complaints within a one-year period, predicting the development of WMSDs with an accuracy of 86.79%. The early detection and accurate prediction of WMSDs are key elements for the prescription of appropriate treatments aimed to prevent the worsening of WMSDs.

Currently, the treatment of WMSDs involves the use of therapeutic modalities including the prescription of analgesic medication, electrical stimulation, exercises to build strength and enhance motor control, and hot/cold therapy. These modalities are aimed to reduce inflammation and pain, promote the healing of damaged tissue and maintain/increase strength and/or joint mobility (Poitras & Brosseau, 2008). An alternative to therapeutic treatments involves the use of ergonomic interventions and educational approaches to adjusts the workers' environment and behaviors. The use of therapeutic interventions provide pain relief only for a short-term, while ergonomic intervention provides better long-term results for treating WMSDs (Kim & Hong, 2013). The implications of developing WMSDs are not only a problem for the public health professionals, but they also create a significant financial burden to workers, employers and insurance systems (Sultan-Taieb et al., 2017).

Health and Economic Impact of WMSDs

The WMSDs drastically affect the workers' quality of life, cause considerably negative financial impacts, and are a major cause of all reported work-related diseases (Armstrong et al., 1993). They are the largest category of work-related illnesses in the United States and other High Income countries, accounting for one third of all reported occupational diseases (Bernard & Putz-Anderson, 1997; Sjøgaard et al., 1995). According to the US Department of Labor, the annual incidence rate was 34 cases of WMSD diagnosed per 10,000 workers in 2014, translating into approximately 1000 cases daily (Statistics, 2015). There is a high incidence of days away from work among construction workers, a population commonly affected by upper extremity WMSDs, resulting in direct and indirect costs to the workers and their employers (Abas et al., 2018).

The American Federation of Labor and Congress of Industrial Organizations (AFL-CIO) estimated that WMSDs accounted for 31.7% of the total number of reported injuries and illnesses involving absence from work in 2015, making them the primary source of injuries and illnesses in the workplace (Organizations, 2017). The economic impact of WMSDs was estimated by the Centers for Disease Control and Prevention (CDC) between $45-54 billion annually in 2016 based on costs for compensation and loss of productivity and wages (Prevention, 2016). As reported by United States Census bureau, WMSDs predominantly affect construction workers between the ages of 25 to 54 years old (Table 2). Because most of the workers affected by WMSDs are between 25 and 54 yeast old, better working conditions including preventive measures are needed to reincorporate workers as early as possible and having a favorable prognosis for the WMSDs.

Table 2. Distribution of WMSDs including construction workers of different age groups from 2003 to 2014. Adapted from (Bureau, 2018).

	2003-2007		2008-2010		2011-2014	
Age (Years)	Number	Percent	Number	Percent	Number	Percent
16-19	2100	1.2	430	0.6	860	1
20-24	17690	10.1	4870	7	5100	6.2
25-34	51670	29.4	21060	30.1	20410	24.7
35-44	53310	30.3	20030	28.6	23170	28
45-54	37240	21.2	16780	24	21990	26.6
55-64	11240	6.4	6100	8.7	9510	11.5
65+	880	0.5	310	0.4	410	0.5
Age Not Reported	1850	1.1	400	0.6	1180	1.4

Identification of Prognostic Factors in Upper Extremity WMSDs

The identification of early prognostic factors suggesting a return to work in patients with upper extremity WMSDs is of the utmost importance. These factors, including age, changes in pain, frequency of pain and recurrence of pain, could lead to the identification of workers recovering quickly, leading to shorter or minimal interventions, or they could also identify workers requiring more thorough interventions and requiring longer time to recover before returning to work. Hogg-Johnson and Cole (2003), suggest that focusing on a small set of prognostic factors during the first month would help to categorize the urgency and the type of treatment needed. One of these small set of factors includes the assessment of inflammation to assess the potential risk for the development or worsening of upper extremity WMSDs (Christian, 2014).

The Inflammatory Response

Inflammation is the response of the immune system to an irritant, including injury, pathogens, and the consequence of radiation and chemicals. The signs of inflammation may include redness in the affected area, swelling, heat, pain, and potential loss of function. The inflammatory response involves the action of different cells from the immune system. There is a release of multiple inflammatory mediators that drive a cascade of processes aimed to increase the blood flow to the affected area, recruitment of additional immune cells, and healing of damaged tissue (Care, 2018). Studying the mediators of inflammation can reveal valuable tools that could be screened prior to injury that may prevent further damage to the tissue and the development or worsening of WMSDs.

Biomarkers of Inflammation

The WHO defines a biomarker as a process or structure that can be used to measure or predict the outcome of disease in the body (WHO, 2001). Biomarkers include physical functions such as blood pressure and pulse, and biochemical molecules including proteins, nucleic acids (i.e. DNA, RNA, miRNA), lipids, peptides, whole cells and sugars (Strimbu & Tavel, 2010). The biochemical molecules can be identified in different biological samples including blood plasma or serum, tissues, saliva, urine, sputum and stool (Henry & Hayes, 2012). The identification and study of biomarkers of inflammation present in WMSDs caused by repetitive motion could help in the development of a screening tool to evaluate an individual's risk of developing WMSDs. There is limited research focusing specifically on cause-and-effect relationships between inflammatory markers and the risk of WMSDs in human subjects performing repetitive motion tasks. Therefore, this dissertation aims to explore a relationship between repetitive use of the upper limbs and the expression of inflammatory biomarkers commonly detected in patients with WMSDs.

Chapter 2: Literature Review

Background

Work-related Musculoskeletal Disorders (WMSDs) account for the largest category of totally preventable workplace injuries and costs outlaid by worker compensation programs in the United States. Fatigue, repetitive stress, and overuse can cause musculoskeletal imbalances over time that the body is no longer able to recover from. Some of the more common sources of these imbalances include poor posture, a lack of physical fitness or health, overly heavy applied force, and a high number of repetitions causing fatigue or injury (Bureau, 2019). Because of the persistent pain and reduced movement often associated with WMSDs, these conditions may lead to forced early retirement, an overall reduction in accumulated wealth, and challenges participation in social and family roles (Organization, 1985). This problem is better described by examining the incidence rates of conditions associated with WMSDs and the professions commonly affected.

Incidence rates

WMSDs have been the source of one of the largest reported types of injuries and illnesses in the United States, greatly affecting workers performing repetitive manual tasks. In 2017, there were 282,750 cases of non-fatal WMSD injuries and illnesses (incidence rate of 28.6 cases per 10,000 full-time workers) that accounted for more than 32% of days away from work. Although the incidence for tendonitis was the lowest at 0.5 cases/100,000 full-time workers (FTW), it resulted in the highest average median days away from work at 33 days. This was followed by fractures (8.7 cases/100,000 FTW at 31 median days) and carpal tunnel syndrome (0.6

cases/100,000 FTW at 30 median days, respectively). The greatest incidence rates for WMSDs were observed in individuals with highly physically demanding positions such as laborers and freight/stock/material movers (24,800 cases with an incidence rate of 117.6 cases/100,000 FTW), nursing assistants (18,090 cases with an incidence rate of 166.3 cases/100,000 FTW), and light truck/delivery service drivers (8,680 cases with an incidence rate of 105.7 cases/100,000 FTW) (Statistics, 2018). As with other diseases, different risk factors have been associated with the development and progression of WMSDs.

Risk Factors for Work-Related Musculoskeletal Disorders (WMSDs)

Several risks have been associated with increases in WMSDs development and severity including individual predisposition, physical, biomechanical, and psychosocial conditions. Some of the major work-related risk factors contributing to upper extremity WMSDs include repetitive patterns of motion including: rapid pacing of movements, forceful manual exertion, heavy lifting, concentrated mechanical pressure, insufficient recovery time, constant awkward positioning of the shoulders, elbows or wrists, and the use of vibrating tools (Punnett & Wegman, 2004). The performance of forceful or repetitive hand-intensive activities, especially those happening in fixed or awkward positions, those involving vibration, or those performed in cold temperatures have been associated with the initiation and exacerbation of WMSDs of the hand and wrist (Barr, Barbe, Clark, & therapy, 2004). Also, inflammation, degeneration in musculotendinous tissues, and fibrosis have been observed in subjects with upper extremity WMSDs, however, the time period where these features were developed in the disease process has not been well-established (Barbe, Barr, & immunity, 2006; Carp, Barbe, Winter, Amin, & Barr, 2007; Rechardt et al., 2011;

Riondino et al., 2011). To this end, a review of the relationship between exposure to work-related risk factors and the development of WMSDs is of particular importance.

Repetition and maximal exerted force

The precise relationship that exists between work-task demand and overuse injury of upper and lower limbs resulting in WMSDs are often not clearly defined and may vary greatly due to the type, frequency, and duration of the task. Researchers have attempted to generate criteria delineating upper limits for acceptable ranges of movements and force categorized by task and based on psychophysical outcomes (Barr & Barbe, 2002). Attempts to clarify this limit were described by Silverstein, Fine, Armstrong, and Medicine (1986) who performed a job analysis of industrial workers seeking to quantify the upper and lower acceptable repetition rates of a task, suggesting that acceptable upper and lower repetition rates could be defined as any task with a cycle rate less than or greater than 30 seconds, respectively. Although differences exist between procedures to estimate the force of a task, there is a consensus suggesting that a task requiring less than 15% of the maximum grip force can be classified as insignificant to low exertion, whereas a task that requires a force greater than 15% of the maximum grip force is classified as high exertion (Barr & Barbe, 2002). The exertion of force while performing a work task may be directly caused by the manipulation of heavy objects.

Manipulation of heavy loads

The manipulation of heavy loads by manual workers that require a high expenditure of muscular force may result in fatigue or acute overload of the shoulder and lower back muscles.

Jobs that require repeated application of high musculoskeletal force to the system can contribute to the risk of tissue damage and acute overloading. Choobineh, Tabatabaee, and Behzadi (2009), suggest that there is a need for the reduction in manual handling during heavy loading in order to reduce the level of exposure to risk factors for and to prevent development of WMSD in manual labor jobs. Degenerative disorders of the lower back may develop if force-loading happens over a long duration of time. Static loading can lead to irreversible structural changes in the musculoskeletal system if sufficient recovery time is not allowed (Allahyari, Hedayati, Khalkhali, & Ghaderi, 2014). Linked to the number of repetitions and the exerted force, the specific movement of the limbs and the general body position must be considered while analyzing the work task.

Body posture

Ergonomic factors including body posture can significantly contribute to the initiation and progression of WMSDs. For example, sitting at work for more than 95% of the time is positively correlated with neck pain, as is performing tasks requiring a minimum flexion of the neck at 20° greater than 70% of the work period (Ariëns et al., 2001). Whether unnatural shoulder postures, especially flexion and abduction, associate with WMSDs is currently under debate. Groups such as Tittiranonda, Burastero, and Rempel (1999) and Punnett and Bergqvist (1997) report that flexion and abduction of the shoulder beyond the neutral range are associated with WMSD symptoms of the upper limbs and neck. Conversely, Marcus et al. (2002) report that unnatural postures of the shoulder were not associated with shoulder, neck, hand, or arm symptoms, although working with an angle <121° at the inner elbow did result in an increased risk for musculoskeletal

symptoms. Extreme wrist positions are also considered a risk factor for WMSDs of the hands and wrists, suggesting that tasks producing wrist extension greater than 20° increase the risk for the development of carpal tunnel syndrome (CTS) (Bernard & Putz-Anderson, 1997; Liu et al., 2003; Malchaire, Cock, & Robert, 1996; Viikari-Juntura & Silverstein, 1999). Concurrent to the exposure to risk factors involving physical stress, psychosocial factors have also been linked to WMSDs.

Psychosocial and other risk factors

Stress in and out of the workplace, job control, job demands, and a lack of social support are all psychosocial factors that influence an individual's likelihood of developing WMSDs. Current or past comorbid medical conditions including systemic diseases that affect the musculoskeletal system, traumatic injuries affecting a body part, and circulatory system disorders or diseases can also play a role in WMSDs. A female bias has been observed in WMSDs development, however, this is industry-dependent. Finally, obesity and age can be compounding factors that may increase the impact of other risk factors on the severity of WMSDs (Council, 2001). A better understanding of these risk factors, especially those associated with the inflammatory process, will facilitate the development and application of preventive strategies to reduce the overall incidence and number of WMSDs worldwide.

Musculotendinous Injury and Inflammation in the Development of WMSDs

The performance of forceful or repetitive tasks can lead to musculotendinous injuries due to repeated compression, over-stretching, ischemia, overexertion, and friction ultimately resulting

in an inflammatory response as summarized in Figure 1 (Barbe et al., 2006). The overall desired outcome of inflammation is ultimately the repair or replacement of the injured tissue with regenerated healthy tissue. However, when inflamed and injured tissue is repeatedly subjected to a repetitive task, chronic or systemic inflammation, a hurtful cycle of injury, fibrosis, or even breakdown of the tissue may occur. This pathway often results in chronic pain and possible loss of mechanical function (Banasik, 2000). While the inflammatory response is the body's initial response to injury, chronic inflammation can make the affected tissue more susceptible to injury (Barr et al., 2004).

Inflammation progression to fibrosis

One of the major challenges when studying workers performing repetitive manual tasks is the difficulty in determining causality between tissue damage and the physiological changes resulting from physical stress. The concept of "temporary" inflammation, in which continuous, short-term inflammation contributes to the development or progression of fibrosis (Wynn & Ramalingam, 2012), has been supported by multiple clinical studies evaluating biopsies of patients suffering from tenosynovial thickening resulting in the development of carpal tunnel syndrome (CTS). Hirata et al. (2005) evaluated CTS participants in cohorts based on the duration of their symptoms: less than 3, 4 – 7, 8 – 12, and more than 12 months. Patients presenting symptoms of less than 3 months primarily experienced edematous tissue changes. Those with 4 – 7 months of symptoms had increased expression of vascular endothelial growth factor (VEGF) and prostaglandin E2 (PGE2). Finally, patients with greater than 7 months of symptoms presented with fibrotic changes, suggesting that inflammatory processes had shifted to chronic inflammation, and

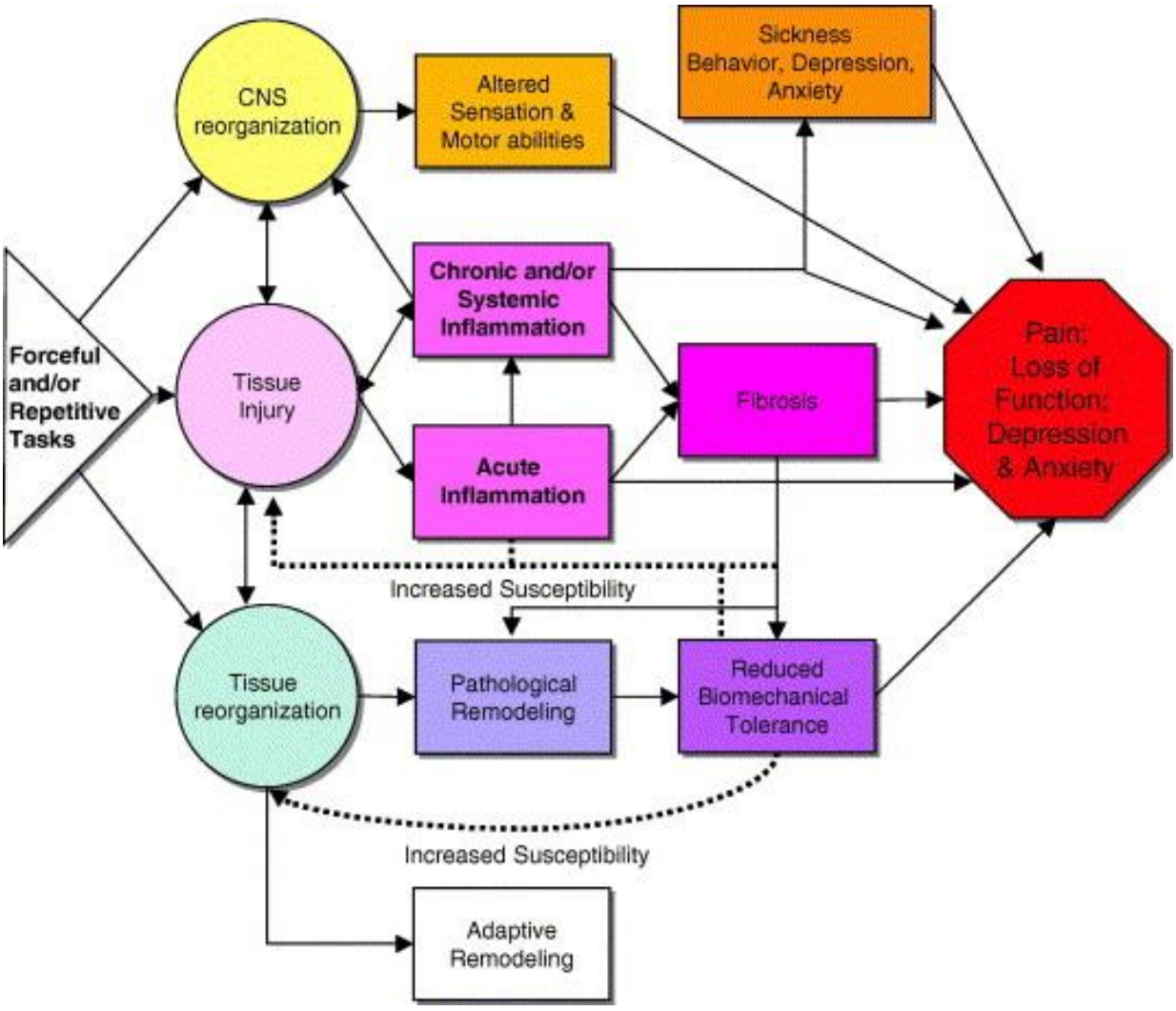

Figure 1. Progression of Work-Related Musculoskeletal Disorders (WMSDs). After a forceful or repetitive movement producing tissue injury there is an inflammatory response to repair the damaged tissue. If an inflamed tissue is continuously overused, repair may be impaired, leading fibrosis, pain and possible loss of mechanical function (Barbe et al., 2006).

fibrotic replacement (Hirata et al., 2005). Some of these findings were supported by Freeland, Tucci, Barbieri, Angel, and Nick (2002), who also observed increases in the expression of PGE2 and interleukin 6 (IL-6), another pro-inflammatory cytokine, in tenosynovial tissues from 41 patients diagnosed with idiopathic carpal tunnel syndrome that had abnormal electrophysiologic findings following carpal tunnel release surgery (Freeland et al., 2002).

Tenosynovial tissue studies by Freeland and Hirata revealed that CTS disease pathology likely begins with an early inflammatory response followed by unsuccessful attempts at healing, ultimately culminating in fibrosis (Freeland et al., 2002; Hirata et al., 2005). Similar investigations of trapezius muscle biopsy samples from male and female workers with intermittent or uninterrupted myalgia for at least one year which were diagnosed with chronic tissue overuse syndromes have been performed. These patients also presented with evidence of myopathic changes including an increased incidence of type II myofibers consistent with muscle injury, damaged and torn type I muscle fibers, and loss of muscle fibers due to denervation and ischemia in the absence of inflammation (Larsson, Björk, Elert, Lindman, & Gerdle, 2001). Biopsy samples from the dorsal interosseous muscle were collected from 29 females diagnosed with painful chronic overuse syndrome (Dennett and Fry (1988). The biopsies revealed similarly pathologic changes in the muscular structure including an increase in type I fibers and decreases in, and hypertrophy of, type II fibers. An increase in perivascular mononuclear inflammatory cells was also observed, suggesting an inflammatory state consistent with that described by Hirata et al. Importantly, all of the noted changes were associated with the severity of the syndrome (Dennett & Fry, 1988).

The disparities that existed amongst these early studies regarding the presence or absence of inflammation has since been clarified by more recent work implicating differences in the types of responses according to anatomical site, the level of exposure, and the type of the task being performed. For example, the relationship between exposure and tissue damage was explored by Cannon et al. (1989), who evaluated muscle biopsy samples from five males between 21 and 29 years old. The participants were asked to complete a 45-minute downhill run at 75% of their maximal aerobic capacity (VO_2 max). Muscle biopsy samples were obtained 45 minutes immediately after cessation of the exercise and again 5 days later. Immunohistochemical staining of muscle biopsies from the *vastus lateralis* showed an increase in IL-1β expression, small increases of Interleukin 1 Alpha (IL-1α) and Tumor Necrosis Factor Alpha (TNFα) after completing the exercise on day 5, indicating that inflammatory cytokines can be detected in muscle tissue shortly after exercise even in the absence of stress related to infection (Cannon et al., 1989). This progression from inflammation into fibrosis is mediated by cytokine activity affecting different mechanisms and the level of tissue overuse.

Mediators of inflammation and their relationship with tissue overuse

The inflammatory mediators PGE2 and VEGF enhance cytokine activity, vasodilation, edema, and proliferation of smooth muscle and endothelial cells in chronic inflammation, respectively (Barbe et al., 2006). A cytokine with both anti-inflammatory and pro-inflammatory properties, IL-6 is an early responder cytokine not normally detected in peripheral blood. Expression of IL-6 is tightly regulated in serum, except in conditions of infection, cellular stress, or trauma. During low-grade inflammation, IL-6 may be anti-inflammatory and induce

upregulation of the IL-1 receptor antagonist (IL-1RA) and soluble Tumor Necrosis Factor receptor (TNF-R) expression in peripheral circulation. The most common pro-inflammatory properties of IL-6 include mediating acute phase responses to physiologic injury and inducing cell proliferation and growth (Biffl, Moore, Moore, & Peterson, 1996).

Other mediators of inflammation include C-reactive proteins (CRPs). The type of associations and cause-effect relationships that exist between levels of overuse exposure, tissue damage severity, and inflammatory cytokines and C-reactive CRPs in the early stages of musculoskeletal injuries of the upper extremities has been explored (Carp et al. (2007). Tissue samples were analyzed for the expression of CRP and inflammatory cytokines in 9 healthy subjects experiencing pathologic symptoms within the upper extremities caused by overuse over approximately 12 weeks. Subjects were divided into groups based on disease severity as determined using the Upper-Body Musculoskeletal Assessment (UBMA). CRP expression levels were strongly correlated and inflammatory cytokines IL-6, IL-1β, and Tumor Necrosis Factor Alpha (TNFα) were moderately correlated to higher UBMA scores, indicating that early during overuse, inflammatory responses may contribute to the onset and development of musculoskeletal injuries (Carp et al., 2007). Some of the most common injuries in the workplace happen in the tendons of the upper extremities (Collins, Janse Van Rensburg, & Patricios, 2011)

Tendon overload

The force development function of the skeletal muscle fibers, as well as the force transforming role of the attached tendons to produce movements, is well understood (Huxley,

1969; Kawakami, Ichinose, & Fukunaga, 1998; Maas, Baan, & Huijing, 2001; McCall, Byrnes, Dickinson, Pattany, & Fleck, 1996). In the mechanically overloaded tendon, collagen turnover and remodeling promote healing. However, continuous loading and insufficient healing time may lead to the development of permanent tendon damage (Kjær, 2004). Animal models of rotator cuff injury have also been studied to assess the complex relationships that exist between overload and intrinsic tendon injury or extrinsic tendon compression (Carpenter, Flanagan, Thomopoulos, Yian, & Soslowsky, 1998). Rotator cuff tendon samples were collected from experimental and control rats after four and eight weeks of overuse. The experimental animals were divided into two cohorts in which half were subjected to overuse plus either an *intrinsic* or *extrinsic* injury in the left shoulder and only overuse in the right shoulder. Control rats received no treatment. Those tendons exposed to overuse and overuse plus injury had increased levels of collagen disorganization and pathologic changes in cellular shape compared to control tendons. Rotator cuff tendons subjected to overuse and injury received a worse histological grade (amount of tumor cells) than those tendons that were overused only. The tissue moduli (the amount of force per unit of area) was significantly lower in animals eight weeks after overuse when compared to both control and four weeks of overuse. Therefore, overuse causes damage to the supraspinatus tendon of the rotator cuff regardless of the presence of previous injury, specifically (Carpenter et al., 1998).

