Biomarkers of Physiological Damage and their Potential for Work-Related Musculoskeletal Disorder Risk Assessment

Marc Christian

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Maury A. Nussbaum, Chair

Michael J. Agnew

Michael L. Madigan

Deborah E. Young-Corbett

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Abstract

Work-related musculoskeletal disorders (WMSDs) continue to present a substantial personal and economic burden. Biomarkers, in providing objective measures of physiological changes, may offer advantages over current tools for WMSD risk assessment. Existing work has identified biomarkers of cartilage and muscle damage, and demonstrated responsiveness to various forms of physical activity and biomechanical loading. Here, three studies were complete to further assess the occupational relevance/utility of three selected biomarkers: Cartilage Oligomeric Matrix Protein (COMP), Interleukin-6 (IL6), and Creatine Kinase (CK). First, the effects of age, obesity, gender, and diurnal variation was investigated. Significant effects of time, age, and gender were evident, as well as some interactive effects, for COMP and CK, but not IL6. Second, biomarker levels were compared between individuals in occupations having relatively high and low WMSD risk. IL6 levels were greater in the high-risk group, while COMP levels demonstrated an oscillatory pattern, and CK levels did not vary between groups. Third, physical demands were imposed on the lumbar spine during a repetitive flexion/extension task, under conditions with different loading and frequency. IL6 levels varied significantly over time and between added load levels, while CK levels varied over time and was influenced by load and frequency. These studies demonstrate important features of biomarkers; that personal confounding factors need to be considered, that select biomarkers may be sensitive to occupational risk factor exposure, and particularly to task parameters in lifting activities involving the lower back. Further, these studies reveal important information concerning the relevance of the selected biomarkers, favorable time points for biomarker collection, and approximate biomarker levels expected between occupations and exposure to common risk factors. These results support the use of biomarkers in occupational settings for assessing exposure and WMSD risk imposed by common risk factors. Sensitivity to exposure levels is an important precursor to risk prediction, however prospective work is needed to verify predictive validity.

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1. Introduction

Work-related musculoskeletal disorders (WMSDs) continue to present a substantial economic burden for employers, workers' compensation, and the medical system, and adverse health effects on employees. In 2011 alone, about 3 million workplace injuries were recorded (Bureau of Labor Statistics, 2011b). Among these, overexertion injuries in particular account for the majority of WMSDs (~25%), whose associated annual direct costs are about $13 billion (Liberty Mutual Research Institute for Safety, 2013), and with indirect costs estimated as high as $200 billion (da Costa and Vieira, 2010). Diverse tools have been developed to assess workplace tasks, but discrepancies between prevailing theories of injury causation and risk assessment methods suggest limitations in their sensitivity and specificity.

Current theory suggests that overexertion injuries are caused primarily by an inability of one or more tissues to withstand a given load (Coenen et al., 2012a; Kumar, 2001). Such a direct relationship, though, is complicated by potential decreases in tissue tolerance over time (Marras, 2000) and the complex influences of diverse personal factors such as psychological state, age, gender, and obesity (Steering Committee for the Workshop on Work-Related Musculoskeletal 1998). Many tissue tolerance limits stem from *in vitro* studies, such as of muscles, vertebrae, and connective tissues (Brinckmann and Hilweg, 1989; Noyes, 1977; Sato et al., 1984). Despite general agreement that the etiology of overexertion injuries is multivariate in nature (da Costa and Vieira, 2010; Marras, 2000; Waters et al., 1993), few current exposure and risk assessment methods account for multiple causal pathways. Compounding the difficulty of determining causation, sub-clinical WMSDs often lack pathological symptoms,

making their identification prior to injury more difficult (Vällfors, 1985). Additional risk assessment tools that can account for known risk factors, while also detecting sub-clinical physiological changes, would enhance our ability to predict WMSD risk and could help in the future to substantially reduce the prevalence and adverse impacts of WMSDs.

Biomarkers that are specific to the biological tissue(s) of interest may help overcome some limitations of current WMSD risk assessment methods, since they are directly related to physiological injury. As such, they can help describe the presence and/or progression of sub-clinical tissue damage (see Figure 1.1) prior to developing a WMSD, and thus may be used to enhance our ability to predict future injury risk. By examining the exposure-response relationships between physiological demanding occupational tasks and secreted biomarkers it may be possible to better predict WMSD risk. While there is evidence that personal factors can confound biomarker measurements, there are very few empirical studies that have described such effects. Furthermore, biomarker levels have been associated with the level of physical work, but few controlled studies have demonstrated a causal relationship with exposure to known WMSD risk factors. The following paragraphs summarize several limitations of current exposure and WMSD risk assessment methods, emphasizing discrepancies due to confounding factors as well as commenting on their validity. Then, biomarkers are introduced as an alternative, relevant research gaps are highlighted, and arguments are given regarding how the proposed work addresses current research needs.