Repetitive muscular strain cycle studies were performed every other day for four weeks as 5 sets of 10 repetitions with a 30 second rest period in between sets within female rats. Using electron microscopy to analyze the muscle samples of female rats exposed to muscular strain, investigators focused on the fast-stretching and slow twitching responses due to induced muscular strains. They observed that fast-stretching of the muscle led to the production of fibrosis, whereas

slow-stretching did not. Furthermore, slowly stretched muscles contained connective tissue "struts" connecting adjacent myofibers. These data indicate that slow-stretch straining produces positive structural adaptations, whereas fast muscular strains lead to the generation of morphological changes analogous to tendinopathies such as fibrosis (Stauber, Knack, Miller, Grimmett, & Medicine, 1996).

Other groups have also evaluated the effects of muscular strain on tendon structures. Histological analysis of Achilles tendon samples in male rats subjected to one hour of eccentric exercise at a rate of 30 contractions per minute three times per week for 11 weeks showed increases in the number of nerve filaments as well as hypervascularization and inflammation in the epitendon and the peritendon. Interestingly, no change was observed in the distribution of Type I and Type II collagen, and the changes associated with chronic tendinosis were not developed after 11 weeks of activity using the selected load. These data support the hypothesis that the initial phases of tissue damage are inflammatory rather than degenerative (Messner, Wei, Andersson, Gillquist, & Räsänen, 1999).

Injuries in the shoulder, caused by overuse of the supraspinatus tendon, were evaluated by Perry, McIlhenny, Hoffman, Soslowsky, and Surgery (2005). Rats were subjected to downhill treadmill runs for 1 hour daily for 5 days per week at a speed of 17 m/min. The rats were divided into groups based on duration of treatment: 3 days, 1 week, 2 weeks, 8 weeks, and 16 weeks. Increased expression in the pro-inflammatory enzyme cyclooxygenase-2 (COX-2) and tendon hypervascularization (Perry et al., 2005) was observed in all groups, consistent with previously cited studies demonstrating an association between inflammation and repetitive tendon overuse.

Rabbit models have also been exploited to explore tendon loading in the lower and upper extremities and their association with inflammation and early changes in the tissues (Backman, Boquist, Fridén, Lorentzon, and Toolanen (1990). To better understand chronic Achilles tendon paratendonitis with tendinosis in a rabbit model, animals were induced to exercise on a machine designed to produce passive flexions and extensions of the ankle as the rabbits were kicking. The *triceps surae* muscles were actively contracted using electrical stimulation by means of surface electrodes. The exercise protocol consisted of two hours of activity at a rate of 150 flexions and extensions per minute, 3 times per week, for 5-6 weeks. Achilles tendon samples were analyzed histologically using light microscopy and showed degenerative changes, infiltration of inflammatory cells, increased numbers of capillaries, fibrosis in the paratendon and edema. Outcomes from this experiment served as the basis for subsequent research demonstrating that tendon damage has an inflammatory component (Backman et al., 1990). J. M. Archambault, Herzog, and Hart (1997) refined the Backman et al. (1990) model by prolonging the exercise protocol to 11 weeks. Tissue analysis revealed the presence of inflammation in the paratendon accompanied by an increase in the inflammatory cytokines IL-1 and TNFα, along with tendon matrix transcript molecules such as proteoglycans and Type I collagen. Furthermore, when the kicking protocol was extended to 11 weeks, there were no signs of injury or inflammation in the muscle or in the tendon after the conclusion of the protocol. This indicates that inflammation in the tissue, as well as tissue remodeling may occur early in musculoskeletal pathology, prior to obvious signs of decline in function and tissue damage. More importantly, repair of the damaged tissue may be completed early if the loads are adequately low (J. M. Archambault et al., 1997).

In summary, experiential evidence indicates that an inflammatory response initiates the healing process following tendon overuse. If the tendon can heal, inflammation subsides and the tissue returns to its healthy operational state. Should a tendon be unable to heal successfully due to continuous overuse or excessive loading, the tendon structure is damaged and becomes fibrotic, inflammation is reduced, and long-term damage results. Aspects contributing to WMSD damage include the location of the anatomical site affected, the level of overload exposure, and the type of the task being performed as well as variations in the duration of the inflammatory response.

Theoretical Framework

Mechanism of tendon injury

Industrial workers performing tasks characterized by repetitive manual movements have an increased prevalence of MSDs, particularly those that involve degenerative and mechanical injuries of the tendons caused by inflammation or degradation to the tendon and the surrounding tissue. Although tendon injuries include a wide variety of conditions, two of the most common observed pathologies include tendonitis and tendinosis. Tendonitis refers specifically to the damage of a tendon caused by inflammation. Over time, if the damage to the tendon is irreparable or occurs repeatedly during the healing process, this inability to heal is referred to as tendinosis (Baring, Emery, Reilly, & Rheumatology, 2007; Huang, Qureshi, & Biundo Jr, 2000). The most common joint impacted by these tendinopathies in this class of worker is the shoulder (Frost, Andersen, & Medicine, 1999; Ohisson et al., 1995; Ohlsson et al., 1994). Continuous repetitive work, physical effort of the upper extremities, and uncomfortable postures may damage shoulder tissue, specifically the supraspinatus muscle and its respective tendon (Frost et al., 2002). Pathogenic mechanisms involved in the development of shoulder tendinitis can include either or both intrinsic or extrinsic mechanisms (Fu, Harner, Klein, & research, 1991). Intrinsic mechanisms can result from frequent and repetitive levels of high intramuscular pressure in the muscles of the rotator cuff. This pressure damages the microcirculation of the inferior portion of the supraspinatus tendon, causing inflammation and eventual degradation (Järvholm, Styf, Suurkula, Herberts, & physiology, 1988). Extrinsic mechanisms result from faulty mechanical elevation of the arm, causing impingement or tearing of the superior portion of the supraspinatus tendon between the inferior surface of the acromion and the humeral head (Neer II & Surgery, 2005). Both intrinsic

and extrinsic mechanisms can provoke inflammatory and mechanical injury to the tendons through the release of signaling molecules such as cytokines, chemokines and growth factors that stimulate the immune system (Evans, 1999).

Cytokines and matrix metalloproteinases

The release of cytokines/chemokines and growth factors brings about the recruitment of neutrophils, lymphocytes, platelets, macrophages, and other inflammatory cells to the site of injury, initiates production, movement and vascularization of fibroblasts and tenocytes and stimulates the synthesis of new collagen fibers (Molloy, Wang, & Murrell, 2003). The tenocytes respond to the applied loads by increasing the intracellular concentrations of calcium (Ca^{2+}) and adenosine triphosphate (ATP), altering the content of cytoplasmic filaments and their organization, activating intracellular signaling pathways, secreting of metalloproteinases (MMPs), and changing gene and protein expression (Joanne M Archambault, Elfervig-Wall, Tsuzaki, Herzog, & Banes, 2002; Banes, Horesovsky, et al., 1999; Banes et al., 1995; Banes, Weinhold, et al., 1999; M. Lavagnino et al., 2015; Michael Lavagnino et al., 2015; Magra, Hughes, El Haj, & Maffulli, 2007). Both tenocytes and ligament cells secrete cytokines and ATP, causing phenotypic changes that can accelerate matrix degeneration (M. Lavagnino et al., 2015).

Cytokines and matrix metalloproteinases act as mediators in multicellular organisms that can affect cell communication, modulate the mechanical state of the extracellular matrix, and differentially regulate type I collagen and elastin expression (Kamimura, Ishihara, & Hirano, 2003; M. Lavagnino et al., 2015). Elevated levels of cytokines and other mediators are implicated in the

progression of tendinopathic conditions as they have been detected in ruptured and painful tendons. Interleukin 6 (IL-6), a cytokine with a central role in tissue injury and inflammation, has also been shown to be expressed in higher levels after cyclical stretches of human tendon fibroblasts (Skutek, van Griensven, Zeichen, Brauer, & Bosch, 2001). Interleukin 1β (IL-1β), another pro-inflammatory cytokine, mediates the inflammatory response in connective tissues including tendons (Bankers-Fulbright, Kalli, & McKean, 1996) and increases the expression and secretion of MMPs including MMP-1, MMP-2, MMP-3, MMP-9 and MMP-13 at the site of tendon injury (J. Archambault, Tsuzaki, Herzog, & Banes, 2002). MMPs are a unique group of enzymes that contain zinc and depend on calcium as a cofactor to function. After exercise, they digest and degrade collagen and elastin, regulating collagen turnover, promoting tendon remodeling and repair (Del Buono, Oliva, Osti, & Maffulli, 2013). To date, 25 MMPs have been identified in vertebrate organisms, of which 22 have human homologues. The classification of MMPs is based on substrate specificity and structural domain and are named according to the sequential number designated to the vertebrate MMPs. (Zhang & Nothnick, 2005). The release and interactions of inflammatory biomarkers associated to tendon load are summarized in figure 2.

The theoretical framework of this dissertation was grounded in experimental research established in the fields of immunology, physiology, and tendon biology. Based on the research that has been done to date, we identified associations that exist between overuse and repetitive use injuries and inflammatory markers which ultimately culminate in pain, loss of function and lost workdays. Often, subjects are evaluated late in the disease process after inflammation and damage have already occurred. Development of an early screening tool would allow for detection of individuals *at risk* of developing WMSDs *before* damage occurs rather than after. Limited research

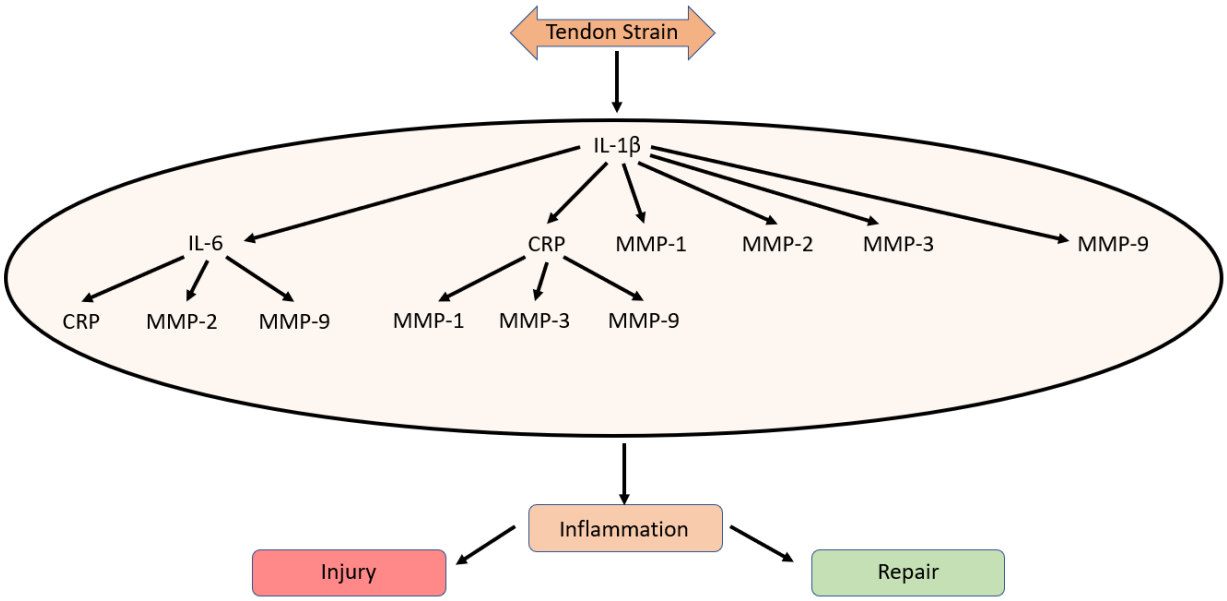

Figure 2. Release of inflammatory biomarkers associated to tendon load. After the tendon is exposed to tendon strain there is an increase in the expression of IL-1β inside in the tendon. The increase in IL-1β cause an increase in CRP, IL-6 and MMPs. Increase IL-6 and CRP also contribute to the increase in MMPs. The increase in inflammatory biomarkers start an inflammatory response to repair or cause injury to the tendon.

has been done regarding concentration changes of inflammatory biomarkers in circulating peripheral blood of healthy individuals performing repetitive manual lifting tasks. Therefore, the purpose of this study was to investigate peripheral blood samples for changes in inflammatory markers within healthy individuals undergoing repeated yet controlled strenuous use of the upper extremities. This study was based on a pre-experimental, single-subject, repetitive-measures design in healthy male subjects between 18-25 years old without any history of tendon injury, inflammatory or autoimmune disorder. Quantitative levels of inflammatory biomarkers were assessed using cellular and molecular methods and compared to self-reported scales of perceived exertion, heart rate variability, and pressure pain threshold testing to identify associations. Preliminary data generated by this research will inform about the feasibility of developing an early-warning rapid test for populations of workers at risk of developing WMSDs.

Objectives and Research Questions

The strain produced by repetitive mechanical loading of the tendons stimulate the expression of selected inflammatory biomarkers, including CRPs, cytokines and MMPs, that may potentially initiate a sub-clinical inflammatory response. Therefore, examining the expression of selected inflammatory biomarkers while performing repetitive work cycles with the upper extremities in a sample of healthy males can provide information about associations between mechanical tendon load and early markers of inflammation, which have been associated with an individual's risk of developing a WMSD. Other physiologic factors including muscular strength and heart rate variability may also be associated with the expression of biomarkers of inflammation. Based on this information, the following research aims were formulated to evaluate two questions: can repetitive mechanical strain initiate a degree of tendon loading sufficient to stimulate an inflammatory response at a sub-clinical level? and can these biomarkers of inflammation be identified in peripheral blood?

The first aim sought to analyze peripheral blood serum samples in order to quantify changes in a subset of inflammatory markers from a cohort of healthy males aged 18-25 at baseline, day 3, day 5 and one week following the completion of a daily repetitive manual task for five consecutive days, and to identify whether associations existed between the duration of exposure to the repetitive task and expression levels of the inflammatory biomarkers. The question we asked was:

- What were the expression levels of the inflammatory markers IL-6, IL-1β, MMP-1, MMP-2, MMP-3, MMP-9, and CRP in peripheral blood before, during, and after the completion of a repetitive manual task daily for 1800 cycles for 5 consecutive days?

The second aim sought to determine the associations between baseline values of muscular strength, perceived exertion, heart rate variability, muscular activity while performing the task, and the expression of inflammatory biomarkers before, during, and one week after the completion of the repetitive manual task.

- What is the relationship between measures of muscular strength, perceived exertion, heart rate variability, PPT and inflammatory marker expression of IL-6, IL-1β, MMP-1, MMP-2, MMP-3, MMP-9, and CRP?

The third aim sought to evaluate similarities and differences between the inflammatory responses of subjects undergoing the same repetitive task and parameters of heart rate variability, perceived exertion, muscular strength, and pressure pain threshold.

- Was the inflammatory response similar in all participants receiving the same exposure? and if not,
- What factors may have differentially impacted the expression of inflammatory markers IL-6, IL-1β, MMP-1, MMP-2, MMP-3, MMP-9, and CRP?

Based on the literature, the following hypotheses were developed: 1) Increased expression of inflammatory biomarkers are measurable in peripheral blood as the exposure to the manual task increases; 2) Positive correlations exist between inflammatory biomarker expression and measurements of perceived pain and perceived exertion, while a negative correlation exist between inflammatory biomarker expression and muscular strength, and no correlations exist between heart rate variability and inflammatory biomarker expression; 3) Inflammatory biomarker changes will be homogenous amongst participants exposed to the same task at each time point.

Chapter 3: Methods and Procedures

Participants

A total of 17 participants were recruited and 16 were included in the final analysis (a participant had to drop out of the study due to an injury not related to the study). All participants were part of a college age student convenience sample from the University of Texas at El Paso. Given the repetitive measures nature of the study, all participants were considered as part of a cohort and each participant served as their own control. All the procedures included in the study were approved by the Human Subjects Institutional Review Board (IRB) at the University of Texas at El Paso. All the participants gave written consent prior to the beginning of the study.

To achieve the main goal of our research, we identified a sample population that minimized possible inflammatory effects of factors other than tendon overload. We included healthy young males (18 – 25 years old) with no history of diagnosed WMSDs or upper extremity surgery of the upper limbs within the past year (Staff et al., 2010), not taking anti-inflammatory medication (Vane & Botting, 1987), and not classified as overweight (BMI < 25) (Cevenini et al., 2010; Chung et al., 2009; Johnson et al., 2007). To reduce the effects of physical activity on the expression of the biomarkers, participants performing high intensity training (i.e. Olympic weightlifting, power lifting, CrossFit, or high-intensity interval training) were not considered for the study (Nimmo, Leggate, Viana, & King, 2013). Participants engaged in regular physical activity were asked not to change their routine (i.e. do not start a new exercise modality, and not stop the current exercise modality. The sample population included college students from the College of Health Sciences, College of Liberal Arts, and College of Engineering at The University of Texas at El Paso.

Recruitment and Written Consent

All participants were recruited using the University's digital advertising system. Digital flyers were displayed in the digital bulletin boards at the Undergraduate Learning Center, the College of Liberal Arts and the College of Health Sciences. Invitations were also provided in person to the students in the Kinesiology and Public Health Sciences programs. Students that accepted the invitation to participate were asked to complete a screening questionnaire to determine their eligibility based on the inclusion criteria. After we reviewed the questionnaires, participants were contacted to clarify answers if necessary. Those participants meeting inclusion criteria were subsequently notified by email and were invited to participate. Before the start of the study, the participants were asked to meet with the research assistant in the research ergonomics research lab in the College of Health Sciences and Nursing Building. The research assistant explained the purpose of the study and the procedures were explained, all the participant's questions were addressed prior to signing for providing written consent.

Instruments

Screening questionnaire

The screening questionnaire (Appendix A) was completed by all individuals and was designed to assess whether participants would meet inclusion criteria for participation. The instrument was composed of 24 questions that covered the following information: age, ethnicity, physical requirements of their job (if employed), exposure to repetitive or forceful manual tasks, exercise routine (including type of exercise), history of injuries or tendinopathies, surgeries of the

upper extremities, recurring or persistent pain, history of anti-inflammatory medications used, and history of conditions preventing the donation of blood samples. To facilitate answering of the questions and to gather necessary information pertinent to the study, all questions pertaining to exercise, injuries and medications provided a list of the most common answers as well as a space to write in answers not listed.

Anthropometric measurements, blood pressure and heart rate

Anthropometric data was obtained for every participant at the beginning of the study. Body weight (in kilograms) and height (in meters) were measured using a Detecto® scale (Global Industrial) with a stadiometer. Body Mass Index (BMI) was calculated using the standardized formula (Health, NA) listed below.

$$BMI = \frac{body\ weight\ (kg)}{height^2(m)}$$

Resting blood pressure was obtained with sphygmomanometer and stethoscope (American Diagnostics Corporation) (Pickering et al., 2005) and the resting heart rate measured using the Polar H10 heart rate chest strap (Polar Electro) wirelessly connected to an iPad (Apple) running the Heart Rate Variability (HRV) logger application (Marco Altini) after 5 minutes of quiet rest while the participants were sitting down (Markovics, Lauznis, Erins, Minejeva, & Kivlenieks, 2018). The predicted maximum heart rate was calculated with the following equation: *Max HR = 220 - age* (Prevention, 2015; Tanaka, Monahan, & Seals, 2001).

Rating of perceived exertion

To determine whether the level of perceived exertion aligned with physiological markers such as HRV and muscular activity, Borg's *Rating of Perceived Exertion* scale (RPE Scale) was used to collect data regarding the participant's perceived effort during the performance of the task (G. A. Borg, 1982). The scale is divided in different numerical color-coded sections based on the levels of perceived effort, ranging from no exertion at all to very light (6 – 10), light to hard (11 - 15), and very hard to maximal exertion (16 – 20). This scale has been correlated to heart rate, making it an accurate and reliable tool for the measurement of perceived exertion (G. Borg, Hassmén, & Lagerström, 1987). The measurements of perceived effort were collected every two minutes while the participant performed the manual task and 5 minutes into the resting period. The participant was asked to choose the number on the 15-grade scale that corresponded with his rating of perceived exertion (Figure 3).

Borgs Rating of Perceived Exertion	
6	No Exertion at All
7	
8	Extremely Light
9	Very Light
10	
11	Light
12	
13	Somewhat Hard
14	
15	Hard
16	
17	Very Hard
18	
19	Extremely Hard
20	Maximal Exertion

Figure 3. Borg's 15-grade scale for rating of perceived exertion (RPE Scale). Adapted from G. A. Borg (1982).

Pressure pain threshold

The basal measurements of pressure pain threshold (PPT) were taken from the *extensor carpi radialis longus* and *anterior tibialis* muscles using the JTech muscle tester (JTech Medical Industries) with the algometer attachment. Briefly, the muscle of interest was located and the site was marked using a pen. The tip of the algometer was then placed into the muscle at the marked site and increasing uniform pressure was applied until the participant stopped the test by clicking the end-test device. The applied force in Newtons (N) was recorded for the dominant and non-dominant extremities. All measurements were taken in triplicate and the average maximal force was used in the analysis. If the Coefficients of Variation (CV) between measurements were greater than 15%, the measurements were repeated (Ylinen, Nykänen, Kautiainen, & Häkkinen, 2007).

Muscular strength

Measurements of muscular strength at the shoulder and wrist were taken at baseline using the JTech muscle tester (Jtech Medical Industries) with the curved and flat pad attachments, respectively. Shoulder flexion measurements were taken with the participant sitting down with the back straight. Participants were then asked to flex the shoulder at a 90° angle. The muscle tester would be placed with the curved pad just proximal to the participant's elbow, while the participant performed an isometric contraction by pushing up against the provided resistance for 2-3 seconds.

Wrist extension measurements were taken with the participant sitting with the back straight, the forearm resting on a table, and the wrist at the edge of a table to allow the subject to extend the wrist at a 0° angle. The flat pad would be placed above the dorsal side of the

participant's hand, while the participant performed an isometric contraction by pushing up against the provided resistance for 2-3 seconds. The applied force in Newtons (N) was recorded for the dominant and non-dominant extremities. All measurements were taken in triplicates and the average maximal force was used for analysis. If the CV between measurements was greater than 10%, the measurements were repeated (Izquierdo, Häkkinen, Gonzalez-Badillo, Ibanez, & Gorostiaga, 2002).

Repetitive manual task to mechanically strain shoulder and forearm tendons

The participants were asked to complete a simple manual task for 5 consecutive days using only the dominant hand. The task consisted of moving a 0.5 lb. ball up and down three platform levels positioned at varying heights: level 1 was positioned so the shoulder was flexed at a 45° angle, level 2 at a 68° angle, and level 3 at a 90° angle. The participants first grasped the ball with the hand in a neutral position and the wrist at a 0° angle (Figure 2). The participants were asked to complete 1,800 cycles by moving the ball up or down to a different level which was considered one cycle. A digital metronome was used to set the pace at 72 beats per minute (BPM) where each beat of the metronome signaled the participant to move the ball up or down one level. Additionally, the 1,800 cycles were divided into three stages: two stages of 576 cycles (8 minutes) each and the third of 648 cycles (nine minutes). The participants were given 10 minutes of rest between the three stages.

Figure 4. Location of the three different levels: shoulder at about 45°, 68°, and 90° angle. Participants moved a 0.5 lb. ball up and down until 1,800 cycles were completed.

Heart rate variability

Measurements of heart rate variability were obtained by placing a Polar H10 heart rate sensor (Polar) on the participant's chest to record cardiac activity through the duration of the task comprised of 25 minutes of upper limb activity and 20 minutes of quiet rest. Data was recorded using the HRV logger app (Marco Altini) and exported to the Kubios HRV premium analysis (Kubios) software to obtain HRV measurements. Electrocardiogram (ECG) signal was transformed for the analysis of the differences in contiguous R-R intervals using the root mean square of the standard deviation (RMSSD) and the low and high frequency were obtained (figures 5 and 6). All HRV measurements were divided into resting and manual task stages. The best five minutes of each recorded data segment were used in the analysis included (Tarvainen et al., 2014).

Electromyography

Non-invasive electromyography data from the upper limbs was obtained by placing Noraxon dual EMG electrodes (Noraxon USA) in the anterior deltoid and the *extensor carpi radialis* muscles of the dominant arm. The anterior deltoid of the non-dominant arm was collected as a control. Surface EMG data were used to monitor muscular activity while the participants performed the simple manual task. Muscular activation was maintained below 15% of the maximal voluntary contraction (MVC) throughout the activity to simulate the muscular activity of manual workers and to reduce the risk of injury. Surface EMG recordings were obtained simultaneously in three different channels and were sampled at 1,500 Hertz (Hz). Recorded data was rectified using a bandpass with a high frequency of 100 Hz and a low frequency of 15 Hz (Merletti & Di Torino, 1999).

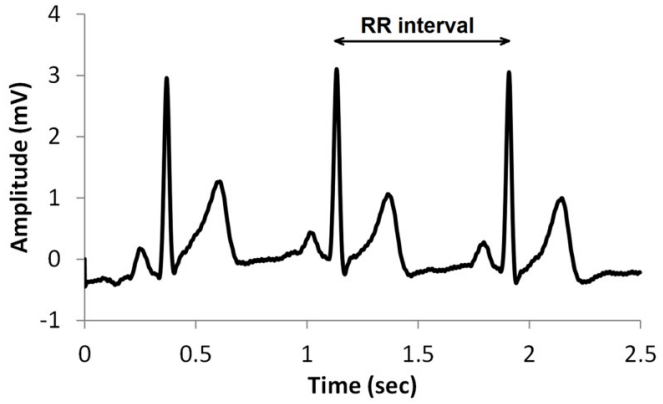

Figure 5. The assessment of HRV involves the measurement of the difference in time between consecutive R waves of the ECG (Makivić, Nikić Djordjević, & Willis, 2013).

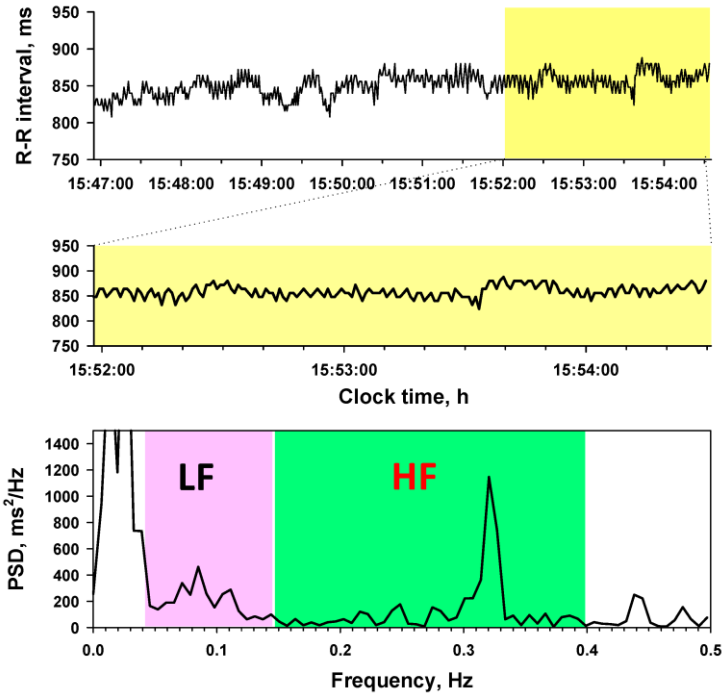

Figure 6. Transformed ECG data using the RMSSD to analyze changes in HRV. After data has been transformed, low and high frequencies can be measured to assess physiological stress (Makivić et al., 2013).

Blood samples

Venous blood samples were collected from the antecubital area of the arm prior to the beginning the task (baseline), on days three and five after completion of the task, and one week following the end of the task assignment. Each blood donation consisted of an approximate 30 milliliter (mL) sample of whole blood collected in three red top vacutainer tubes and one yellow top vacutainer tube (Becton, Dickinson and Company). Blood samples were incubated at room temperature for 25 minutes to allow the blood to clot, then centrifuged at 3,200 RPM for ten minutes using the Adams Compact II centrifuge (Clay Adams). Serum was removed and frozen at -80^{o}C until analysis (Thavasu, Longhurst, Joel, Slevin, & Balkwill, 1992).

Luminex multiplex analysis

Expression levels of IL-6, IL-1β, MMP-1, MMP-2, MMP-3, MMP-9, and CRP were quantified using MILLIPLEX® XMAP magnetic bead-based multi-analyte panels at baseline, day three, day five, and five days post-task. Blood serum was diluted 1:40000 for CRP analysis, 1:100 for IL-6 and IL-1β analysis in Millipore dilution buffer (EMD Millipore), blood serum was not diluted for MMP analysis. Multi-analyte panels were analyzed using the Millipore Luminex 200 instrument and xPONENT 3.1 software. Total analyte concentrations were calculated using MILLIPLEX® 5.1 analysis software (Millipore Sigma).

C-Reactive protein enzyme-linked immunosorbent assay

Expression of CRP in blood serum at baseline, day three, day 5 and five days after the end of the task was quantified using Enzyme-linked Immunosorbent Assay (ELISA) according to the manufacturer's guidelines (Aviva System Biology). Samples were diluted a 100-fold and absorbance was measured at 450 nm using the Promega GloMax Discover (Promega, Inc) system. Total analyte concentration was calculated based on a curve using standards of known concentration.

IL-1β and IL-6 enzyme-linked immunosorbent assay

The expression of IL-1β and IL-6 was also quantified in serum at baseline, day three, day five, and five days after the end of the task using ELISA according to manufacturer's suggested guidelines (R&D systems). Samples were measured undiluted and absorbance was measured at 450 nm on the Promega GloMax Discover (Promega, Inc) system. Total analyte concentration was calculated based on a curve using standards of known concentration.

COX-2 enzyme-linked immunosorbent assay

Expression of COX-2 was quantified in serum at baseline, day three, day five, and five days after the end of the task using ELISA according to manufacturer's suggested guidelines (R&D systems). Samples were undiluted and absorbance and was measured at 450 nm on the Promega GloMax Discover (Promega, Inc.) system. Total analyte concentration was calculated based on a curve using standards of known concentration.

Procedures

Before the start of the study, a research assistant explained the purpose and procedures of the study to each participant on the first visit, any questions and concerns were addressed, and the participant was asked to sign the consent form. After obtaining consent, anthropometric, PPT, muscular strength measurements and a 30-cc blood sample were obtained. The blood samples were processed by centrifugation at 2500 rpm for 20 min, after which the serum was collected and stored at -80°C. After obtaining all the baseline measurements, the participants were asked to select the most convenient time of day to perform the manual task each day. To minimize the effects of collection time in the expression of the biomarkers of inflammation, the participants completed the task at a similar time each day (Marinac et al., 2015).

On the first day of the manual task, the research assistant prepared the subject for HRV and surface EMG measurements by placing the polar heart rate monitor in the participant's chest and the surface electrodes in the participant's shoulders and *extensor carpi radialis longus*. The research assistant asked the participant to stand in front of the steps and grasp the 0.5 lb. ball to adjust the height of the steps. Once the steps were at the appropriate height, the metronome was set to 72 beats per minute and the participant was asked to start moving the ball up and down following the rhythm of the metronome. The participants were asked to grasp the ball throughout the performance of the task in order to maintain an isometric hold and to continuously repeat the cycles for 8 consecutive minutes before given a 10-minute break. After the 10-minute break, the participants resumed the cyclic task for a second 8-minute period, followed by a second 10-minute break. Finally, subjects were asked to complete the last stage of 9 minutes for a total of 25 minutes

(1,800 cycles) per day. The research assistant asked the participants to evaluate their perceived exertion every two minutes while performing the task and in the middle of the break.

The participants repeated the same procedures for all five days of the study. On days three and five, a blood sample was obtained after the completion of the task. The blood was processed and stored at -80°C. After the blood donation on day five, the participants were asked to rest for one week and return for a final blood donation. Following the blood donation, participants were acknowledged and received financial compensation for their participation in the study.

Once all the blood samples were obtained, the blood serum was analyzed for the expression of biomarkers of inflammation. The expression of CRP, IL-6, IL-1β, MMP-1, MMP-2, MMP-2 and MMP-9 was assessed using the Luminex Multiplex assays following the manufacturer's recommendations. The expression of COX-2 was assessed using ELISA following the manufacturer's recommendations. Secondary assessment of CRP, IL-6, and IL-1β was perfomed to verify questionable readings obtained from the Luminex assays. High sensitivity CRP, IL-6, and IL-1β ELISAs were performed following the manufacturer's recommendations. Data was collected, coded and exported into Statistical Package for the Social Sciences version 25 (SPSS, International Business Machines) for statistical analysis.

Statistical Analysis

Data was coded using a participant number and by the removal of any identifiers and compiled in a Microsoft Excel worksheet before being exported to SPSS.

Descriptive analysis of variables was conducted using SPSS software. Non-parametric tests were performed to account for the skewed nature of the data and the low number of participants. To test our first and third hypotheses, Friedman test analysis was used to check for significant differences in the expression of biomarkers of inflammation at different collection times. If significant differences were identified, a Wilcoxon signed rank test was used to identify those specific differences. Furthermore, analysis of covariance (ANCOVA) was used to identify the possible effect of different covariates on the expression of inflammatory biomarkers. To test the second hypothesis, a Spearman Rank-Order Correlation Coefficient was used to determine whether significant correlations existed between the expression of the different biomarkers of inflammation and between the expression of the biomarkers of inflammation and measurements of strength, perceived exertion, heart rate variability and PPT. Significance was recognized as $p \leq 0.05$.

Chapter 4: Results

This chapter sought to present data collected to answer the following research questions: 1) can inflammation be initiated in healthy males following the performance of a repetitive task? and 2) are the markers of that inflammatory process measurable in peripheral blood? In order to answer these questions, we collected anthropometric, clinical and laboratory data from 16 participants, and performed statistical analyses designed to identify changes between the inflammatory markers and clinical measurements.

Participant characteristics

A total of 23 individuals expressed interest in participating in the research. After reviewing their eligibility, 17 participants met the inclusion criteria and were contacted to be part of the study. Unfortunately, one participant suffered a skateboard accident and was prescribed anti-inflammatory medication, therefore, he was not included for the final analysis of the data. The participants' anthropometric data is detailed in Table 3. Values are reported as means ± standard deviation (SD). Some of the key factors included age (21 ± 1.57 years old), weight and height (75.5 ± 12.15 kg and 1.75 ± 0.01 meters), BMI (24.75 ± 3.26 kg/m^2), blood pressure (124/74 ± 9/6 mmHg), maximum heart rate (199 ± 1.6 bpm), dominant and non-dominant shoulder flexion (207.06 ± 51.24 N and 204.25 ± 50.53 N), wrist extension (161.25 ± 36.52 N and 173.56 ± 35.67 N), arm PPT (68.94 ± 15.85 N and 70.31 ± 21.43 N) and leg PPT (73.88 ± 23.91 N and 74.38 ± 21.97 N; figures 7 and 8).

Table 3. Mean and standard deviation of participants' descriptive data

Variable	Mean ± SD
Age (years)	20.94 ± 1.57
Weight (kg)	75.5 ± 12.15
Height (m)	1.75 ± 0.09
BMI (kg/m^2)	24.75 ± 3.26
Estimated Maximum Heart Rate (220 – age; BPM)	199.06 ± 1.57
Right Shoulder Flexion (N)	207.06 ± 51.24
Left Shoulder Flexion (N)	204.25 ± 50.53
Right Wrist Extension (N)	161.25 ± 36.52
Left Wrist Extension (N)	173.56 ± 35.67
Pressure Pain Threshold Dominant Arm (N)	68.94 ± 15.85
Pressure Pain Threshold Dominant Leg (N)	73.88 ± 23.91
Pressure Pain Threshold Nondominant Arm (N)	70.31 ± 21.43
Pressure Pain Threshold Nondominant Leg (N)	74.38 ± 21.97

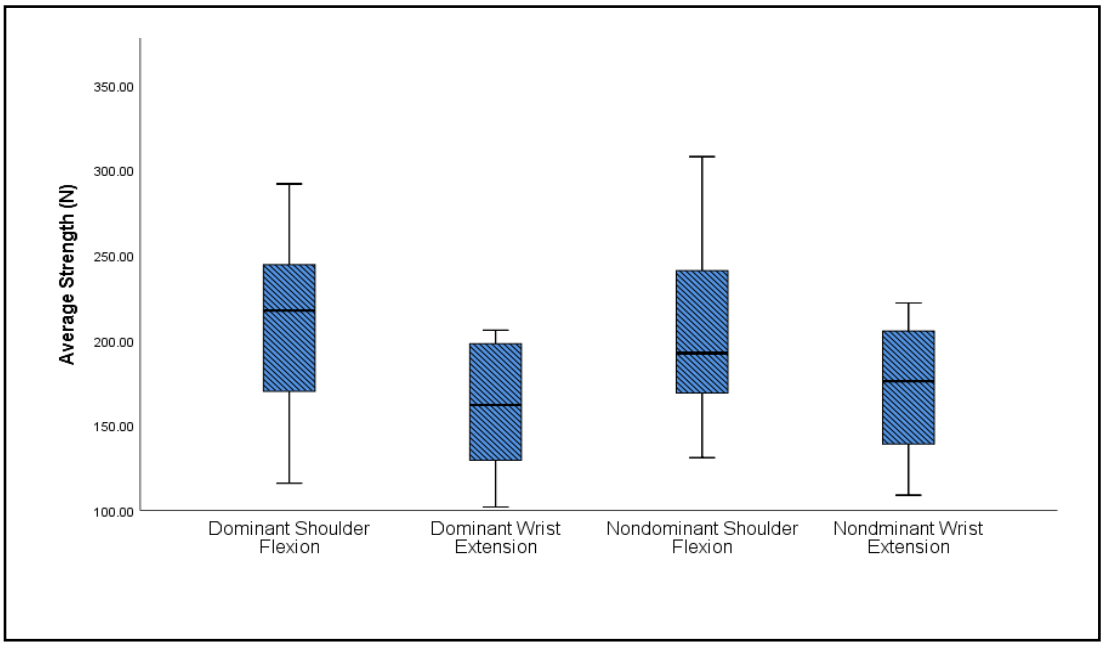

Figure 7. Differences in shoulder flexion and wrist extension strength of the upper limbs. There were no significant differences in shoulder flexion and wrist extension strength.

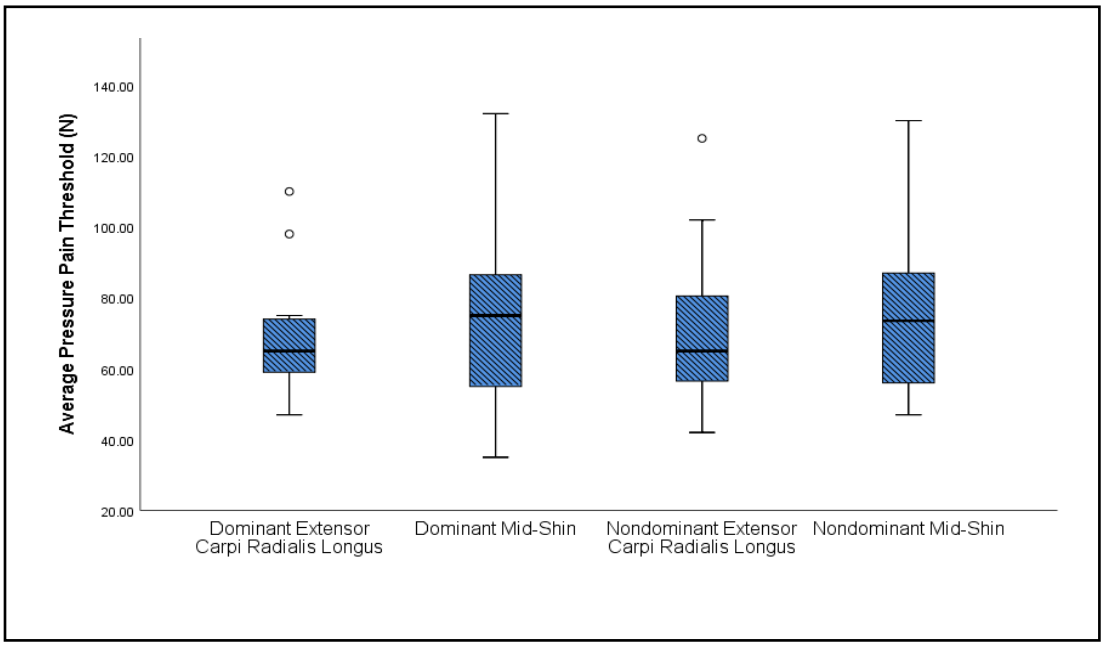

Figure 8. Differences in PPT of the upper and lower limbs. There were no significant differences in PPT of the upper and lower limbs.

Fatigue

The main goal for performing the repetitive task was to induce tendon load in the participants to determine whether the load was sufficient to induce detectable changes in a select group of inflammatory biomarkers. In order to assess whether inflammatory biomarker changes were reflected by tendon and not muscle activity, muscle fatigue was assessed using the percent (%) difference in median frequency and mean amplitude between the first and last minutes of the task (Cifrek, Medved, Tonković, & Ostojić, 2009; Dale, 2009). There were no changes above 10% identified in either median frequency or mean amplitude, suggesting that muscle fatigue was not induced by the task. Therefore, we extrapolated that the observed changes in expression of inflammatory biomarkers were likely to be a result of tendon load (Figure 9 and 10 and Table 4).

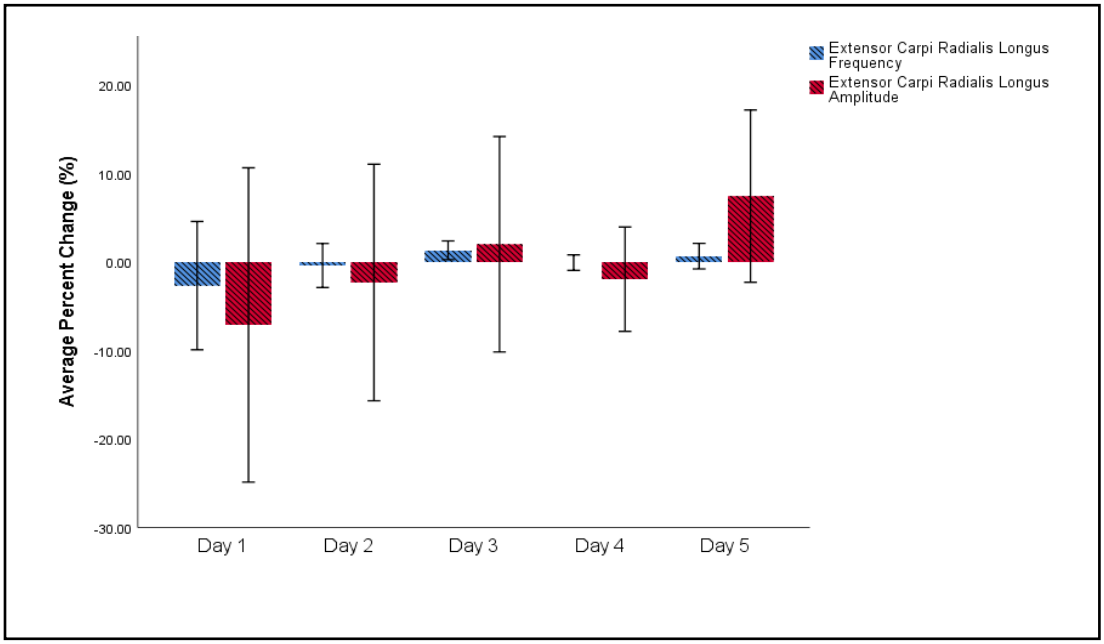

Figure 9. Percent change in median frequency and mean amplitude in the *extensor carpi radialis longus* muscle. There were no changes above 10% for any of the days.

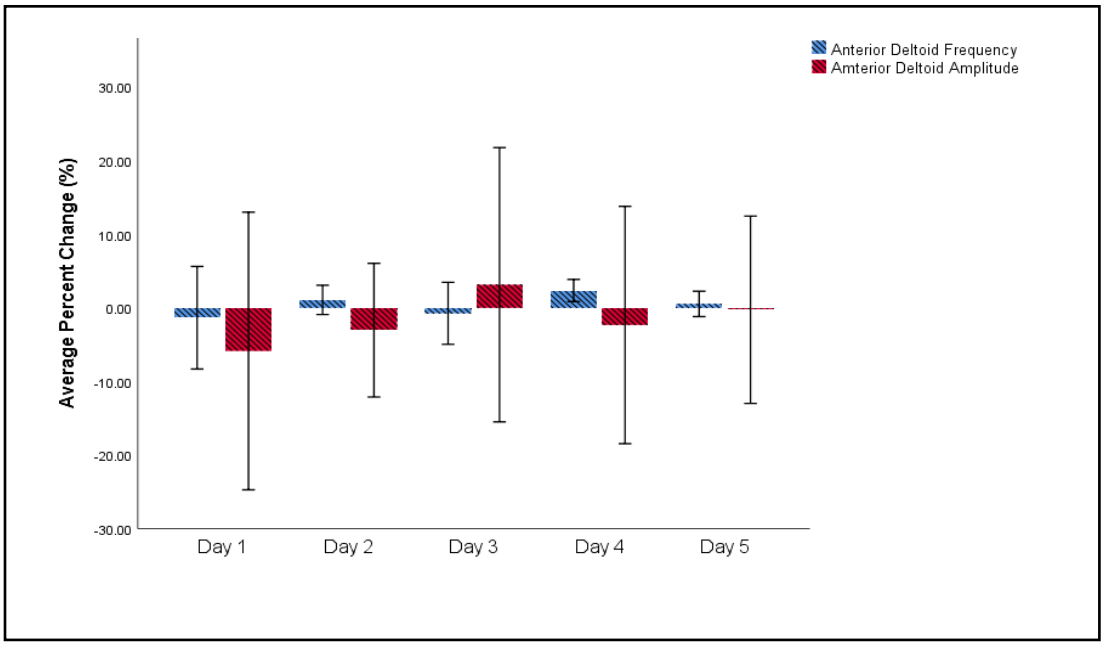

Figure 10. Percent change in median frequency and mean amplitude in the *extensor carpi radialis longus* muscle. There were no changes above 10% for any of the days.

Table 4. Median frequency and mean amplitude percent changes of the *extensor carpi radialis longus* and anterior deltoid muscles.

	Extensor Carpi Radialis Longus		Anterior Deltoid	
	Median Frequency (%)	Mean Amplitude (%)	Median Frequency (%)	Mean Amplitude (%)
Day 1	-2.66	-7.11	-1.34	-5.86
Day 2	-0.39	-2.31	1.08	-3.03
Day 3	1.33	2.01	-0.74	3.14
Day 4	-0.06	-1.94	2.35	-2.33

Inflammatory Biomarkers in Healthy Males Undergoing Repetitive Task Performance

Weight and BMI affect expression of C-reactive protein in healthy males

No significant differences ($\rho = 0.751$) in blood serum CRP concentration were identified amongst any of the collection times using Luminex methodology: baseline (0.0044 ± 0.0052 mg/L), day 3 (0.0039 ± 0.0035 mg/L), day 5 (0.0036 ± 0.00424 mg/L), and follow-up (0.0058 ± 0.00844 mg/L) (Figure 11 and Tables 5 and 6). Interestingly, all values were significantly lower than the clinically significant threshold for CRP at 10 mg/L (Riese, Vrijkotte, Meijer, Kluft, & De Geus, 2002). Therefore, a second analysis for CRP concentration in blood serum was performed using high sensitivity ELISAs to verify the validity of the results. All subsequent statistical analyses were performed using CRP concentrations obtained using high sensitivity ELISAs.