Figure 1.1 Conceptual model of the progression of WMSDs and the use of biomarkers in occupational settings. Current evidence supports the use of biomarkers for assessing levels of sub-clinical tissue damage. Following work to determine predictive ability, biomarkers levels may also be used to assess WMSD risk associated with specific occupations, tasks, etc.

1.1 Current Methods for Assessing Exposure and WMSD Risk

Commonly used methods for assessing exposure to risk factors and WMSD risk can be broadly classified into three categories (Dempsey, 1998); psychophysical, physiological, and physical (or biomechanical). The primary occupational use of these is to, respectively, determine workload perceptions or limits, assess metabolic demands and/or fatigue, and estimate mechanical loads. As noted earlier, prevailing theories of injury causation highlight the central role of loads exceeding tissue tolerance, and each of these categories focuses on a different loading mode. Many exposure assessment tools can be related directly to risk assessment, while other methods have incorporated multiple criteria to determine WMSD risk. The following paragraphs discuss these methods, focusing on the theory and commenting on their validity.

Psychophysical approaches are exposure and risk assessment tools based on the theory that perceived capabilities and discomfort are a reflection of individual physical and/or physiological tolerances (Ehrenstein and Ehrenstein, 1999). Application of psychophysical methods for determining WMSD risk assumes such risk can be inferred by comparing task demands (e.g., weights being lifted) to 'safe' levels obtained from experiments. However, the validity of this assumption may be questionable, particularly since personal factors can confound perceived intensities of exertions and discomfort ratings (Chen et al., 2002; Whaley et al., 1997). A study which prospectively followed high and low risk populations demonstrated non-significant risk ratios for discomfort ratings as predictors of future musculoskeletal pain (Reenen et al., 2008). When using the Revised NIOSH Lifting Equation (RNLE, discussed below), a method incorporating psychophysical, physiological, and physical methods, 60% of jobs classified as high-risk were judged to be acceptable by workers (Marras et al., 1999). In another study, though, ratings of perceived exertion were highly correlated with biomechanical demands, but only when within-subject variability was included in the model (Nussbaum and Lang, 2005). A recent study demonstrated that ratings of perceived exertion significantly correlated with indices of muscular loading (Jakobsen et al., 2013). Psychophysiological methods allow for realistic task simulations, but lack consistently demonstrated validity and may be highly variable between people, and thus appear to have limited applicability for determining the presence of sub-clinical physiological injury and predicting WMSD risk.

Physiological methods incorporate objective measurements to determine the effects of exposure to risk factors on several outcomes. Motivating the use of several

physiological methods is evidence that changes in some measurements can indicate general and/or localized fatigue, and potentially the level of injury risk. One study, for example, associated peak and cumulative muscle loading with the presence of WMSDs and the number of days off work (Village et al., 2005). An 11-year prospective study associated excess occupationally-related physical activity, as measured by oxygen consumption, with significant progression of atherosclerosis (Krause et al., 2007). Maximum exertions for various muscles, as measured by electromyography, were able to predict exhaustion time for a task, and showed good agreement between muscles and with psychophysical perceived exertions (Nussbaum et al., 2001). Unfortunately, electromyography does not differentiate well between concentric and eccentric muscle contractions, where the latter is associated with more muscle damage (Linnamo et al., 2000). While these methods appear to assess task exposure well, the relationship to sub-clinical physiological damage remains unclear, since exact causal pathways of changes in physiological measurements have not been elicited, and subject-specific parameters may confound measurements (McCully and Hamaoka, 2000).

Physical methods may overcome limitations of the other methods since these measures are objective and directly related to established metrics for physiological damage (Chaffin and Park, 1973). This allows them to both assess exposure to risk factors and predict WMSD risk. Both cadaver and epidemiological studies have helped to establish task parameters, such as load and frequency, that likely contribute to overexertion injuries (Marras, 2000; Yoganandan et al., 1994). In combination with physiological methods, biomechanical models can help determine physical task parameters or exposures, such as cumulative and peak spinal loads, and the frequency and force of

wrist movements, aspects of tasks that are associated with an increased risk of developing, respectively, low back (Waters et al., 2006) and wrist disorders (Silverstein et al., 1986).