Analysis of CRP concentration in blood serum using ELISA also resulted in no significant differences ($\rho = 0.896$) between any of the collection times: baseline (1.54 ± 1.3 mg/L), Day 3 (1.72 ± 1.16 mg/L), day 5 (1.46 ± 1.5 mg/L), and follow-up (6.19 ± 7.15 mg/L) (Figure 12 and Tables 5 and 6). Given the difference in body composition, muscular strength and PPT of the participants, an ANCOVA was performed to investigate the influence of those covariates in the expression of CRP. The ANCOVA resulted in a significant influence of all the covariates in mean CRP concentration (Table 7). However, after adjusting mean CRP concentration to account for the influence of the covariates, only weight ($\rho = 0.03$) and BMI ($\rho = 0.02$) significantly affected mean CRP differences between collection points. After adjusting by weight, there was a significant difference ($\rho = 0.05$) between baseline (1.65 mg/L) and follow up (6.3 mg/L). Adjusting by BMI

resulted in a significant difference between baseline (1.64 mg/L) and follow up (1.35 mg/L; $\rho = 0.04$), day 3 (1.59 mg/L) and follow up (1.34 mg/L; $\rho = 0.04$), and day 5 (1.59 mg/L) and follow up (1.35 mg/L; $\rho = 0.03$; Tables 8 and 9). Interestingly, COX-2 levels were measured by analyte-specific ELISA but all were found to be below the assay limit of detection in the serum of all the participants and were not included further in this study.

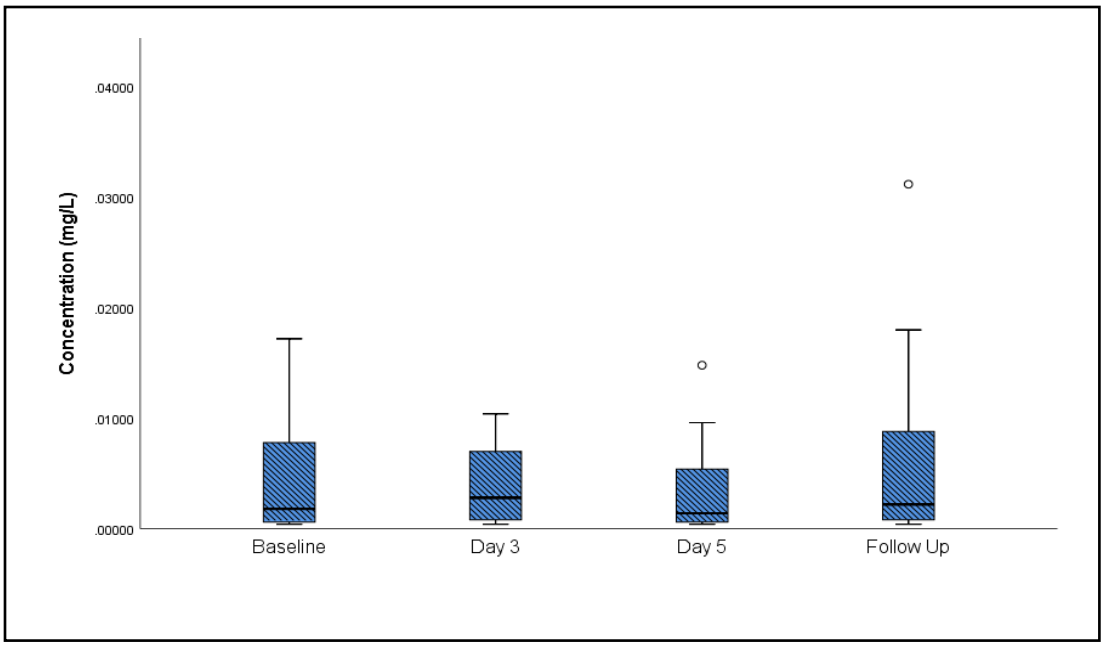

Figure 11. Average CRP concentration by collection time measured by Luminex. There was no significant difference between any of the collection times.
° Outliers

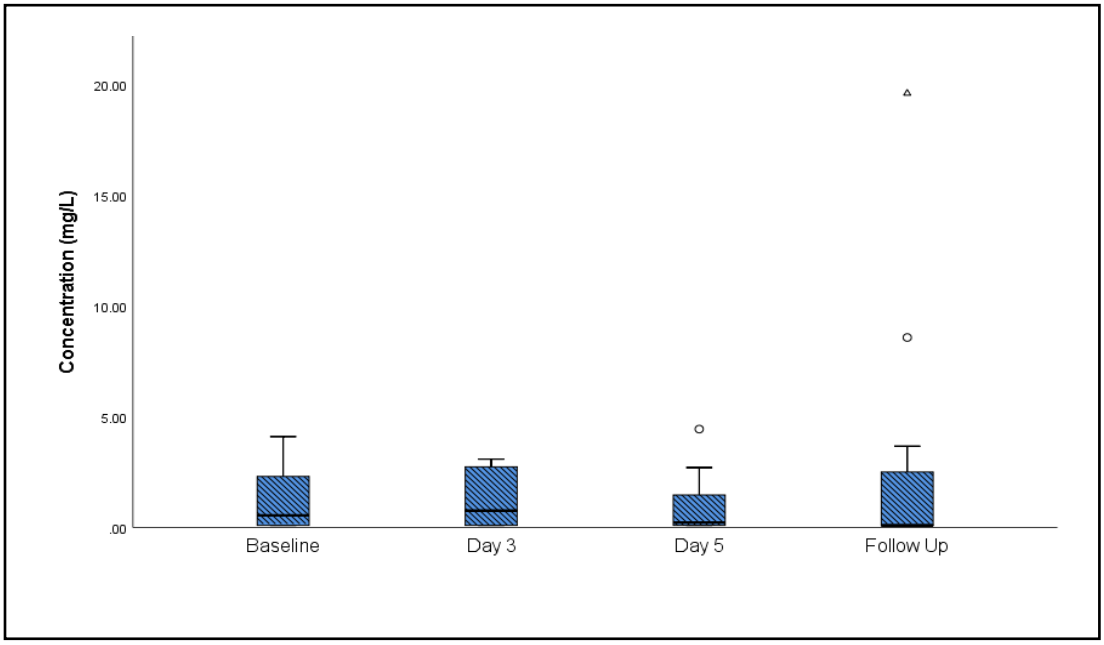

Figure 12. Average CRP concentration by collection time measured by ELISA. There was no significant difference between any of the collection times.
° Outliers
Δ Extreme Outliers

Table 5. Average CRP concentration and standard deviations by assessment method.

	Luminex		ELISA	
Collection Time	Mean	Std. Deviation	Mean	Std. Deviation
CRP Baseline (mg/L)	0.0044	0.0052	1.54	1.31
CRP Day 3 (mg/L)	0.0039	0.0035	1.72	1.16
CRP Day 5 (mg/L)	0.0036	0.0042	1.46	1.5
CRP Follow Up (mg/L)	0.0058	0.0084	6.19	7.15

Table 6. Friedman test analysis of CRP concentrations measured by Luminex and ELISA.

Test Statistics	Luminex	ELISA
Chi-Square	1.21	0.6
Degrees of Freedom	3	3
Asymptotic Significance	0.75	0.9

Table 7. ANCOVA for CRP concentration.

Covariate	Significance
Weight (kg)	0.03*
Height (m)	0.04*
BMI (kg/m^2)	0.02*
Shoulder Muscular Strength (N)	0.04*
Wrist Muscular Strength (N)	0.04*
Arm PPT (N)	0.04*

* Significant $\rho < 0.05$

Table 8. Pairwise comparisons with ANCOVA adjusted means.

		Weight (kg)		Height (m)		BMI (kg/m^2)	
Comparisons		Adjusted Mean	Significance	Adjusted Mean	Significance	Adjusted Mean	Significance
Baseline	Day 3	1.65	1	1.8	1	1.59	1
	Day 5	1.44	1	1.42	1	1.35	1
	Follow Up	6.3	0.05*	6.05	0.07	6.36	0.04*
Day 3	Baseline	1.55	1	1.58	1	1.64	1
	Day 5	1.44	1	1.42	1	1.35	1
	Follow Up	6.3	0.07	6.05	0.11	6.36	0.04*
Day 5	Baseline	1.55	1	1.58	1	1.64	1
	Day 3	1.65	1	1.8	1	1.59	1
	Follow Up	6.3	0.06	6.05	0.07	6.36	0.03*
Follow Up	Baseline	1.55	0.05*	1.58	0.07	1.64	0.04*
	Day 3	1.65	0.07	1.8	0.11	1.59	0.04*
	Day 5	1.44	0.06	1.42	0.07	1.35	0.03*

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.
b. * Significant $\rho < 0.05$

Table 9. Pairwise comparisons with ANCOVA adjusted means.

		Shoulder Strength (N)		Wrist Strength (N)		Arm PPT (N)	
Comparisons		Adjusted Mean	Significance	Adjusted Mean	Significance	Adjusted Mean	Significance
Baseline	Day 3	1.72	1	1.73	1	1.69	1
	Day 5	1.44	1	1.4	1	1.51	1
	Follow Up	6.19	0.06	6.22	0.06	6.16	0.06
Day 3	Baseline	1.55	1	1.56	1	1.55	1
	Day 5	1.44	1	1.4	1	1.51	1
	Follow Up	6.19	0.09	6.22	0.09	6.16	0.09
Day 5	Baseline	1.55	1	1.56	1	1.55	1
	Day 3	1.72	1	1.73	1	1.69	1
	Follow Up	6.19	0.08	6.22	0.07	6.16	0.08
Follow Up	Baseline	1.55	0.06	1.56	0.06	1.55	0.06
	Day 3	1.72	0.09	1.73	0.09	1.69	0.09
	Day 5	1.44	0.08	1.4	0.07	1.51	0.08

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Inflammatory cytokines IL-6 and IL-1β significantly decreased in human males after completing repetitive task

Analysis of IL-6 concentration in blood serum using Luminex resulted in no significant differences ($\rho = 0.65$) between any of the collection times: baseline (20.07 ± 16.52 pg/mL), day 3 (20.27 ± 33.37 pg/mL), day 5 (19.62 ± 19.15 pg/mL), and follow up (18.21 ± 21.79 pg/mL) (Figure 13 and Tables 10 and 11). The analysis of IL-6 concentrations using the Luminex assay resulted in several readings below the minimum threshold for detection, therefore a second analysis using ELISA was performed.

Concentrations of IL-6 in blood serum measured by ELISA at baseline (3.77 ± 1.6 pg/mL), day 3 (2.93 ± 0.93 pg/mL), day 5 (2.79 ± 0.84), and follow up (3.56 ± 1.68 pg/mL) resulted in significant differences between baseline and day 3 ($\rho = 0.009$), day 5 ($\rho = 0.002$) and no significant differences between the other conditions (Figure 14 and Tables 10, 11 and 12). Similar to CRP analysis, ANCOVA was performed to assess the influence of covariates, resulting in no significant influence of the covariates in mean IL-6 concentration (Table 13).

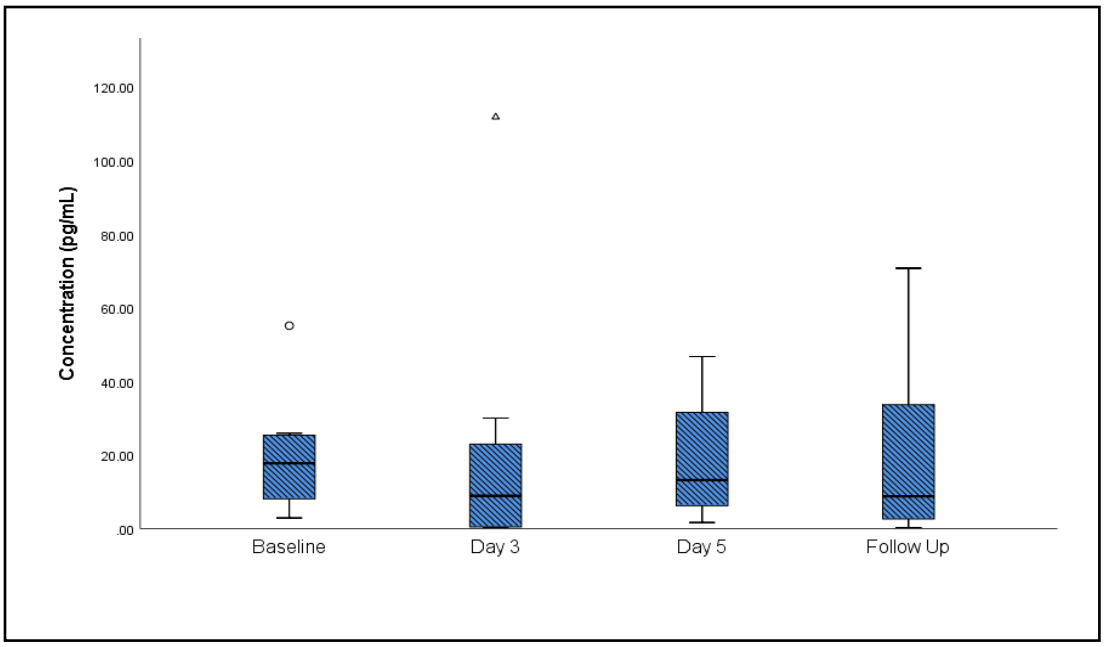

Figure 13. Average IL-6 concentration by collection time measured by Luminex. There was no significant difference between any of the collection times.
° Outliers
Δ Extreme Outliers

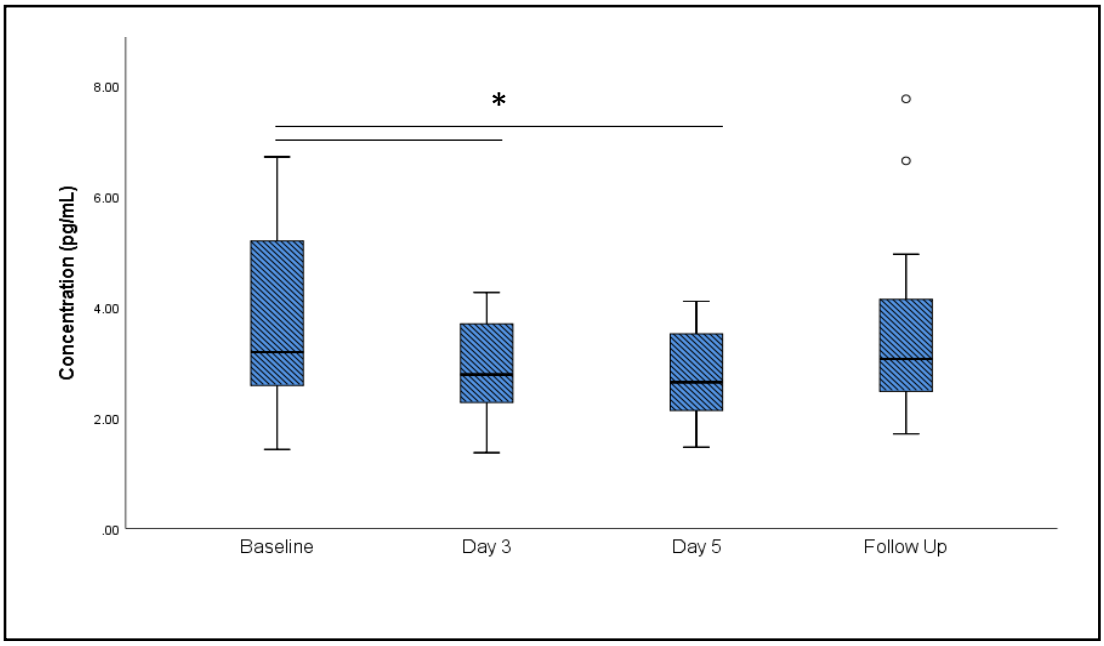

Figure 14. Average IL-6 concentration by collection time measured by ELISA. There was a significant difference between baseline and day 3 and day 5.
° Outliers
* Significant Difference ($p<0.05$)

Table 10. Average IL-6 concentrations and standard deviations.

	Luminex		ELISA	
Collection Time	Mean	Std. Deviation	Mean	Std. Deviation
IL-6 Baseline (pg/mL)	20.07	16.51	3.77	1.6
IL-6 Day 3 (pg/mL)	20.27	33.77	2.93	0.93
IL-6 Day 5 (pg/mL)	19.62	19.15	2.79	0.84
IL-6 Follow Up (pg/mL)	18.21	21.79	3.56	1.68

Table 11. Friedman test analysis of IL-6 concentrations measured by Luminex and ELISA.

Test Statistics	Luminex	ELISA
Chi-Square	1.65	10.88
Degrees of Freedom	3	3
Asymptotic Significance	0.65	0.01

Table 12. Wilcoxon signed-rank test analysis of IL-6 concentrations measured by ELISA.

Collection Times		Z	Asymptotic Significance
Baseline	Day 3	-2.612	0.009*
	Day 5	-3.155	0.002*
	Follow Up	-0.491	0.623
Day 3	Baseline	-2.612	0.009*
	Day 5	-1.474	0.14
	Follow Up	-1.138	0.255
Day 5	Baseline	-3.155	0.002*
	Day 3	-1.474	0.14
	Follow Up	-1.396	0.163
Follow Up	Baseline	-0.491	0.623
	Day 3	-1.138	0.255
	Day 5	-1.396	0.163

* Significant $\rho < 0.05$

Table 13. ANCOVA for IL-6 concentration.

Covariate	Significance
Weight (kg)	0.12
Height (m)	0.11
BMI (kg/m^2)	0.1
Shoulder Muscular Strength (N)	0.12
Wrist Muscular Strength (N)	0.11
Arm PPT (N)	0.11

Similar to IL-6, IL-1β concentrations in blood serum were first analyzed using Luminex assays then validated by ELISA. Blood serum concentrations at baseline (7.83 ± 6.29 pg/mL), day 3 (3.71 ± 1.95 pg/mL), day 5 (6.6 ± 7.79 pg/mL), and follow up (4.46 ± 5.24 pg/mL) resulted in no significant differences ($\rho = 0.87$) between the different collection times (Figure 15 and Tables 14 and 15). The use of the Luminex assay resulted in several readings below the minimum threshold for detection. Therefore, a secondary analysis using ELISA was performed to assess IL-1β in blood serum.

The measurement of blood serum concentration of IL-1β using ELISA at baseline (5.14 ± 1.5 pg/mL), day 3 (4.78 ± 1.49 pg/mL), day 5 (4.7 ± 1.53 pg/mL), and follow up (4.44 ± 1.15 pg/mL) resulted in significant differences between baseline and day 3 ($\rho = 0.02$), day 5 ($\rho = 0.039$), and follow up ($\rho = 0.015$; Figure 16 and Tables 14, 15 and 16). There were no significant differences identified amongst day 3, day 5 and follow up. The influence of covariates was assessed using ANCOVA which resulted in no significant influences amongst any of the covariates (Table 17).

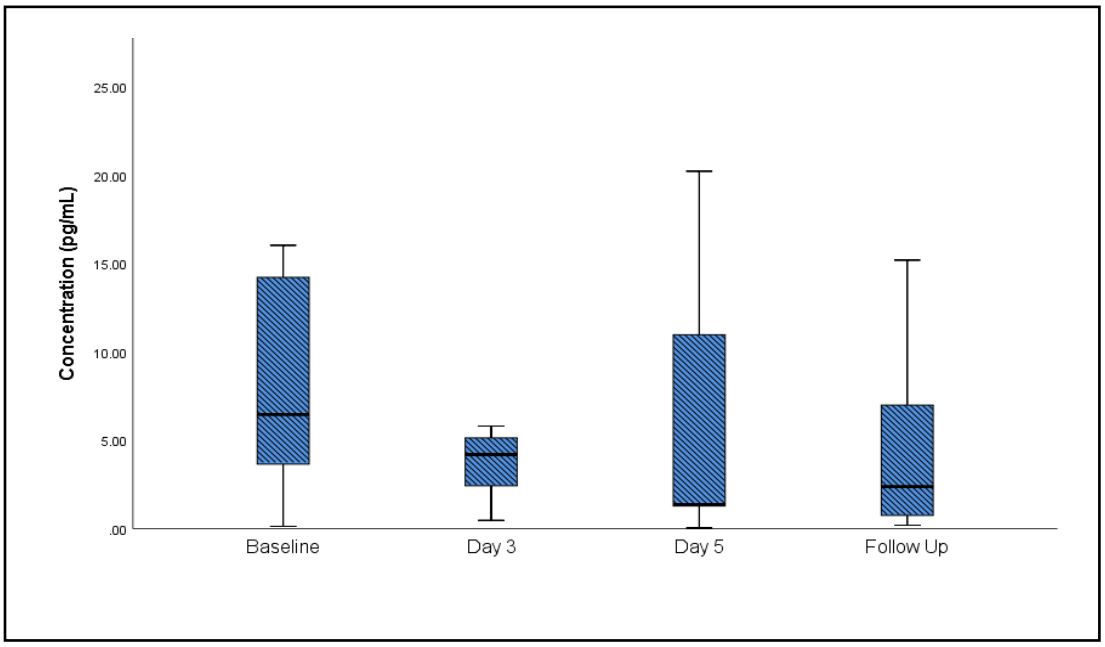

Figure 15. Average IL-1β concentration by collection time measured by Luminex. There was no significant difference between any of the collection times.

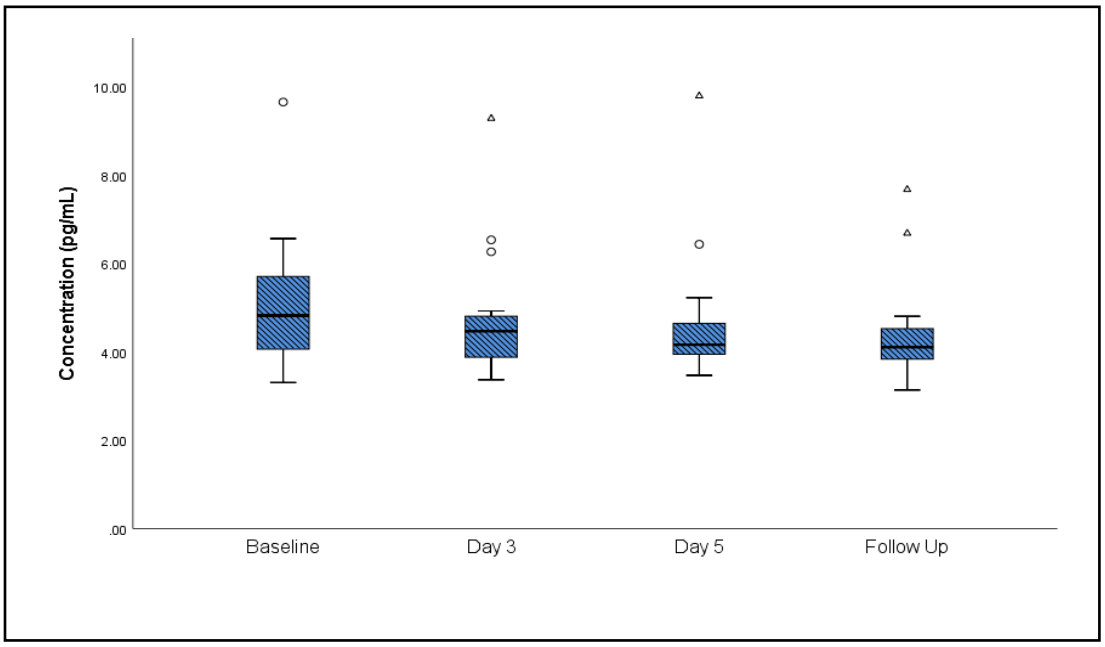

Figure 16. Average IL-1 concentration by collection time measured by ELISA. There was a significant difference between baseline and day 3, day 5 and follow up.
° Outliers
Δ Extreme Outliers

Table 14. Average IL-1β and standard deviations.

	Luminex		ELISA	
Collection Time	Mean	Std. Deviation	Mean	Std. Deviation
Baseline (pg/mL)	7.83	6.29	5.14	1.5
Day 3 (pg/mL)	3.71	1.95	4.78	1.49
Day 5 (pg/mL)	6.6	7.79	4.7	1.53
Follow Up (pg/mL)	4.46	5.24	4.44	1.15

Table 15. Friedman test analysis of IL-1β concentrations measured by Luminex and ELISA.

Test Statistics	Luminex	ELISA
Chi-Square	0.72	9.3
Degrees of Freedom	3	3
Asymptotic Significance	0.87	0.03

Table 16. Wilcoxon signed-rank test analysis of IL-1β concentrations measured by ELISA

Collection Times		Z	Asymptotic Significance
Baseline	Day 3	-2.327	0.02*
	Day 5	-2.068	0.039*
	Follow Up	-2.43	0.015*
Day 3	Baseline	-2.327	0.02*
	Day 5	-0.414	0.679
	Follow Up	-1.603	0.109
Day 5	Baseline	-2.068	0.039*
	Day 3	-0.414	0.679
	Follow Up	-1.603	0.109
Follow Up	Baseline	-2.43	0.015*
	Day 3	-1.603	0.109
	Day 5	-1.603	0.109

* Significant $\rho < 0.05$

Table 17. ANCOVA for IL-1β concentration.

Covariate	Significance
Weight (kg)	0.57
Height (m)	0.41
BMI (kg/m^2)	0.58
Shoulder Muscular Strength (N)	0.59
Wrist Muscular Strength (N)	0.53
Arm PPT (N)	0.59

Significant differences in MMP concentrations derived from performance of simple manual task

Comparative analysis of blood serum MMP-1 levels at baseline (4.26 ± 3.14 ng/mL), day 3 (3.72 ± 2.59 ng/mL), day 5 (4.28 ± 3.1 ng/mL), and follow-up (4.36 ± 2.96 ng/mL) resulted in significant differences between day 3 and day 5 ($\rho = 0.064$) and day 3 and follow up ($\rho = 0.061$; Figure 17 and Tables 18, 19, and 20). The ANCOVA resulted in no significant influence of any of the covariates (Table 21).

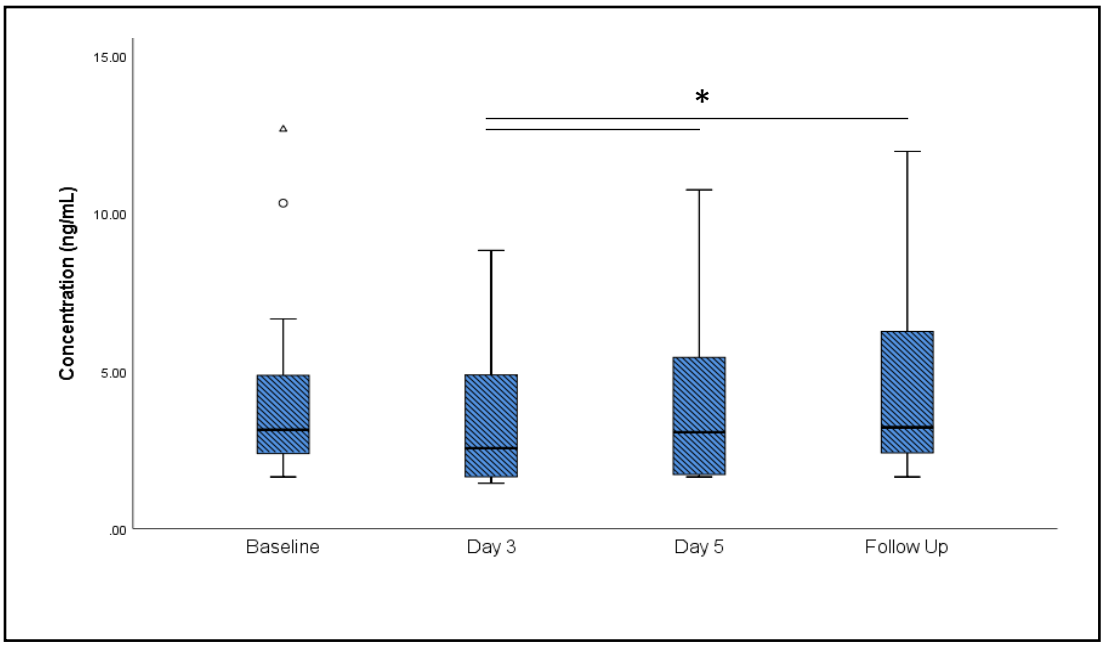

Figure 17. Average MMP-1 concentration in blood serum. There was a significant difference between day 3 and day 5, and between day 3 and follow up.
° Outliers
Δ Extreme Outliers
* Significant Difference ($p<0.05$)

Table 18. Average MMP-1 concentrations and standard deviations.

Collection Time	Mean	Std. Deviation
MMP-1 Baseline (ng/mL)	4.26	3.14
MMP-1 Day 3 (ng/mL)	3.72	2.59
MMP-1 Day 5 (ng/mL)	4.28	3.1
MMP-1 Follow Up (ng/mL)	4.36	2.96

Table 19. Friedman test analysis of MMP-1 concentration.

Test Statistics	
Chi-Square	11.9
Degrees of Freedom	3
Asymptotic Significance	0.008

Table 20. Wilcoxon signed-rank test analysis of MMP-1 concentrations.

Collection Times		Z	Asymptotic Significance
Baseline	Day 3	-1.59	0.112
	Day 5	-1.022	0.307
	Follow Up	-1.396	0.163
Day 3	Baseline	-1.59	0.112
	Day 5	-1.853	0.064
	Follow Up	-1.874	0.061
Day 5	Baseline	-1.022	0.307
	Day 3	-1.853	0.064
	Follow Up	-0.534	0.594
Follow Up	Baseline	-1.396	0.163
	Day 3	-1.874	0.061
	Day 5	-0.534	0.594

Table 21. ANCOVA for MMP-1 Concentration.

Covariate	Significance
Weight (kg)	0.9
Height (m)	0.92
BMI (kg/m^2)	0.87
Shoulder Muscular Strength (N)	0.92
Wrist Muscular Strength (N)	0.92
Arm PPT (N)	0.92

The analysis of MMP-2 concentrations in blood serum at baseline (149.45 ± 41.99 ng/mL), day 3 (135.5 ± 34.09 ng/mL), day 5 (146.97 ± 41.84 ng/mL), and follow-up (152.05 ± 44.98 ng/mL) resulted in no significant differences ($\rho = 0.07$) between any of the conditions (Figure 18 and Tables 22 and 23). The ANCOVA resulted in no significant influence of any of the covariates (Table 24).

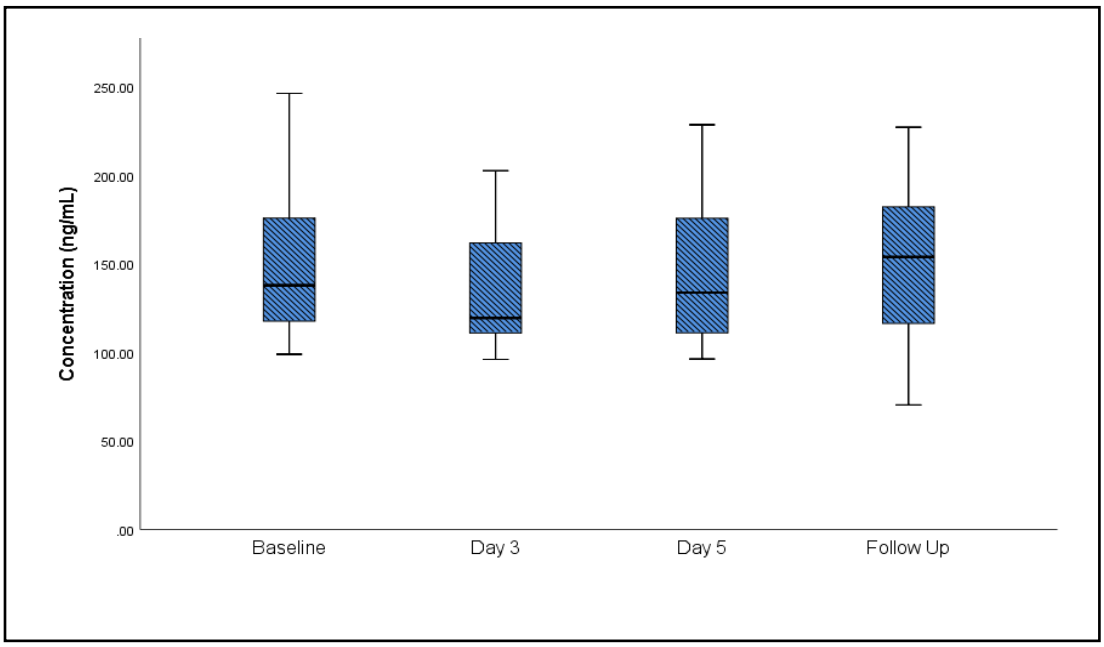

Figure 18. Average MMP-2 concentration by collection time. There was no significant difference between any of the collection times.

Table 22. Average MMP-2 concentration and standard deviation.

	Mean	Std. Deviation
MMP-2 Baseline (ng/mL)	149.45	41.99
MMP-2 Day 3 (ng/mL)	135.5	34.09
MMP-2 Day 5 (ng/mL)	146.97	41.84
MMP-2 Follow Up (ng/mL)	152.05	44.98

Table 23. Friedman test analysis of MMP-2 concentration.

Test Statistics	
Chi-Square	7.13
Degrees of Freedom	3
Asymptotic Significance	0.07

Table 24. ANCOVA for MMP-2 concentration.

Covariate	Significance
Weight (kg)	0.68
Height (m)	0.66
BMI (kg/m^2)	0.63
Shoulder Muscular Strength (N)	0.66
Wrist Muscular Strength (N)	0.67
Arm PPT (N)	0.67

Analysis of average MMP-3 values in serum at baseline (47.43 ± 66.26 ng/mL), day 3 (33.62 ± 13.39 ng/mL), day 5 (33.47 ± 12.6 ng/mL), and follow up (85.19 ± 107.28 ng/mL) resulted in significant differences between baseline and follow up (ρ =0.03), day 3 and follow up (ρ = 0.004), and day 5 and follow up (ρ = 0.006; Figure 19 and Tables 25, 26, and 27). The ANCOVA assessment resulted in no significant influence of any of the covariates (Table 28).

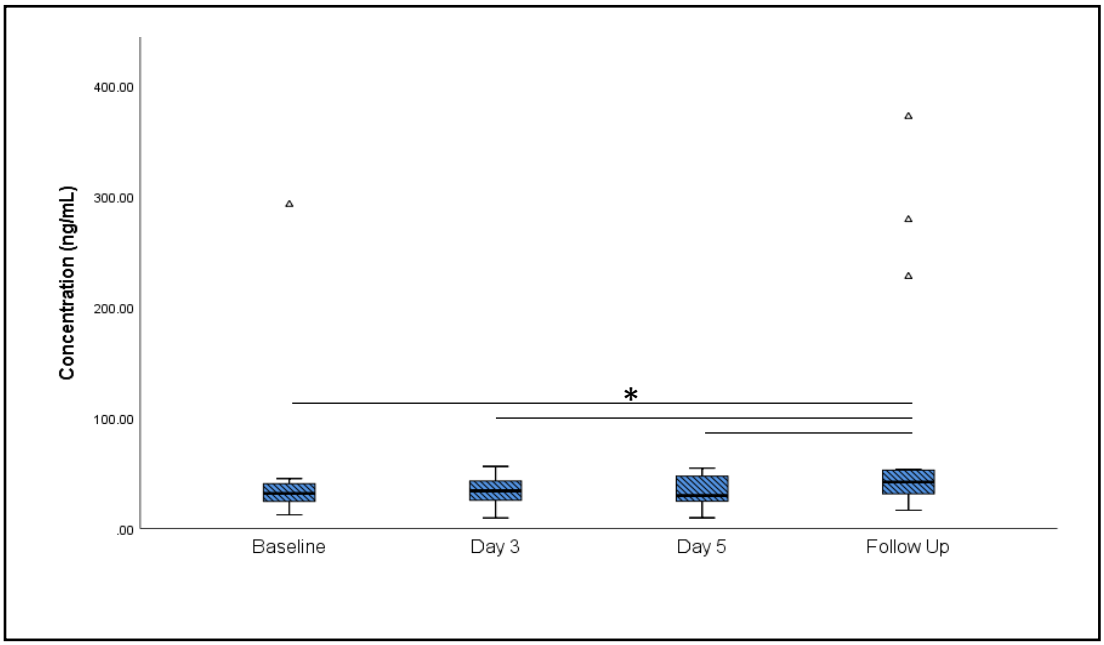

Figure 19. Average MMP-3 concentration by collection time. There was a significant difference between baseline and follow up, day 3 and follow up, and day 5 and follow-up.
$^{\Delta}$ Extreme Outliers
* Significant Difference ($p<0.05$)

Table 25. Average MMP-3 concentration and standard deviation.

	Mean	Std. Deviation
MMP-3 Baseline (ng/mL)	47.43	66.26
MMP-3 Day 3 (ng/mL)	33.62	13.39
MMP-3 Day 5 (ng/mL)	33.47	13.6
MMP-3 Follow Up (ng/mL)	85.19	107.28

Table 26. Friedman test analysis of MMP-3 concentration.

Test Statistics	
Chi-Square	12.98
Degrees of Freedom	3
Asymptotic Significance	0.005

Table 27. Wilcoxon signed-rank test analysis of MMP-3.

Collection Times		Z	Asymptotic Significance
Baseline	Day 3	-0.621	0.535
	Day 5	-0.465	0.642
	Follow Up	-2.172	0.03*
Day 3	Baseline	-0.621	0.535
	Day 5	-0.052	0.959
	Follow Up	-2.844	0.004*
Day 5	Baseline	-0.465	0.642
	Day 3	-0.052	0.959
	Follow Up	-2.741	0.006*
Follow Up	Baseline	-2.172	0.03*
	Day 3	-2.844	0.004*
	Day 5	-2.741	0.006*

* Significant $\rho < 0.05$

Table 28. ANCOVA for MMP-3 concentration.

Covariate	Significance
Weight (kg)	0.086
Height (m)	0.086
BMI (kg/m^2)	0.086
Shoulder Muscular Strength (N)	0.084
Wrist Muscular Strength (N)	0.078
Arm PPT (N)	0.086

Average MMP-9 concentration in blood serum at baseline (86.90 ± 43.43 ng/mL), day 3 (72.99 ± 16.06 ng/mL), day 5 (74.21 ± 28.67 ng/mL), and follow up (85.53 ± 43.88 ng/mL) resulted in no significant differences ($\rho = 0.62$) between any of the collection times (Figure 20 and Tables 29 and 30). The ANCOVA assessment resulted in no significant influence of any of the covariates (Table 31).

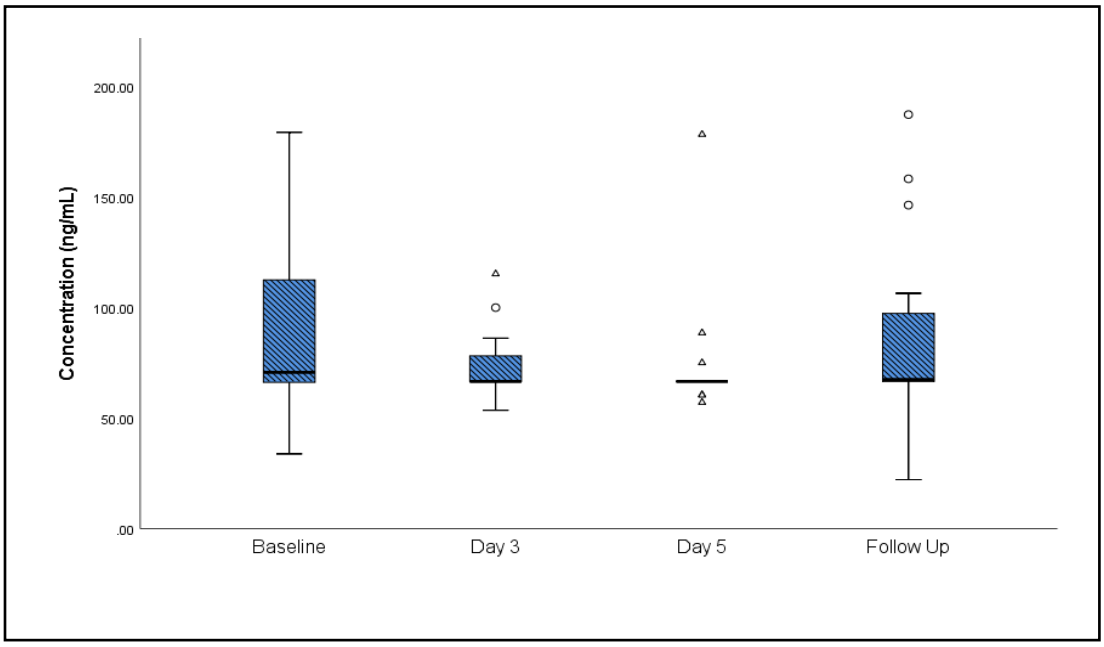

Figure 20. Average MMP-9 concentration by collection time. There was no significant difference between any of the collection times.
° Outliers
Δ Extreme Outliers

Table 29. Average MMP-9 concentration and standard deviation.

	Mean	Std. Deviation
MMP-9 Baseline (ng/mL)	86.9	43.43
MMP-9 Day 3 (ng/mL)	72.99	16.06
MMP-9 Day 5 (ng/mL)	74.21	28.67
MMP-9 Follow up (ng/mL)	85.53	43.88

Table 30. Friedman test analysis of MMP-9 concentration.

Test Statistics	
Chi-Square	1.78
Degrees of Freedom	3
Asymptotic Significance	0.62

Table 31. ANCOVA for MMP-9 concentration.

Covariate	Significance
Weight (kg)	0.552
Height (m)	0.555
BMI (kg/m^2)	0.559
Shoulder Muscular Strength (N)	0.539
Wrist Muscular Strength (N)	0.551
Arm PPT (N)	0.559

Measurement of Physical Parameters Identify Significant Changes in Perceived Exertion

Measurements of perceived exertion were divided into resting and task RPE. For analysis, the mean of two resting and three task RPE periods per day were used for analysis. The average RPE at rest for day 1 (7.28 ±1.41), day 2 (7.34 ± 1.68), day 3 (7.25 ± 1.82), day 4 (7.22 ± 1.79), and day 5 (7.38 ± 1.88) showed no significant differences between any of the collection times (Figure 18 and Tables 32 and 33). The average RPE while performing the task for day 1 (11.43 ± 2.39), day 2 (11.13 ± 2.5), day 3 (10.33 ± 2.39), day 4 (10.13 ± 2.48), and day 5 (10.06 ± 2.62) showed significant differences between day 1 and day 3 ($p = 0.03$), day 4 ($p = 0.012$), and day 5 ($p = 0.016$), between day 2 and day 3 ($p = 0.006$), day 2, day 4 ($p = 0.005$), and day 5 ($p = 0.009$; Figure 21 and Tables 32 and 33). These data suggest that the level of perceived exertion is reduced as the duration of the repetitive task increases.

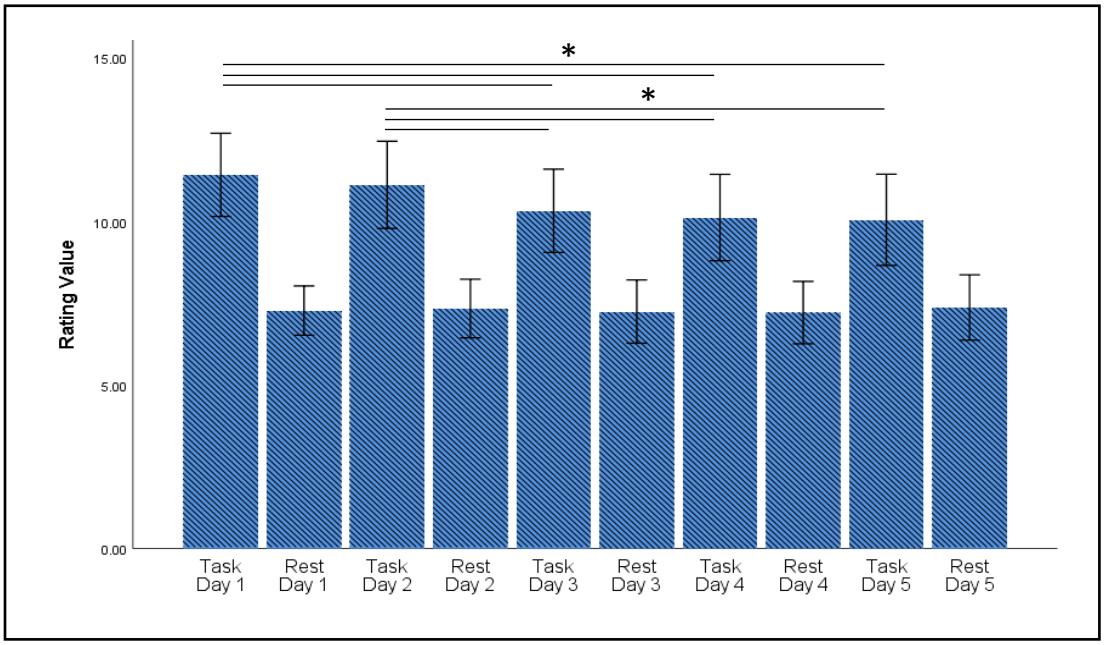

Figure 21. Average RPE values. There was a significant difference between task RPE at day 1 and task RPE at days 3, 4, and 5, and between task RPE day 2 and task RPE days 3, 4, and 5.

Table 32. Average resting and task RPE values and standard deviation.

	Rest		Task	
Collection Time	Mean	Std. Deviation	Mean	Std. Deviation
RPE Day 1	7.28	1.41	11.43	2.39
RPE Day 2	7.34	1.68	11.13	2.5
RPE Day 3	7.25	1.82	10.33	2.39
RPE Day 4	7.22	1.79	10.13	2.48
RPE Day 5	7.38	1.88	10.06	2.62

Table 33. Repeated measures ANOVA of resting and task RPE.

		Rest		Task	
Collection Times		Mean Difference	Significance	Mean Difference	Significance
Day 1	Day 2	-0.06	1	0.31	1
	Day 3	0.03	1	1.1	0.03
	Day 4	0.06	1	1.31	0.01
	Day 5	-0.09	1	1.38	0.02
Day 2	Day 1	0.06	1	-0.31	1
	Day 3	0.09	1	0.79	0.01
	Day 4	0.13	1	1	0.01
	Day 5	-0.03	1	1.07	0.01
Day 3	Day 1	-0.03	1	-1.1	0.03
	Day 2	-0.09	1	-0.79	0.01
	Day 4	0.03	1	0.21	1
	Day 5	-0.13	1	0.28	1
Day 4	Day 1	-0.06	1	-1.31	0.01
	Day 2	-0.13	1	-1	0.01
	Day 3	-0.03	1	-0.21	1
	Day 5	-0.16	0.96	0.07	1
Day 5	Day 1	0.09	1	-1.38	0.02
	Day 2	0.03	1	-1.07	0.01
	Day 3	0.13	1	-0.28	1
	Day 4	0.16	0.96	-0.07	1

* Significant $\rho < 0.05$

Heart Rate Variability is not Significantly Changed at Rest or During the Performance of a Repetitive Task

Similar to the observed measurements of RPE, HRV measurements were divided into two groups: resting HRV and task HRV. The assessment of HRV at rest for day 1 (48 ± 28.06 ms), day 2 (39.78 ± 12.45 ms), day 3 (45.67 ± 23.98 ms), day 4 (45.07 ± 21.72 ms), and day 5 (42.2 ± 24.4 ms) resulted in no significant differences between any of the collection times (Figure 19 and Tables 34 and 35). The average HRV while performing the task at day 1 (29.18 ± 21.71 ms), day 2 (21.52 ± 8.36 ms), day 3 (25.53 ± 15.51 ms), day 4 (24.77 ± 10.72 ms), and day 5 (22.96 ± 10.24 ms) resulted in no significant difference in HRV while performing the task at different collection times (Figure 22 and Tables 34 and 35).