More generally, field studies have identified factors such as lumbar moment and shear, hand force, and trunk velocity as associated with increased WMSD risk in the low back (Marras et al., 1995; Norman et al., 1998). A recent prospective study of 56 distribution center workers demonstrated that cumulative spine loading predicted clinically meaningful declines in low-back function with a sensitivity of 72% (Marras et al., 2014). In a comparison of five common risk assessment tools, 67 occupational tasks were evaluated and which had highly variable estimated risk levels (Lavender et al., 1999). Further, results from epidemiological studies typically provide only associations between risk factor exposures and WMSD risk. In practice, the use of generalized loads and frequencies though, cannot account for potential confounding or moderating effects, such as age and gender (Pintar et al., 1998), thus limiting predictive ability. As with physiological methods, physical task assessment appears to assess exposure well, but may be limited in ability to detect physiological damage, and thus predict WMSD risk.

Although the reviewed methods can be used as risk assessment tools, they are primarily utilized to assess exposure. By combining evidence from psychophysiological, physiological, and physical methods, risk assessment tools have been developed that account for numerous modes of risk factor exposure. The RNLE, for example, incorporates several lifting task parameters to determine risk relative to a task that is acceptable to 75% of females, has a maximum energy expenditure of 4.7 kcal/min, and a maximum spinal compressive force of 3400kN (Waters et al., 1993). In a study

comparing multiple risk assessment methods for assessing risk of 93 vehicle production tasks, the RNLE had the lowest threshold for identifying tasks as medium and high risk (Lavender et al., 1999). Despite a demonstrated sensitivity for identifying high risk tasks of 73% (Marras et al., 1999) and significant odds ratios for 'high-risk' tasks (Waters et al., 1999), a one cross-sectional study demonstrated that tasks classified as 'high risk' did not result in significantly higher WMSD rates (Waters et al., 2011b). A recent prospective study followed 256 workers from diverse manufacturing facilities and found that composite peak and peak lifting index significantly predicted self-reported low back pain and risk for seeking medical treatment (Garg et al., 2013a; Garg et al., 2013b). Again though, at high levels of exposure, risk estimates were lower than at medium exposure levels. Another recent prospective study found no significant association of composite lift index to low back pain in a multivariate model, but an odds ratio of 5.8 for lift indices above 2 (Lu et al., 2014). While the RNLE appears to predict risk for low back pain well at medium exposure levels, variable risk predictions at extremes of exposure call into question its predictive validity. Finally, certain RNLE factors may not actually influence risk as predicted (Adams et al., 2010).

As other examples of exposure tools, the Rapid Upper Limb and Entire Body Assessment tools (RULA and REBA, respectively), were designed to more generally code individual body segments and musculoskeletal groups, and provide an estimate of risk and the urgency of attention (Hignett and McAtamney, 2000; McAtamney and Corlett, 1993). Using highly trained observers one can achieve consistent and reliable task assessments (Yen and Radwin, 2002), but unfortunately risk estimates may not be reliable (Brodie and Wells, 1996). Some tools are much more specific to single body

area, such as the Hand Activity Level (HAL), which calculates a risk index based on a description of the movement and normalized peak force exerted (Drinkaus et al., 2005). The HAL has shown general agreement with psychophysical methods (Johnson and Nussbaum, 2003), and recent evidence suggests the HAL may be able to the predict risk of Carpal Tunnel Syndrome (Bonfiglioli et al., 2013). Two recent prospective studies followed a cohort of manufacturing workers over six years and demonstrated that the HAL did not significantly predict lateral epicondylitis (Garg et al., 2014), and only predicted flexor tendon entrapment of the digits when dichotomized, not as a continuous variable (Kapellusch et al., 2013). Unfortunately, another study showed that tasks deemed as acceptable, by the HAL, still resulted in many hand and wrist WMSDs (Franzblau et al., 2005), resulting in low sensitivity. One exposure assessment tool that has shown promise for risk assessment is the Strain Index (Moore and Garg, 1995). Higher levels of exposure derived from this tool have been found to be monotonically associated with injury outcomes (Moore et al., 2001; Rucker and Moore, 2002). The same prospective studies discussed above demonstrated that the Strain Index significantly predicted both lateral epicondylitis (Garg et al., 2014) and flexor tendon entrapment of the digits (Kapellusch et al., 2013). In a survey of general WMSDs a dichotomized, not continuous, Strain Index significantly predicted hand and arm symptoms (Gerr et al., 2013). The Michigan 3-Dimenstional Static Strength Prediction Program (3DSSPP) predicts risk based on both strength and biomechanical factors, and is arguably one of the most popular risk assessment methods as it can be applied to a wide variety of industrial tasks (Fischer, 2011). Outputs of this tool include a percentage of capable population, estimates of spinal compressive forces, and muscle