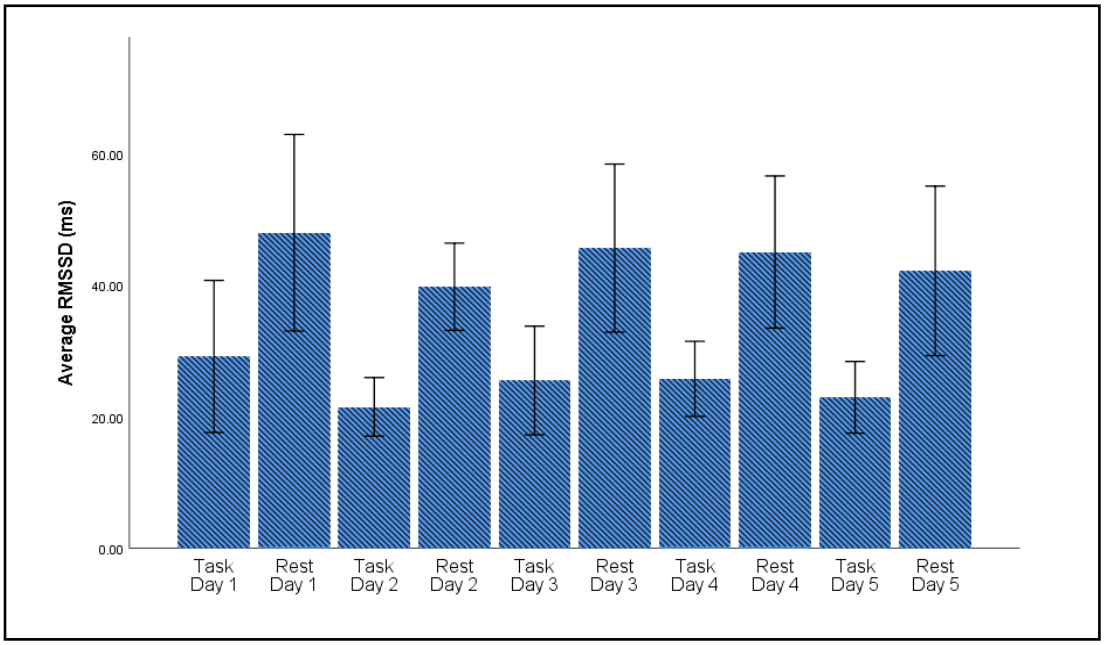

Figure 22. Average HRV measured as RMSSD. There was no significant difference in HRV between any of the collection times.

Table 34. Average and task HRV and standard deviation.

	Rest		Task	
Collection Time	Mean	Std. Deviation	Mean	Std. Deviation
HRV Day 1 (ms)	48	28.06	29.18	21.71
HRV Day 2 (ms)	39.78	12.45	21.52	8.36
HRV Day 3 (ms)	45.67	23.98	25.53	15.51
HRV Day 4 (ms)	45.07	21.72	25.77	10.72
HRV Day 5 (ms)	42.2	24.20	22.96	10.24

Table 35. Repeated measures ANOVA of resting and task HRV.

		Rest		Task	
Collection Times		Mean Difference	Significance	Mean Difference	Significance
Day 1 (ms)	Day 2	8.22	1	7.66	1
	Day 3	2.34	1	3.64	1
	Day 4	2.94	1	3.4	1
	Day 5	5.8	1	6.21	0.97
Day 2 (ms)	Day 1	-8.22	1	-7.66	1
	Day 3	-5.88	1	-4.01	1
	Day 4	-5.28	1	-4.26	0.46
	Day 5	-2.42	1	-1.45	1
Day 3 (ms)	Day 1	-2.34	1	-3.64	1
	Day 2	5.88	1	4.01	1
	Day 4	0.6	1	-0.24	1
	Day 5	3.46	1	2.57	1
Day 4 (ms)	Day 1	-2.94	1	-3.4	1
	Day 2	5.28	1	4.26	0.46
	Day 3	-0.6	1	0.24	1
	Day 5	2.86	1	2.81	1
Day 5 (ms)	Day 1	-5.8	1	-6.21	0.97
	Day 2	2.42	1	1.45	1
	Day 3	-3.46	1	-2.57	1
	Day 4	-2.86	1	-2.81	1

Peak low and high frequencies of the HRV frequency domain were also analyzed for each of the five days of the manual task. Similar to HRV RMSSD, measurements were divided into resting and task frequencies for each day. Average low and high frequency for rest at day 1 (0.09 ± 0.01 Hz and 0.2 ± 0.05 Hz), day 2 (0.09 ± 0.02 Hz and 0.21 ± 0.06 Hz), day 3 (0.09 ± 0.03 Hz and 0.21 ± 0.07 Hz), day 4 (0.09 ± 0.03 Hz and 0.2 ± 0.04 Hz), and day 5 (0.09 ± 0.02 Hz and 0.21 ± 0.05) resulted in no significant differences between any of the collection times (Figure 23 and Tables 36, 37, 38). Furthermore, average low and high frequency during the task at day 1 (0.09 ± 0.01 Hz and 0.21 ± 0.05 Hz), day 2 (0.08 ± 0.01 Hz and 0.22 ± 0.05 Hz), day 3 (0.09 ± 0.01 Hz and 0.2 ± 0.04 Hz), day 4 (0.08 ± 0.01 Hz and 0.21 ± 0.05 Hz), and day 5 (0.08 ± 0.01 Hz and 0.22 ± 0.04 Hz) resulted in no significant differences between any of the collection times. These data suggest that heart rate variability does not increase before, during, or after the performance of a repetitive task (Figure 24 and Tables 36, 37, 38).

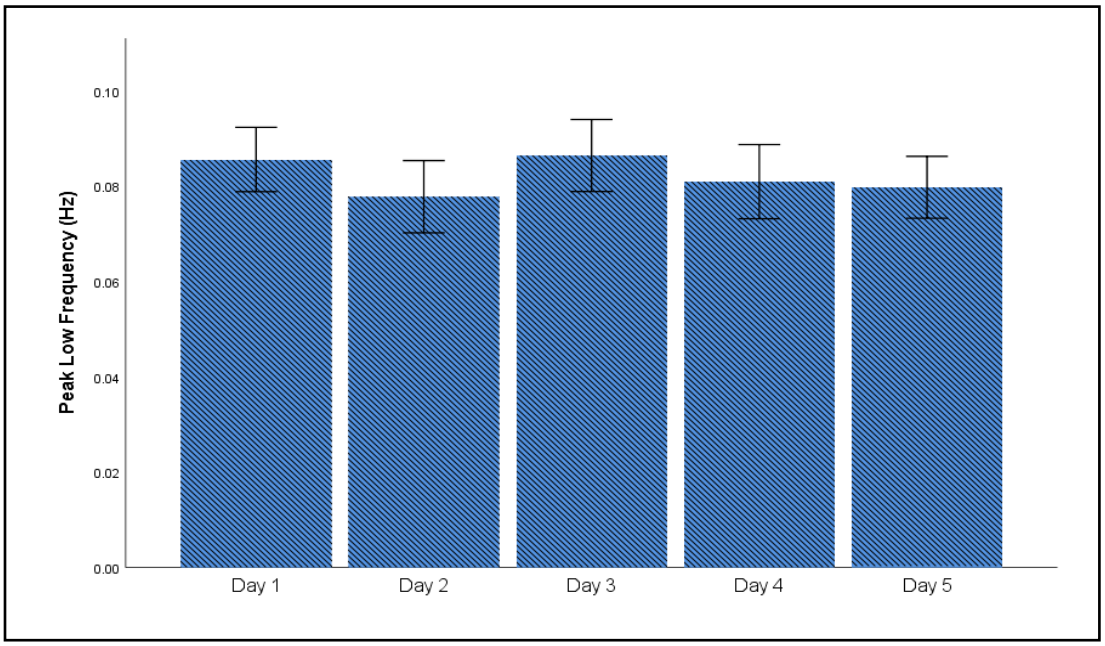

Figure 23. Average peak low frequency. There was no significant difference in low frequency between any of the collection times.

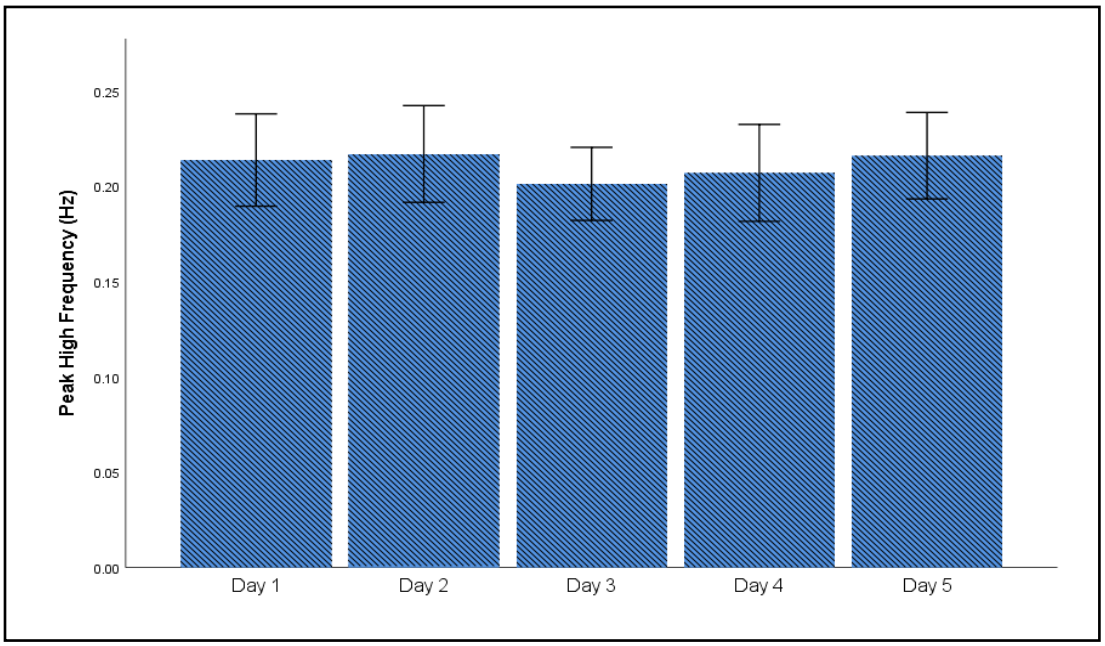

Figure 24. Average peak high frequency. There was no significant difference in high frequency between any of the collection times.

Table 36. Mean low and high frequency and standard deviation

	Low Frequency (Hz)				High Frequency (Hz)			
	Rest		Task		Rest		Task	
Collection Time	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
Day 1	0.09	0.01	0.09	0.01	0.2	0.05	0.21	0.05
Day 2	0.09	0.02	0.08	0.01	0.21	0.06	0.22	0.05
Day 3	0.09	0.02	0.09	0.01	0.21	0.07	0.2	0.04
Day 4	0.09	0.03	0.08	0.01	0.2	0.04	0.21	0.05
Day 5	0.09	0.02	0.08	0.01	0.21	0.05	0.22	0.04

Table 37. Repeated measures ANOVA for peak low frequency

		Rest		Task	
Collection Times		Mean Difference	Significance	Mean Difference	Significance
Day 1 (Hz)	Day 2	0	1	0.01	0.21
	Day 3	0	1	0	1
	Day 4	0	1	0.01	1
	Day 5	0	1	0.01	0.62
Day 2 (Hz)	Day 1	0	1	-0.01	0.21
	Day 3	0	1	-0.01	0.21
	Day 4	0	1	0	1
	Day 5	0	1	0	1
Day 3 (Hz)	Day 1	0	1	0	1
	Day 2	0	1	0.01	0.21
	Day 4	0	1	0.01	1
	Day 5	0	1	0.01	1
Day 4 (Hz)	Day 1	0	1	-0.01	1
	Day 2	0	1	0	1
	Day 3	0	1	-0.01	1
	Day 5	0	1	0	1
Day 5 (Hz)	Day 1	0	1	-0.01	0.62
	Day 2	0	1	0	1
	Day 3	0	1	-0.01	1
	Day 4	0	1	0	1

Table 38. Repeated measures ANOVA for peak high frequency.

		Rest		Task	
Collection Times		Mean Difference	Significance	Mean Difference	Significance
Day 1 (Hz)	Day 2	0	1	0	1
	Day 3	-0.01	1	0.01	1
	Day 4	0	1	0.01	1
	Day 5	-0.01	1	0	1
Day 2 (Hz)	Day 1	0	1	0	1
	Day 3	0	1	0.02	1
	Day 4	0.01	1	0.01	1
	Day 5	0	1	0	1
Day 3 (Hz)	Day 1	0.01	1	-0.01	1
	Day 2	0	1	-0.02	1
	Day 4	0.01	1	-0.01	1
	Day 5	0	1	-0.02	1
Day 4 (Hz)	Day 1	0	1	-0.01	1
	Day 2	-0.01	1	-0.01	1
	Day 3	-0.01	1	0.01	1
	Day 5	-0.01	1	-0.01	1
Day 5 (Hz)	Day 1	0.01	1	0	1
	Day 2	0	1	0	1
	Day 3	0	1	0.02	1
	Day 4	0.01	1	0.01	1

Correlation Analysis

Following analysis of statistical differences and the influence of covariates were analyzed for serum markers (inflammatory cytokines and MMPs), perceived exertion and HRV, we sought to determine whether correlations existed amongst them. The identification of relevant associations could identify a group of surrogate markers that could be used as a marker of risk during pre-injurious tendon damage.

Significant correlations between CRP expression and expression of IL-6 and MMP-9 after completing simple task for 3 days

We sought to determine whether changes in CRP levels correlated with other inflammatory biomarkers or physical measures. No relevant correlations were identified between CRP and IL-1β, MMP-1, MMP-2, and MMP-3. Interestingly, a positive correlation was identified between CRP and IL-6 at day 3 ($r = 0.71$) and follow up ($r = 0.83$) indicating that as CRP levels rise the level of IL-6 also increases. A positive correlation was also identified between CRP and MMP-3 at day 3, indicating that as CRP increases MMP-3 increases (Figures 25, 26, and 27 and Table 39).

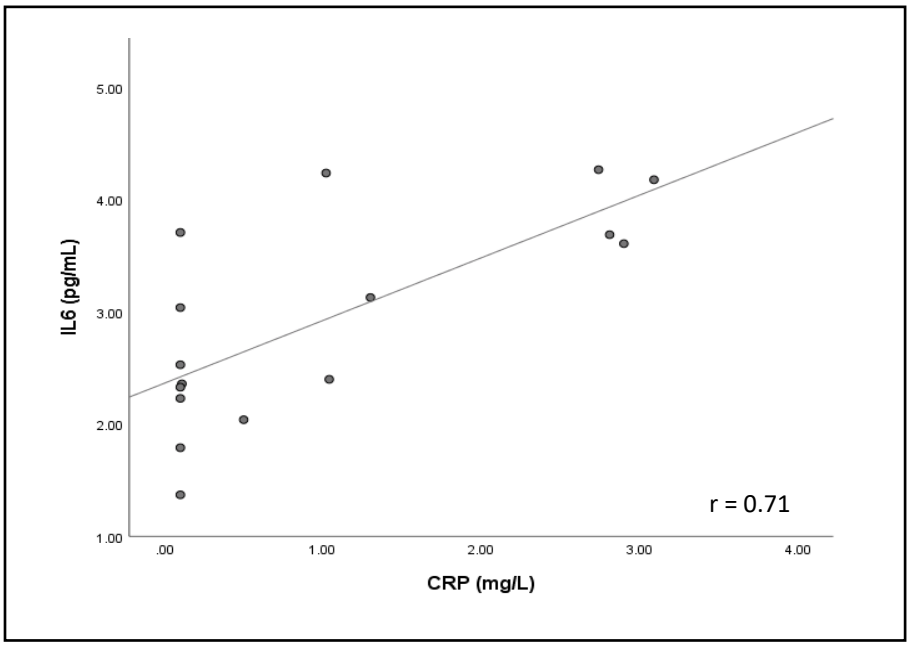

Figure 25. Correlation between CRP and IL-6 at day 3. There was a positive correlation between CRP and IL-6 at day 3.

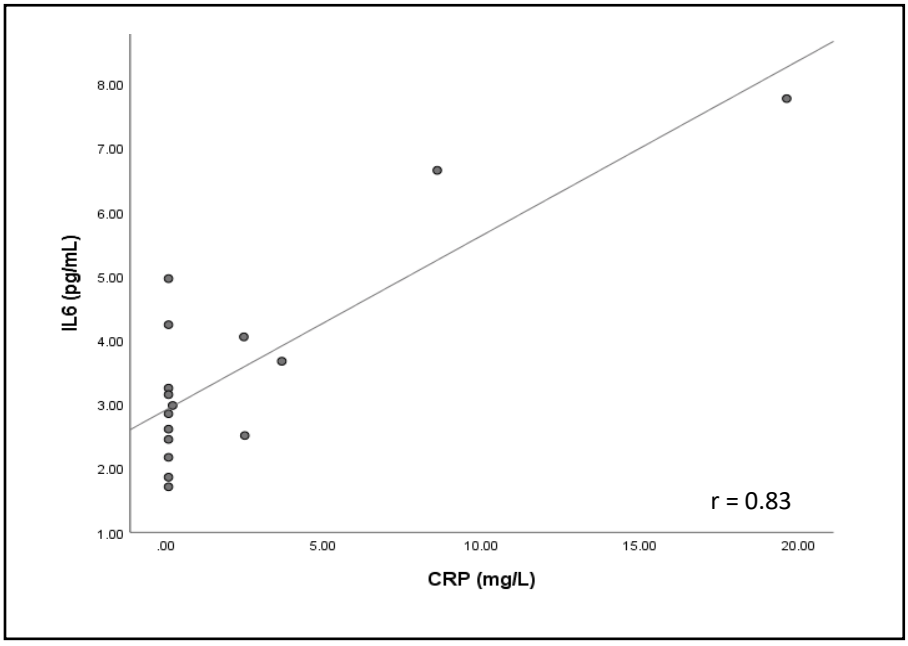

Figure 26. Correlation between CRP and IL-6 at follow up. There was a positive correlation between CRP and IL-6 at follow up.

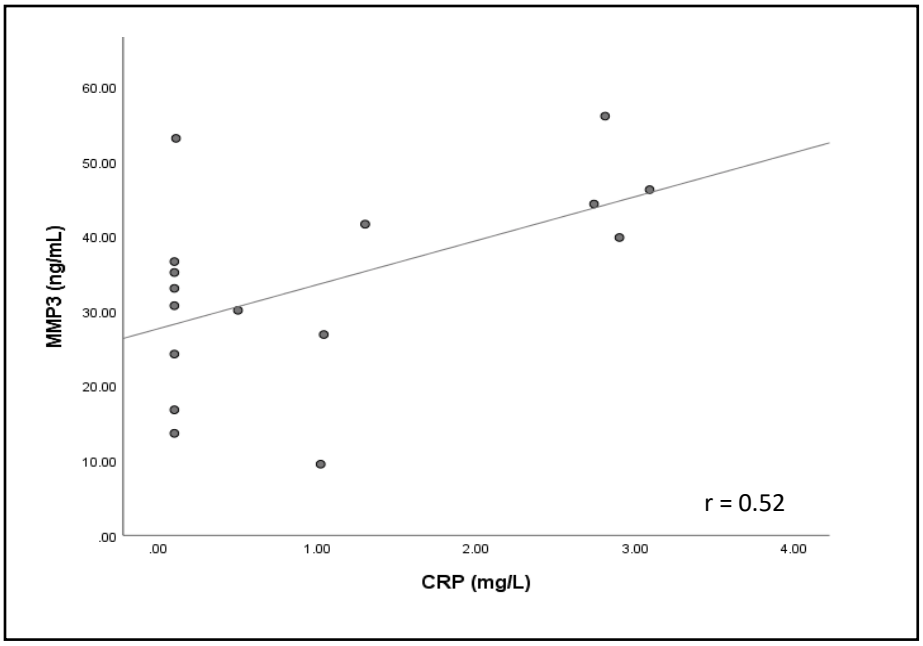

Figure 27. Correlation between CRP and MMP-3 at day 3. There was a positive correlation between CRP and MMP-3 at day 3.

Table 39. Correlations between CRP and inflammatory cytokines and MMPs.

		CRP (mg/L)			
Collection Time		Baseline	Day 3	Day 5	Follow Up
IL-6 (pg/mL)	Baseline	-0.316	-0.326	-0.345	-0.253
	Day 3	-0.087	-0.063	-0.162	-0.111
	Day 5	-0.093	-0.065	-0.158	-0.122
	Follow Up	-0.014	0.029	-0.138	0.013
IL-1β (pg/mL)	Baseline	0.448	0.598*	0.42	-0.035
	Day 3	0.712**	0.714**	0.62*	0.27
	Day 5	0.597*	0.71**	0.477	0.136
	Follow Up	0.283	0.26	0.138	0.831**
MMP-1 (ng/mL)	Baseline	-0.316	-0.326	-0.345	-0.253
	Day 3	-0.087	-0.063	-0.162	-0.111
	Day 5	-0.093	-0.065	-0.158	-0.122
	Follow Up	-0.014	0.029	-0.138	0.013
MMP-2 (ng/mL)	Baseline	-0.316	-0.326	-0.345	-0.253
	Day 3	-0.087	-0.063	-0.162	-0.111
	Day 5	-0.093	-0.065	-0.158	-0.122
	Follow Up	-0.014	0.029	-0.138	0.013
MMP-3 (ng/mL)	Baseline	0.448	0.598*	0.42	-0.035
	Day 3	0.712**	0.714**	0.62*	0.27
	Day 5	0.597*	0.71**	0.477	0.136
	Follow Up	0.283	0.26	0.138	0.831**
MMP-9 (ng/mL)	Baseline	-0.327	-0.418	-0.292	-0.141
	Day 3	-0.396	-0.489	-0.373	-0.06
	Day 5	-0.286	-0.376	-0.301	-0.02
	Follow Up	-0.381	-0.497	-0.351	-0.182

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Significant correlations between basal levels of IL-6 and MMP-9 and between IL-1β and MMP-9 after completing simple task for 3 days

No relevant correlations were identified between IL-6 and IL-1β, MMP-1, MMP-2 and MMP-9. A positive correlation was identified between IL-6 and MMP-3 at baseline, suggesting that basal levels of IL-6 correspond to basal levels of MMP-3 (Figure 28 and Table 40). No relevant correlations were observed between IL-1β, CRP, IL-6, MMP-1, MMP-2, and MMP-3. A positive correlation was identified between IL-1β and MMP-9 at day 3, suggesting that elevated concentrations of IL-1β correspond to elevated concentrations of MMP-9 after completing a simple task for 3 days (Figure 29 and Table 41).

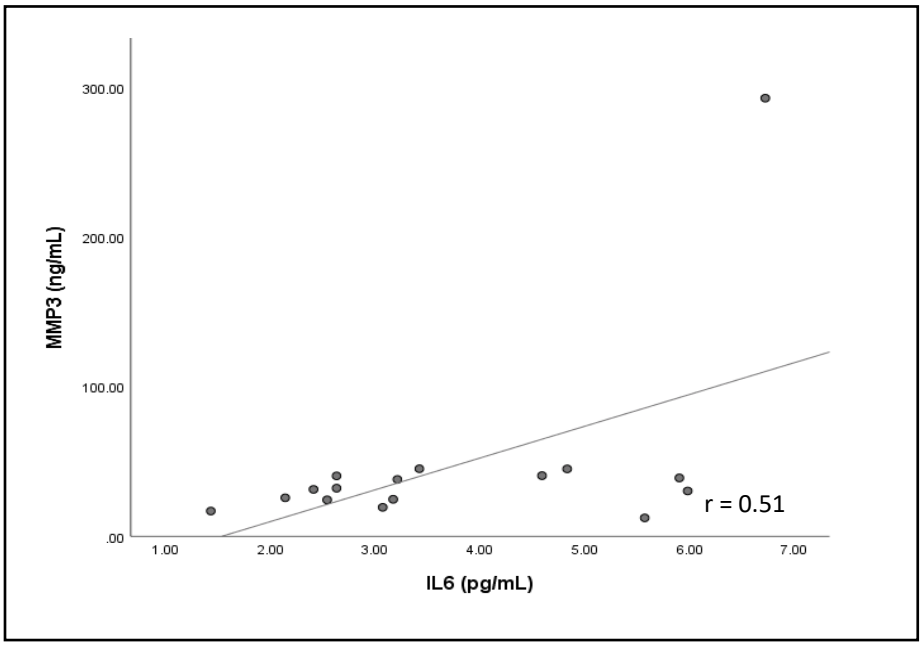

Figure 28. Correlation between IL-6 and MMP-3 at baseline. There was a positive correlation between IL-6 and MMP-3 at baseline.

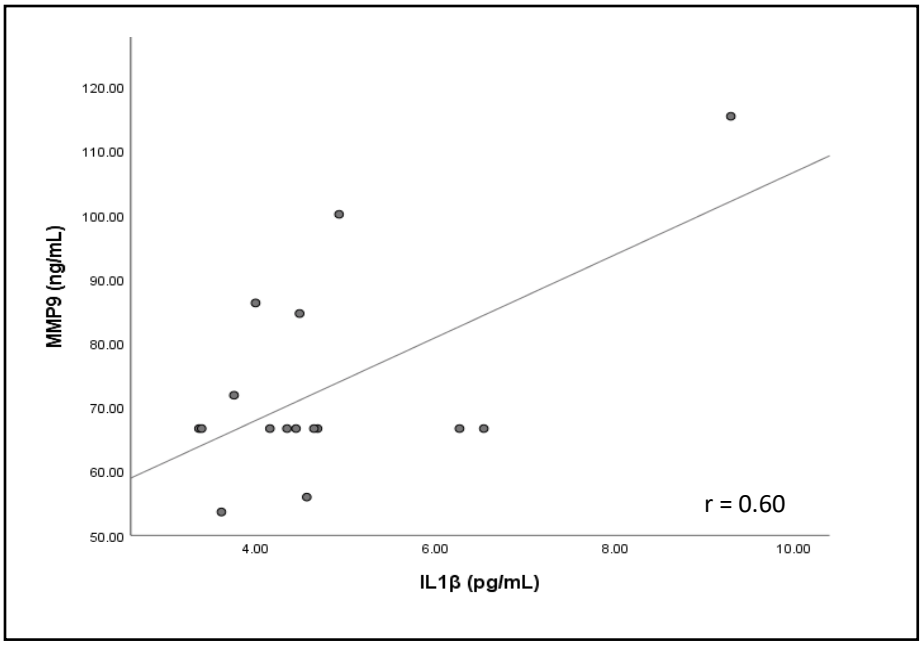

Figure 29. Correlation between IL-1β and MMP-9 at Day 3. There was a positive correlation between IL-1β and MMP-9 at day 3.

Table 40. Correlations between IL-6 and inflammatory cytokines and MMPs.

		IL-6 (pg/mL)			
Collection Time		Baseline	Day 3	Day 5	Follow Up
CRP (mg/L)	Baseline	0.525	0.774**	0.589	0.286
	Day 3	0.286	0.747*	0.701*	0.15
	Day 5	0.22	0.851**	0.506	0.263
	Follow Up	-0.376	-0.092	-0.218	0.915*
IL-1β (pg/mL)	Baseline	-0.362	-0.307	-0.217	-0.318
	Day 3	-0.224	-0.144	-0.046	-0.27
	Day 5	-0.272	-0.218	-0.092	-0.243
	Follow Up	-0.328	-0.212	-0.056	-0.092
MMP-1 (ng/mL)	Baseline	-0.275	-0.13	-0.176	-0.142
	Day 3	-0.27	-0.191	-0.29	0.002
	Day 5	-0.308	-0.156	-0.192	0.081
	Follow Up	-0.35	-0.235	-0.293	-0.179
MMP-2 (ng/mL)	Baseline	-0.165	-0.075	0.048	0.412
	Day 3	-0.324	-0.14	-0.027	0.324
	Day 5	-0.209	0.023	0.115	0.404
	Follow Up	-0.026	0.123	0.135	0.081
MMP-3 (ng/mL)	Baseline	0.514*	-0.097	-0.045	-0.069
	Day 3	0.117	0.289	0.206	0.681**
	Day 5	0.197	0.226	0.237	0.375
	Follow Up	0.118	-0.107	-0.057	0.358
MMP-9 (ng/mL)	Baseline	0.331	0.061	0.128	-0.107
	Day 3	-0.145	-0.292	-0.268	-0.011
	Day 5	-0.153	-0.153	-0.179	0.171
	Follow Up	0.009	-0.254	-0.169	-0.232

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 41. Correlations between IL-1β and inflammatory cytokines and MMPs

		IL-1β (pg/mL)			
Collection Time		Baseline	Day 3	Day 5	Follow Up
CRP (mg/mL)	Baseline	-0.55	-0.418	-0.288	-0.25
	Day 3	-0.524	-0.366	-0.332	-0.177
	Day 5	-0.487	-0.41	-0.352	-0.339
	Follow Up	-0.299	-0.223	-0.259	-0.037
IL-6 (pg/mL)	Baseline	-0.362	-0.224	-0.272	-0.328
	Day 3	-0.307	-0.144	-0.218	-0.212
	Day 5	-0.217	-0.046	-0.092	-0.056
	Follow Up	-0.318	-0.27	-0.243	-0.092
MMP-1 (ng/mL)	Baseline	0.107	0.075	-0.021	-0.007
	Day 3	0.193	0.141	-0.005	0.002
	Day 5	0.154	0.112	0.031	0.124
	Follow Up	0.274	0.194	0.007	0.039
MMP-2 (ng/mL)	Baseline	0.17	0.21	0.296	0.425
	Day 3	0.298	0.26	0.377	0.481
	Day 5	0.211	0.186	0.297	0.452
	Follow Up	0.09	0.034	0.077	0.079
MMP-3 (ng/mL)	Baseline	-0.073	-0.025	-0.047	-0.139
	Day 3	-0.161	-0.03	-0.022	0.068
	Day 5	-0.29	-0.178	-0.186	-0.145
	Follow Up	-0.213	-0.224	-0.194	-0.178
MMP-9 (ng/mL)	Baseline	0.094	0.099	0.147	0.155
	Day 3	0.632**	0.601*	0.609*	0.554*
	Day 5	-0.152	-0.173	-0.137	-0.066
	Follow Up	0.168	0.019	-0.072	-0.004

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Correlations were absent amongst MMPs

We sought to evaluate whether expression of inflammatory cytokines correlated with those of MMP-1, MMP-2, MMP-3 and MMP-9. No correlation was observed between any of the inflammatory markers at all collection points for MMP-1 (Table 42) or MMP-2 (Table 43). Correlation analysis with MMP-3 resulted in the previously described positive correlation with IL-6 at baseline but no other relevant correlations were observed (Table 44). Similarly, a previously mentioned positive correlation between MMP-9 and CRP and IL-1β at day 3 was observed, indicating that MMP-9 decreases as CRP increases, and a corresponding increase in IL-1β as MMP-9 increases (Table 45).

Table 42. Correlations between MMP-1 and inflammatory cytokines and MMPs.

		MMP-1 (ng/mL)			
Collection Time		Baseline	Day 3	Day 5	Follow Up
CRP (mg/L)	Baseline	-0.587	-0.649*	-0.496	-0.609
	Day 3	-0.535	-0.632	-0.381	-0.572
	Day 5	-0.342	-0.437	-0.281	-0.361
	Follow Up	-0.415	-0.259	-0.311	-0.341
IL-6 (pg/mL)	Baseline	-0.275	-0.27	-0.308	-0.35
	Day 3	-0.13	-0.191	-0.156	-0.235
	Day 5	-0.176	-0.29	-0.192	-0.293
	Follow Up	-0.142	0.002	0.081	-0.179
IL-1β (pg/mL)	Baseline	0.107	0.193	0.154	0.274
	Day 3	0.075	0.141	0.112	0.194
	Day 5	-0.021	-0.005	0.031	0.007
	Follow Up	-0.007	0.002	0.124	0.039
MMP-2 (ng/mL)	Baseline	-0.212	-0.059	0.175	-0.102
	Day 3	-0.11	-0.002	0.262	-0.022
	Day 5	-0.237	-0.17	0.052	-0.183
	Follow Up	-0.469	-0.311	-0.215	-0.259
MMP-3 (ng/mL)	Baseline	-0.181	-0.055	-0.226	-0.162
	Day 3	-0.119	-0.026	-0.019	-0.263
	Day 5	0.033	-0.025	-0.132	-0.243
	Follow Up	-0.106	0.018	0.02	-0.163
MMP-9 (ng/mL)	Baseline	-0.353	-0.321	-0.386	-0.377
	Day 3	0.138	0.252	0.245	0.172
	Day 5	-0.21	-0.181	-0.254	-0.185
	Follow Up	0.243	0.204	0.159	0.376

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 43. Correlations between MMP-2 and inflammatory cytokines and MMPs.

		MMP-2 (ng/mL)			
Collection Time		Baseline	Day 3	Day 5	Follow Up
CRP (mg/L)	Baseline	-0.122	-0.221	-0.09	-0.158
	Day 3	-0.193	-0.302	-0.183	-0.415
	Day 5	-0.19	-0.238	-0.232	-0.046
	Follow Up	0.042	-0.044	0.197	-0.033
IL-6 (pg/mL)	Baseline	-0.165	-0.324	-0.209	-0.026
	Day 3	-0.075	-0.14	0.023	0.123
	Day 5	0.048	-0.027	0.115	0.135
	Follow Up	0.412	0.324	0.404	0.081
IL-1β (pg/mL)	Baseline	0.17	0.298	0.211	0.09
	Day 3	0.21	0.26	0.186	0.034
	Day 5	0.296	0.377	0.297	0.077
	Follow Up	0.425	0.481	0.452	0.079
MMP-1 (ng/mL)	Baseline	-0.212	-0.11	-0.237	-0.469
	Day 3	-0.059	-0.002	-0.17	-0.311
	Day 5	0.175	0.262	0.052	-0.215
	Follow Up	-0.102	-0.022	-0.183	-0.259
MMP-3 (ng/mL)	Baseline	-0.011	-0.183	-0.212	-0.054
	Day 3	0.115	0.01	-0.022	-0.281
	Day 5	-0.18	-0.272	-0.201	-0.342
	Follow Up	0.222	0.176	0.121	0.094
MMP-9 (ng/mL)	Baseline	0.118	0.081	0.481	0.429
	Day 3	0.016	0.122	0.083	-0.099
	Day 5	0.201	0.168	0.439	0.331
	Follow Up	-0.451	-0.39	-0.261	-0.438

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 44. Correlation between MMP-3 and inflammatory cytokines and MMPs.

		MMP-3 (ng/mL)			
Collection Time		Baseline	Day 3	Day 5	Follow Up
CRP (mg/L)	Baseline	0.604	0.411	0.201	-0.363
	Day 3	-0.135	0.38	0.336	-0.401
	Day 5	-0.307	0.617	0.13	-0.204
	Follow Up	0.251	0.884*	0.233	0.14
IL-6 (pg/mL)	Baseline	0.514*	0.117	0.197	0.118
	Day 3	-0.097	0.289	0.226	-0.107
	Day 5	-0.045	0.206	0.237	-0.057
	Follow Up	-0.069	0.681**	0.375	0.358
IL-1β (pg/mL)	Baseline	-0.073	-0.161	-0.29	-0.213
	Day 3	-0.025	-0.03	-0.178	-0.224
	Day 5	-0.047	-0.022	-0.186	-0.194
	Follow Up	-0.139	0.068	-0.145	-0.178
MMP-1 (ng/mL)	Baseline	-0.181	-0.119	0.033	-0.106
	Day 3	-0.055	-0.026	-0.025	0.018
	Day 5	-0.226	-0.019	-0.132	0.02
	Follow Up	-0.162	-0.263	-0.243	-0.163
MMP-2 (ng/mL)	Baseline	-0.011	0.115	-0.18	0.222
	Day 3	-0.183	0.01	-0.272	0.176
	Day 5	-0.212	-0.022	-0.201	0.121
	Follow Up	-0.054	-0.281	-0.342	0.094
MMP-9 (ng/mL)	Baseline	0.324	-0.246	-0.071	0.006
	Day 3	-0.118	0.214	0.057	0.375
	Day 5	-0.027	0.053	0.272	0.37
	Follow Up	-0.14	-0.038	0.127	0.209

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 45. Correlations between MMP-9 and inflammatory cytokines and MMPs.

		MMP-9 (ng/mL)			
Collection Time		Baseline	Day 3	Day 5	Follow Up
CRP (mg/L)	Baseline	0.022	-0.583	-0.311	-0.529
	Day 3	-0.229	-0.713*	0.179	-0.202
	Day 5	-0.475	-0.499	-0.265	-0.46
	Follow Up	-0.303	0.165	-0.165	-0.326
IL-6 (pg/mL)	Baseline	0.331	-0.145	-0.153	0.009
	Day 3	0.061	-0.292	-0.153	-0.254
	Day 5	0.128	-0.268	-0.179	-0.169
	Follow Up	-0.107	-0.011	0.171	-0.232
IL-1β (pg/mL)	Baseline	0.094	0.632**	-0.152	0.168
	Day 3	0.099	0.601*	-0.173	0.019
	Day 5	0.147	0.609*	-0.137	-0.072
	Follow Up	0.155	0.554*	-0.066	-0.004
MMP-1 (ng/mL)	Baseline	-0.353	0.138	-0.21	0.243
	Day 3	-0.321	0.252	-0.181	0.204
	Day 5	-0.386	0.245	-0.254	0.159
	Follow Up	-0.377	0.172	-0.185	0.376
MMP-2 (ng/mL)	Baseline	0.118	0.016	0.201	-0.451
	Day 3	0.081	0.122	0.168	-0.39
	Day 5	0.481	0.083	0.439	-0.261
	Follow Up	0.429	-0.099	0.331	-0.438
MMP-3 (ng/mL)	Baseline	0.324	-0.118	-0.027	-0.14
	Day 3	-0.246	0.214	0.053	-0.038
	Day 5	-0.071	0.057	0.272	0.127
	Follow Up	0.006	0.375	0.37	0.209

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Correlations Between Basal Expression of Inflammatory Cytokines, Anthropometric and Strength Measurements

Basal levels of inflammatory cytokines and MMPs correlate with height and BMI

Correlation analysis of baseline concentration of inflammatory cytokines and MMPs and baseline measurements of weight, height, BMI, PPT in the arm, shoulder and wrist strength, resulted in a negative correlation between baseline concentrations of MMP-2 and BMI ($r = -0.552$), suggesting that MMP-2 decreases as BMI increases (Figure 30 and Table 46).

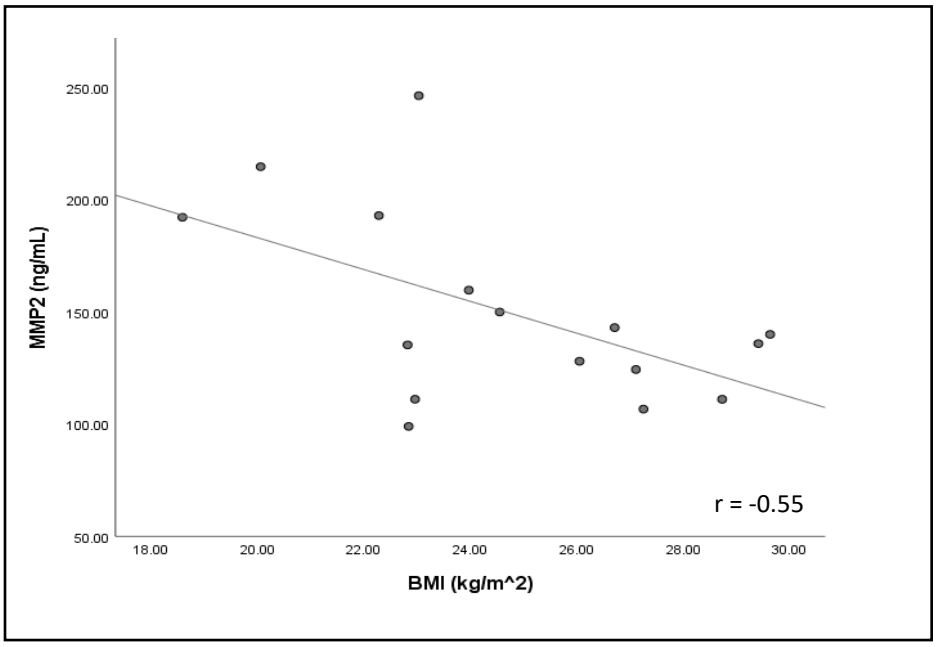

Figure 30. Correlation between MMP-2 at baseline and BMI. There was a negative correlation between MMP-2 at baseline and BMI.

Table 46. Correlations between baseline concentration of inflammatory cytokines and MMPs and baseline anthropometric, PPT and muscular strength measurements.

Baseline	Weight (kg)	Height (m)	BMI (kg/m^2)	Arm PPT (N)	Shoulder Strength (N)	Wrist Strength (N)
CRP (mg/L)	0.134	-0.43	0.581	-0.076	0.47	0.049
IL-6 (pg/mL)	0.249	-0.238	0.494	0.284	-0.047	0.01
IL-1β (pg/mL)	0.157	0.545*	-0.281	-0.001	0.058	-0.345
MMP-1 (ng/mL)	-0.389	-0.065	-0.433	0.155	-0.04	-0.097
MMP-2 (ng/mL)	-0.345	0.165	-0.551*	0.32	0.262	-0.214
MMP-3 (ng/mL)	0.028	-0.15	0.166	0.138	-0.038	0.28
MMP-9 (ng/mL)	0.455	0.3	0.306	0.029	-0.256	0.03

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Basal levels of inflammatory cytokines and MMPs correlated with low frequency HRV and arm and shoulder fatigue

Correlation analysis of changes in inflammatory cytokine, MMPs, RPE, and HRV at day 3 showed a positive correlation between IL-6 and low frequency HRV ($r = -0.561$); Figure 31 and Table 47).

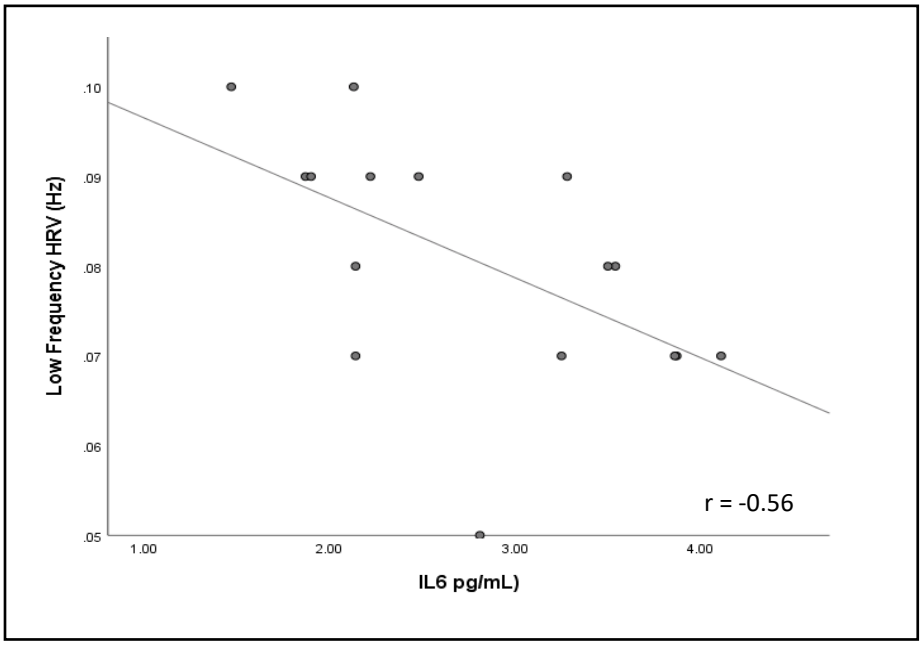

Figure 31. Correlation between IL-6 and low frequency HRV at day 5. There was a negative correlation between IL-6 and low frequency HRV at day 5.

Table 47. Correlations between CRP, inflammatory cytokines, and MMPs and measurements of perceived exertion, HRV (RMSSD), low frequency HRV, and high frequency HRV.

Baseline	RPE	HRV (RMSSD)	Low Frequency HRV (Hz)	High Frequency HRV (Hz)
CRP (mg/L)	-0.001	-0.164	-0.076	-0.148
IL-6 (pg/mL)	0.012	-0.083	-0.561*	-0.137
IL-1β (pg/mL)	0.252	0.07	0.292	0.157
MMP-1 (ng/mL)	0.086	-0.198	-0.369	-0.323
MMP-2 (ng/mL)	-0.328	-0.263	-0.053	-0.434
MMP-3 (ng/mL)	0.348	0.247	-0.408	0.135
MMP-9 (ng/mL)	-0.266	-0.087	-0.018	-0.322

Chapter 4: Discussion

This pre-experimental, single subject, repeated measures study was conducted to examine the association between repetitive use of the upper extremities and circulating levels of inflammatory biomarkers. We used a different sample population than those previously reported in the literature (Quadros, Oliveira, Scienza, & Candemil, 2018). Our population consisted of 16 healthy college students between 18 and 25 years old with a normal BMI to minimize the effects of age- and sex-related conditions and obesity (such as atherosclerosis and diabetes) in the expression of inflammatory biomarkers (Rea et al., 2018). None of the participants had previous exposure to repetitive or forceful movements of the upper extremities, they were not diagnosed with musculoskeletal disorders, and were not currently taking anti-inflammatory medication. The methods used for the analysis of anthropometric, clinical, and inflammatory biomarker expression data was consistent with extensively used methods reported in the literature. The results from the surface EMG analysis showed no signs of muscle fatigue in the sample. This, coupled with the detection of changes in MMPs within peripheral blood samples suggested that changes in the expression of the inflammatory biomarkers was most probably caused by tendon strain and not muscle fatigue. All the previously mentioned conditions give this research a novel approach to a problem that affects the quality of life of manual workers around the world. The results outlined in this dissertation work shed new light on the effects of repetitive manual work on WMSDs, focusing on tendon damage of the upper limbs caused by repetitive tendon strain.

Changes in Biomarker Expression are Associated to Tendon Strain

The main focus of our research was to identify changes in inflammatory biomarker expression associated to tendon strain. In order to assess the effectiveness of the task at eliciting changes in inflammatory biomarker expression associated to tendon strain, we assessed the influence of muscle fatigue using surface EMG data. A study by Cifrek et al. (2009), suggests that muscle fatigue will exhibit a decrease in median frequency and a corresponding increase in mean amplitude greater than 10% between the initial and final minute of the task. These changes in frequency and amplitude represent a decrease in the contractile velocity of the muscles resulting from muscle fatigue. In our study, we introduced two 10-minute rest periods after each of the 8-minute working periods to reduce the load on the muscles in order to prevent muscular fatigue. Our results showed no changes greater than 10% in frequency and amplitude, suggesting that the resting periods were effective at preventing muscle fatigue and that changes in inflammatory biomarker expression were associated to tendon strain.