demands, all of which are measured relative to previously determined ‘safe’ levels (Waters et al., 2011a). To date, no epidemiological studies confirm the validity of 3DSSPP, but in comparison to the RNLE, 3DSSPP rated only 1% of the same simulated tasks as ‘high-risk’, indicating a lack of predictive power (Lavender et al., 1999). Combinatory methods show promise as their multi-modal assessments account for more known WMSD risk factors. Differences in the assessed task related factors likely influence the efficacy of risk assessment methods (Marras et al., 1999). Thus, more epidemiological research is needed to establish which tools are best-suited for a given task, and will result in accurate WMSD risk predictions.

Current methods have contributed to our understanding of task-related risk factors, and a variety of exposure assessment methods and tools exist, enabling direct comparisons between tasks. However, field studies have not confirmed predictive validity for many of these tools as risk assessment methods. Furthermore, for the same task conditions, different tools can give diverging estimates of risk, suggesting limits in their potential for accurate risk assessment (Lavender et al., 1999). There is a lack of empirical studies demonstrating sub-clinical physiological damage following task exposure as measured by many of these tools, and their ability to accurately predict WMSD risk has not been fully assessed. A method that can predict risk while accounting for personal factors, and which gives measures that are a direct result of physiological damage, would help overcome limitations of and enhance the current methods, thus improving our ability to evaluate workplace tasks and accurately predict WMSD risk.

1.2 Biomarkers Related to Physical Demands and Tissue Damage

Biomarkers are defined as molecules or proteins, "[molecules] that can be objectively measured and evaluated as an indicator of a physiological…process" (Jain, 2010, pg. 1), and are thus closely aligned with theories of WMSD causation. Since changes in select biomarkers can indicate sub-clinical physiological injury, they may act as surrogates for the "gold standards" that involve costly imaging technologies and invasive tissue biopsies. Biomarkers applicable to the research projects proposed here were chosen as matching the following criteria: changes in levels are a direct consequence of physiological damage and use; existing literature suggests these biomarker changes can be seen on a systemic level (as opposed to a local, which would involve obtaining biopsies); biomarkers themselves occur as early as possible in biological pathways of damage and/or repair; and there is little evidence suggesting confounding factors besides those considered here.

Through an examination of the literature, three biomarkers were chosen for use here: Cartilage oligomeric matrix protein (COMP), Interleukin-6 (IL6), and Creatine kinase (CK). As indicated below, respective levels of these indicate the amount of cartilage damage, muscle use, and muscle damage. Since these biomarkers are a direct consequence of physiological damage, examining their levels prior and after exposure to physically demanding tasks may enhance our ability to identify sub-clinical physiological damage and predict WMSD risk, thus alleviating some of the limitations associated with current methods.

COMP is a protein ubiquitous to cartilagenous tissue, and whose main purpose appears to be the stabilization of collagen fibers to support the extracellular matrix under mechanical stress (Muller et al., 1998). Cartilage degrades under mechanical stress, rupturing cells, and thus releases COMP into the bloodstream (Neidhart et al., 1997), while a mechanosensitive promoter region responds by inducing COMP synthesis in cartilage tissue (Amanatullah et al., 2012), replenishing the supply and providing additional mechanical strength. A number of studies have demonstrated that COMP levels are sensitive to physical loading, for example during running (Kim et al., 2007, 2009), walking (Mündermann et al., 2005), and even drop landings (Erhart-Hledik et al., 2012). Thus, COMP is considered appropriate to represent the state of cartilagenous tissue, and temporal changes in COMP may help predict future cartilage injury.

Inflammatory cytokines, such as IL6, commonly act as signaling molecules, inducing both pro- and anti-inflammatory effects to aid recovery (Nielsen and Pedersen, 2007). The source of the majority of systemic IL6 has been traced to active muscle (Reihmane and Dela, 2013; Toft et al., 2011), where it is expressed proportionally to the intensity, duration, and mass of muscle used (Pedersen and Febbraio, 2008; Pedersen and Fischer, 2007). The purpose of IL6 is to suppress previously expressed inflammatory proteins (Starkie et al., 2003) and to induce collagen synthesis in tendons to aid recovery (Andersen et al., 2011). IL6 increases with general exercise (Toft et al., 2002; Wallberg et al., 2011), running (Scott et al., 2013), cyclic loading of the lumbar spine in felines (D'Ambrosia et al., 2010) and humans (Splittstoesser et al., 2012; Yang et al., 2011), and in various types of lumbar disc herniation (Takahashi et al., 1996). Changes in IL6 expression have been correlated with the severity of upper-extremity WMSDs

(Carp et al., 2007), making it a promising candidate for identifying sub-clinical muscle damage and inflammation.