Site of Inflammation, Intensity and Duration Effects on CRP and Cytokine Expression

The repetitive tendon strain induced by the task did not significantly change the expression of circulating CRP in our study. However, previous research has reported significant increases in CRP expression immediately, and after 24 hours following, the completion of strenuous exercise (Fallon, 2001; Siegel et al., 2003; Weight, Alexander, & Jacobs, 1991). The absence of significant changes experienced in our research may be attributed primarily due to the intensity and/or duration of the manual task. As we observed, the levels of CRPs began and remained below the clinical threshold (10 mg/L) with a single subject, single time point exception which was likely due to a random external influence. Contrary to the studies from Fallon (2001); Siegel et al.

(2003); Weight et al. (1991), we targeted the tendons of the upper limbs instead of large muscle groups. This may have affected the presence or absence of inflammatory biomarkers in peripheral blood since muscle damage is a greater contributor to the systemic expression of inflammatory biomarkers than tendon strain which is usually more localized (Fallon, 2001).

In general, studies aimed to investigate the inflammation and damage caused by tendon overuse are primarily performed in cell culture or use animal models or diseased tendons, making the collection of tissue or extracellular fluid easier (Carpenter et al., 1998; Dakin et al., 2015; Perry et al., 2005). In our case, the use of an invasive technique such as a micro-dialysis or tendon biopsy was not possible because of the procedural risk of long-term injury to the participants. In addition, we sought to determine whether the inflammatory markers were detectable in peripheral blood and could be associated with MMP markers associated with tendon strain following the performance of a repetitive manual task over a period of 5 days. Our results showed that changes in inflammatory biomarker expression can be measured in peripheral blood, even at a subclinical level of inflammation. These data suggest that a combined use of inflammatory biomarkers such as IL-6, IL-1β and MMPs could be used to screen for pre-injurious tendon loads and to prevent inflammation.

In order to more closely replicate conditions that workers performing manual repetitive tasks may experience, we developed a manual task that activated the shoulder and hand extensors and the average muscular activation used was within the average muscular activation range reported in the current literature. The activity used in our study was designed so that the maximal muscular activation was no more than 15% of the MVC and tested relatively small muscle groups

with relatively small tendons (anterior deltoid and hand extensors).The repetition cycles used in our study were similar to the number of cycles reported in an average 8-hour work shift. The only variable we could not replicate was the time participants were exposed to the task.

Fallon (2001); Siegel et al. (2003); Weight et al. (1991) demonstrated that expression of inflammatory biomarkers is increased in blood immediately after and up to 24 hours following intense physical activities such as marathon and ultramarathon running. In our study, we were able to demonstrate that even a low intensity, short duration activity was sufficient to induce small changes in the expression of IL-6, IL-1β and CRP. In the case of IL-6 and IL-1β specifically, the intensity of the activity and the activation of small muscle groups used may have had a direct effect on their expression. This is evidenced by data that suggests IL-6 expression is directly proportional to the muscle mass damage induced by higher levels of exercise intensity and duration (Febbraio & Pedersen, 2002; Pedersen, Steensberg, & Schjerling, 2001; Ren & Torres, 2009).

Low Intensity Muscular Activity Decreased IL-6 and IL-1β Expression

Changes in inflammatory cytokines after the participants were exposed to the task for 3 and 5 days showed a significant decrease in IL-6 and a similar, albeit non-significant, decrease in IL-1β. These decreases in inflammatory cytokine expression are consistent with the results of Goldhammer et al. (2005), showing that low exertion physical activity reduces the levels of pre-existing inflammatory cytokines. Our results show that low exertion physical activity effectively decreases pre-existing levels of IL-6 and IL-1β as soon as three days after being exposed to a low intensity (<15% MVC) activity. These findings should be further explored in order to assess the potential use as a preventive method for tendon inflammation and injury. The therapeutic use of stretching exercises, characterized by static muscle contractions, have been reported in the past.

Animal models have been shown to decrease inflammation after the performance of an stretching intervention (Corey, Vizzard, Bouffard, Badger, & Langevin, 2012). Also, acute inflammation has been shown to decrease after two weeks of stretching exercise (Berrueta et al., 2016). Therefore, the potential use for low intensity, dynamic muscle contractions simulating a work-related task should be explored to promote prevention and reduction in circulating inflammatory cytokines.

Influence of IL-1β and Fitness on MMP Expression

After analyzing the expression of tendon-associated MMPs, we determined that while the completion of a manual task did not significantly change MMP-2 and MMP-9 expression, expression of MMP-1 and MMP-3 were significantly changed. A continuous increase in MMP-1 levels were maintained up to one week after the completion of the task. These changes are supported by findings from Tsuzaki et al. (2003); Yang, Im, and Wang (2005) suggesting that stretching of the tendon, as well as stimulation of IL-1β, induces increases in MMP-1 and MMP-3. Even though we did not see significant changes in IL-1β in serum, the combination of tendon stretching and the presence of IL-β in the tendon were sufficient to drive an increase in MMP-1 and MMP-3, in turn causing the breakdown of the tendon's extracellular matrix (Pendás, Santamaría, Alvarez, Pritchard, & López-Otín, 1996). The relatively unchanged expression in MMP-2 and MMP-9 in the presence of increased expression of MMP-1 and MMP-3 has been previously reported by Corps, Curry, Buttle, Hazleman, and Riley (2004), who showed that elevated levels of IL-1β drive an increase in MMP-1 and MMP-3 mRNA, while at the same time having no effect on MMP-2 and MMP-9. Also, the type of physical activity performed, as well as the physical fitness of the participants, might have had an influence on the changes in the

expression of MMPs. As suggested by Urso, Pierce, Alemany, Harman, and Nindl (2009), physically trained (fit) individuals tend to have a decreased release of MMPs immediately after a single exercise session compared to those whom are less fit. In the case of our study, the expected increase in MMP-2 and MMP-9 may have also been affected by the participant' fitness.

Use of MMPs Expression as a Surrogate Marker for Pre-injurious Tendon Load

The measurement of MMP expression could serve as a more specific tool to assess tendon load. The changes of the different MMPs signal the beginning of tendon repair by means of collagen turnover. The turnover cycle begins with the collagenase activity of MMP-1, MMP-2 and MMP-3, followed by transition between collagen breakdown and the synthesis of new collagen mediated by MMP-9 (Barbe et al., 2006; Carp et al., 2007; Tsuzaki et al., 2003). The results of our study showcase these transitions from collagen degradation to collagen synthesis as the task progressed. As soon as three days after the participants were introduced to the task, the levels of MMP-3 significantly increased while the levels of MMP-1 and MMP-2 remained relatively unchanged and the levels of MMP-9 decreased. These changes likely represented the beginning of the collagen breakdown needed to start remodeling of the tendon. As the exposure to the task increased, the expression of MMP-1 and MMP-3 significantly increased while the expression of MMP-2 showed a trending increase. Conversely, the expression of MMP-9 continued to decrease, signaling the existing balance between tissue degradation and tissue repair/remodeling (Febbraio & Pedersen, 2002). Finally, after the participants rested for one week, the expression of MMP-1, MMP-2, and MMP-3 remained elevated, while the expression of MMP-9 showed a trend towards a significant increase, signaling the transition from tendon damage to tendon repair (Febbraio & Pedersen, 2002). Even though the results of MMP expression in our study were below the threshold for clinical inflammation, the collagen turnover pattern was evident, going from increases in collagenase activity (MMP-1, MMP-2, and MMP-3) to an increase in collagen synthesis (MMP-9). The measurement of the expression in MMPs could be used to monitor tendon load instead of

relying on mathematical and mechanical estimation models. Measuring the expression of MMPs will provide a better representation of the physiological demands of the task on tendon load.

BMI Significantly Affects Changes in CRP Expression

One major finding is the influence of BMI on CRP expression. Once we accounted for the influence of BMI, we saw a significant decrease in CRP expression one week after the participants completed a manual task for 5 days. This finding is supported by Huffman, Whisner, Zarini, and Nath (2010); Onat, Can, and Hergenç (2008) who found that CRP expression was strongly associated with BMI and waist circumference. However, unlike their cohort, we did not include obese participants (greater than 29.9 kg/m^2) to avoid this known influence in CRP expression. Nonetheless, BMI still had a significant influence on the expression of CRPs even within healthy young males. Since BMI calculations are based solely on the weight and height of the individual, future research should include more specific anthropometric measurements of body composition such as lean and fat mass of the individuals. We expected other significant changes in CRP expression besides a significant decrease after one week of rest, but this was probably not the case because all of the results were below the threshold for clinical inflammation and several measurements yielded results below the detection threshold of the assay. Furthermore, we did not see any effects of covariates in the expression of inflammatory cytokines or MMPs.

Cyclooxygenase-2 (COX-2) Levels in Healthy Participants

Although we screened serum samples of the participants for changes in another marker of inflammation, namely cyclooxygenase-2 (COX-2), we found that this marker was not detectable in healthy young adults after completing a manual task, and therefore was not included in the analysis. Most studies on tendon damage caused by overload use histology and reverse transcription quantitative polymerase chain reaction (RT qPCR) (Oak et al., 2014; Yang et al., 2005) that can be performed on the injured tissue specifically rather than serum biomarkers. Because obtaining a tissue samples for histology was not practical in our study due to the cohort consisting of healthy adults, the use of RT qPCR may be a viable option to measure the expression of PTGS-2, the gene encoding COX-2 from white blood cells in circulating blood in future studies (Berti et al., 2002)

Decreases in RPE Indicate Adaptation to the Manual Task

We included the assessment of RPE as a measure of the intensity of the task. The unchanged RPE reported by the participants at rest indicate that the resting time was adequate. Our results also indicate that having a rest period helped them to complete the 1,800 cycles; this can be seen in participants reporting high RPE values before the rest period, and then reporting low RPE values after rest. Furthermore, participants reported lower RPE values as the study progressed, reflecting the participants' adaptation to the repetitive task. This physiological and psychological adaptation has been reported by Snyder, Jeukendrup, Hesselink, Kuipers, and Foster (1993) and (Belman & Gaesser, 1988), showing that after participants were exposed to a novel

task, the RPE values decreased as the participants became familiarized to the task. This phenomenon was attributed to neuromuscular adaptations as well as to an increased efficiency at repeating the task.

HRV was not Affected by the Intensity of the Task

In our research, we included the measurement of HRV variability in participants while resting and while performing the repetitive task to assess possible effects on the participants' stress levels. The use of HRV has been used as a physiological marker of stress during and after exercise to obtain an understanding of physiological recovery following physical activity (Makivić et al., 2013). Our measurement of HRV reflected the changes between HRV while performing the task and at rest, indicating a recovery during the rest period. We found no adaptations in HRV caused by the physical demands of our repetitive task. Changes in HRV may be experienced if the intensity and duration of the repetitive task is significantly high enough to stress the cardiovascular system (Jurca, Church, Morss, Jordan, & Earnest, 2004; Plews, Laursen, Stanley, Kilding, & Buchheit, 2013).

Significant Correlations Between Biomarkers of Inflammation.

The correlations resulting from comparing CRP and other biomarkers of inflammation showed a positive correlation with IL-6 and MMP-3 at day 3. These correlations are similar to those described by Nakajima et al. (2014) and Mahmoud, El-Ansary, El-Eishi, Kamal, and El-Saeed (2005), although those studies included populations diagnosed with rheumatoid arthritis.

Our results showed the same correlations even at a subclinical level of inflammation. Because our study measured levels of markers at multiple time points, we were able to determine that markers appear to start changing around day 3, showing an increase in CRP and IL-6 expression. We also believe that this change in expression is dependent on the duration of the activity as suggested by Reihmane, Jurka, Tretjakovs, and Dela (2013). Also,

Significant Correlations Between Basal Anthropometric Measurements and Expression of Inflammatory Biomarkers

When we were evaluating the data seeking whether correlations between anthropometric measurements and the expression of biomarkers of inflammation existed, a correlation between IL-1β and height was identified. We believe that this correlation is spurious and was observed due to an extremely small sample size (McNemar, 1947). Correlations were also observed between BMI and MMP-2. Interestingly, Derosa et al. (2008) described a positive direct correlation between BMI and MMP-2, whereas we observed an inverse correlation when we analyzed our data. Besides the small sample size, this inverse correlation may be true for non-obese individuals since a higher BMI in non-obese individual may reflect a greater muscle mass instead of high fat mass (Zimowska, Brzoska, Swierczynska, Streminska, & Moraczewski, 2003).

Significant Correlations Between Cytokines of Inflammation and MMPs with HRV and Fatigue

A correlation between IL-6 and low frequency HRV was observed after completing the manual task for five days. This correlation indicates that an increase in IL-6 after completing the

manual task for five days exerts some level of stress upon the sympathetic nervous system. Low frequency HRV has been assumed to reflect the changes in sympathetic activity (García-González, Vázquez-Seisdedos, & Pallàs-Areny, 2000). Consequently, a lower variability in Low Frequency HRV reflects higher activity of the sympathetic nervous system, in our study, produced by a subclinical inflammatory response represented by an increase in IL-6 expression. This relationship has been previously reported by the findings of von Känel, Nelesen, Mills, Ziegler, and Dimsdale (2008), showing that a decrease in HRV is associated with low-grade systemic inflammation. Our findings can be further explored to develop a non-invasive tool to screen for inflammatory responses using changes in HRV. This tool could be used to detect inflammatory responses at a sub-clinical level.

Limitations of the Study

The major limitation of the study was the small sample size. A larger sample size could have allowed for a more robust statistical analysis of the data, for example, a regression analysis could have been used to determine if a combination of independent variables could potentially affect the dependent variable. Also, a larger sample size would have allowed us to have a greater statistical power and it could have contributed to have stronger statistically significant changes in biomarker expression, as well as statistically significant correlations between variables.

We were also confronted with the limitation of testing inflammatory biomarker expression only in blood serum because of the potential risks of other methods to induce long term damage to the participants. Statistically significant changes in inflammatory biomarker expression might not

be immediately detectable measuring peripheral blood and may not adequately consider factors such as muscle group use and intensity and/or duration of the task. Furthermore, the expression of some biomarkers may require a longer duration of task performance and at increased concentrations in order to be detected in systemic circulation. Measuring changes in the expression of messenger RNA (mRNA) of genes from white cells encoding inflammatory biomarkers could potentially show changes detectable at a genetic but not protein level.

Finally, exposure time and intensity of the manual task was another major limitation we experienced. As previously mentioned in the methods section, we prioritized repetition over intensity, limited the amount of repetitions, and limited the duration of the manual task to prevent injuries to the participants. Since previous studies reported higher expression of inflammatory biomarkers, *in vitro* and *in vivo*, also reported strenuous physical activity and longer exposure to cyclic movements, the intensity and/or duration of the repetitive manual task might have not been sufficient to elicit changes in inflammatory biomarker expression. Future research should consider increasing exposure time to the repetitive manual task.

Conclusion

The importance of understanding early changes in inflammatory biomarkers caused by repetitive cycling movements is an important factor to prevent the development of WMSDs. The detection of early changes in the expression of inflammatory biomarkers, combined with clinical measurements such as HRV and MVC, can serve as the basis for better preventive measures against the development or worsening of MSDS. In this study, a sample of 16 healthy males with no diagnosis of WMSDs were asked to complete five consecutive days of a repetitive manual task, blood serum levels of inflammatory biomarkers, as well as measurements of PPT, RPE, HRV,

muscular strength and MVC were analyzed to find possible associations. We determined that the changes in the expression of inflammatory biomarkers can be identified in peripheral blood even at a sub-clinical inflammatory expression level. The changes in MMP-1, MMP-2, MMP-3 and MMP-9 could be used as potential surrogate markers of pre-injurious tendon load. Low frequency HRV has been shown to correlate to IL-6 expression, suggesting its potential use as a non-invasive method to assess non-injurious tendon inflammation. Finally, there is a similar inflammatory response from all the participants to the completion of the task and none of the participants expressed biomarkers beyond the reference ranges of analytes associated with clinical inflammation. However, differences in percent body fat should be further explored to establish a relationship between adipose tissue and biomarkers of inflammation in healthy, non-obese individuals.

The results of this study can be used to develop better study designs aimed to assess changes in inflammatory biomarker expression after the completion of a repetitive task. Also, it will help in the design of a screening tool to assess the risk of developing WMDS, taking into consideration not only inflammatory biomarker expression in blood, but also considering clinical measurement such as HRV and MVC.

Future Directions

The results of this dissertation research can be used as the basis for the development of a tool for pre-injurious tendon load screening that uses physiological data instead of mathematical or mechanical estimations. Also, data from our study could be used in the design of better educational interventions and to introduce more appropriate rest periods for individuals at a higher risk for the development of WMSDs.

In the physically active population, our data could be used develop a clinical and molecular approach for injury prevention in physically active individuals by designing a more appropriate exercise to rest ratio that also uses surface EMG data to look at muscle strength and muscle fatigue, molecular data to look at tendon load to prevent injury and allow remodeling/repair, and finally to develop an exercise intensity/rest ratio based on muscular activity instead of the usual hear rate percentage or maximal oxygen uptake percentage (% $VO_{2\ max}$).

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Appendix A Screening Questionnaire

Research Study Introduction

We are asking you to take part in a confidential screening questionnaire for a clinical research study. This study will be conducted to identify a possible relationship between heart rate variability and aerobic, anaerobic and muscular strength measurements. This research will be conducted at the University of Texas at El Paso's Interdisciplinary Health Sciences PhD program.

Participation in this screening questionnaire will not force you to participate in the research study, but will inform the researchers of your interest and eligibility to be part of the study. If you qualify for further participation of the study, the research assistants will contact and guide you through the next steps for participation.

This study's aim is to provide data on a possible relationship between heart rate variability and aerobic, anaerobic and strength measurements. specifically, if greater aerobic, anaerobic or strength measurements correlate with a higher heart rate variability.

If you are selected to participate in the research study, you will be one of 20 people interviewed and selected for this research.

Should there be any questions or concerns about the potential safety, validity, security or confidentiality of this research, please feel free to contact the Principal Investigator Daniel Conde (915) 208 6168; daconde@miners.utep.edu.

Please answer the following questions as accurately as possible. All your answers will be kept strictly confidential.

For the questions requiring a short-written answer, please use the spaces provided below the questions, for example Age _20_ years. For the questions requiring checking a box, please mark one or more boxes as indicated by the questions, for example ☑ or ☒

1. Are you between the ages 18 – 30 years old?

   Yes ☐ No ☐

   If Yes, please continue and indicate your age below. If you are not within this age range, we thank you for your cooperation. Please turn in the form to the research assistant.

   Age _____ years

2. What is your race or ethnicity?

Caucasian or White	☐
Hispanic or Latino	☐
Black or African American	☐
Native American or American Indian	☐
Asian / Pacific Islander	☐
Other	☐

3. Do you exercise on regular basis?

   Yes ☐ No ☐

   If No, please proceed to question 7

4. How many times do you exercise per week?

1 – 2 times per week	☐
3 – 4 times per week	☐
5 – 6 times per week	☐
Everyday	☐

5. Approximate how long do you exercise each day?

   Approximately _____ hours _____ minutes

6. What type of exercise do you perform? Please select all that apply or indicate what other exercise you performed

Resistance training or weight lifting	☐
Power lifting (Olympic lifting)	☐
Running	☐
Cycling	☐
Rowing	☐
Pilates	☐
CrossFit	☐
Yoga	☐
Other	☐

If selected "other" please explain _____

7. To the best of your knowledge, have you ever been diagnosed with a musculoskeletal disorder (such as muscle tears, inflammation of tendons, joint inflammation, and/or fractured bones)?

Yes ☐ No ☐

If No, please proceed to question 9

8. What musculoskeletal disorders were diagnosed? Below is a list of common musculoskeletal disorders, please select all that apply or indicate what other musculoskeletal disorder was diagnosed.

Carpal tunnel syndrome	☐
Radial tunnel syndrome	☐
Tendonitis	☐
Tendinosis	☐
Low back pain syndrome	☐
Herniated disc	☐
Tension neck syndrome	☐
Dislocated fractured Bone	☐
Other	☐

If selected "other" please explain _____

9. Within the past year, have you had a surgery (including the shoulder, arm, elbow, forearm, wrist, hand, and lower extremities)?

   Yes ☐ No☐

   If No, please proceed to question 11.

10. What surgery was performed? Below is a list of common surgeries, please select all that apply or indicate what other surgery was performed.

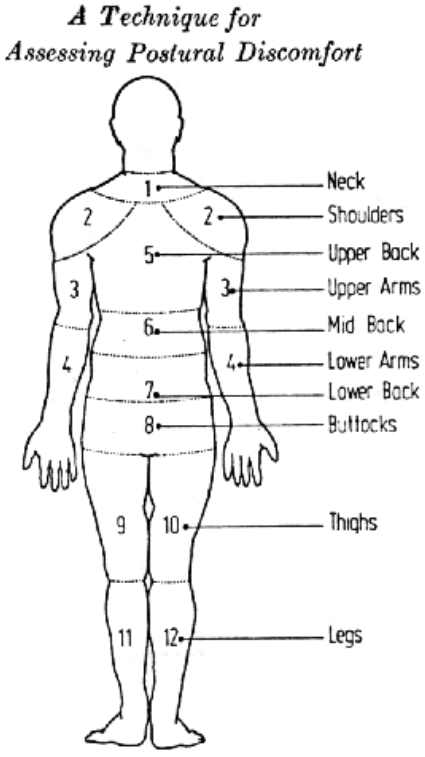
Fractured bone repair	☐
Tendon tear/rupture repair	☐
Muscle tear/rupture repair	☐
Other	☐

   If selected "other" please explain _____

11. During the past 6 months have you experienced persistent pain in any body region?

   Yes ☐ No ☐

   If Yes, please mark the body region in the diagram below. If No, please proceed to question 22.



Body regions.

12. To the best of our knowledge, have you ever been diagnosed with a cardiac condition?

    Yes ☐ No ☐

    If Yes, please indicate what condition. Below is a list of common cardiac conditions, please select all that apply or indicate what other conditions have been diagnosed.

Coronary artery disease	☐
Heart attack	☐
Abnormal heart rhythms or arrhythmias	☐
Heart failure	☐
Heart valve disease	☐
Vascular disease	☐
Cardiomyopathy	☐
Other	☐

    If selected "other" please explain _____

13. Have you ever been diagnosed with a condition that prevents you from exercising at a high intensity?

    Yes ☐ No ☐

<u>Thank you for your participation! The research assistant will review your answers and inform you if you are a candidate to participate in this research</u>

For research assistant Use only

Participant ID Number _____ Date_____

Curriculum Vita

Daniel Conde was born on August 19, 1985 in Guanajuato, Mexico. The first son of Marcela Iwashige, moved to El Paso, Texas after graduating from high school in 2004. In 2010, he earned a Bachelor's in Science of Kinesiology with an Exercise Science concentration and in 2014, he earned a Master's in Science of Kinesiology with an Exercise Physiology concentration from the University of Texas at El Paso. While completing his graduate studies, he presented his research work in several regional, national and international conferences including, the Keystone Symposia on Mitochondria Function Regional Conference, the Society for Industrial and Systems Engineering (SISE), the American College of Sports Medicine (ACSM) National Conference, and the International Commission on Occupational Health (ICOH). Besides working on his research project, he also served as a teaching and research assistant for the Kinesiology, Public Health Sciences, and Physical Therapy Departments and as an adjunct professor for the Speech Language Pathology Program. Daniel has been married to Maiko since August, 2015. In the Fall of 2014, he entered the Interdisciplinary Health Sciences Ph.D. Program.

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