CK has long been used in the medical community as an indicator of heart attacks (Jain, 2010), since it is a muscle-specific enzyme that is released into the bloodstream following eccentrically induced cell rupture (Newham et al., 1986). Consistent with this, transient CK increases have been shown throughout a marathon race proportional to the distance covered (Kim et al., 2009) and in response to repetitive lifting (Splittstoesser et al., 2012; Yang et al., 2011), and are commonly correlated with ratings of delayed onset muscle soreness (Magal et al., 2010; Su et al., 2010). Studies have demonstrated that CK may even be a more reliable indicator of muscle damage than magnetic resonance imaging (Sorichter et al., 1995), and that muscle fatigue indicators predict CK responses (Hody et al., 2013). Such evidence suggests that CK is a useful indicator of muscle damage that, in combination with IL6, indicates the state of muscle tissue.

The criteria mentioned above helped to guide the selection of the three biomarkers noted. Despite this selection, other studies using biomarkers often utilize a large array of molecules. The work of Yang et al. (2011) is arguably most related to the current examination, and which involved exposing participants to a lower back lifting task with different weights. Significant biomarker changes were found for IL6 and CK, but also others such as tumor necrosis factor α (TNFα), interleukin 1β (IL1β) and 10 (IL10), white blood cell count (WBC), and granulocyte counts (GRAN). Unfortunately, many of their selected biomarkers are biologically dependent on each other. TNFα (also commonly measured in other physical task studies) has been shown to be dependent on IL6

production, specifically for non-damaging exercise (Petersen and Pedersen, 2005), in that IL6 inhibits production of TNFα. Conversely, in muscle damage related to physical exercise, TNFα is produced prior to IL6. In choosing IL6 over other cytokines here, it was noted that a slew of cytokines are typically released during the tissue repair process, but IL6 is produced in the muscle *during* exercise (Steensberg et al., 2002), while others are often recruited by repair cells and many other cytokines are typically only observed locally. This repair cascade also explains changes in immune responsive WBC and GRAN counts, suggesting these biomarkers indicate similar physiological properties to IL6. The purposes of COMP and CK are biologically narrowly defined, meaning they likely have few confounding factors, besides those discussed below, making them preferential candidates for biomarkers of cartilage and muscle damage. For these reasons, COMP, IL6, and CK were selected, as changes in levels are a direct consequence of physiological damage and use, literature suggests these biomarker changes can be seen on a systemic level, they occur as early as possible in biological pathways of damage and/or repair, and there is little evidence suggesting confounding factors besides those considered here. Together these three biomarkers give insight into the tissues most commonly associated with WMSDs and, in combination with current methods, should enhance the ability to accurately predict WMSD risk.

1.3 Limitations of Biomarkers

A careful review of the literature has yielded three biomarkers, noted above, that together can indicate the physiological state of cartilagenous and muscle tissues

associated with overexertion WMSDs. Despite previously observed changes in biomarkers following exposure to WMSD risk factors, the complexity of biological signaling pathways suggests that COMP, IL6, and CK may be influenced by other confounding factors besides physical stress, specifically age, obesity, and gender. There is also established medical literature and scientific evidence that may explain how these factors influence levels of these selected biomarkers.

Numerous health decrements are associated with the process of ageing, such as a decreased muscle capacity (Degens and Korhonen, 2012), bone mineral content (Gomez-Cabello et al., 2012), general low-grade systemic inflammation (Pedersen et al., 2000), and an overall impaired tissue recovery ability (Uciechowski and Rink, 2009). This evidence suggests increased baseline levels of IL6. Another study demonstrated that older adults had higher baseline levels of IL6 and a blunted peak value of CK compared to younger adults following eccentric exercise (Toft et al., 2002). While there is no evidence that increased age influences COMP levels, it has been suggested as a biomarker indicating the presence of age-associated arthritis (Verma and Dalal, 2013; Zivanovic et al., 2011). Quantifying whether there are age-related differences in biomarkers is important, given the wide age range of the working population.

Obesity, defined as a BMI≥30 (World Health 2011a), is another factor that can contribute to differences in biomarker levels. Biomechanical approaches suggest increased joint loads with increasing obesity, and there is medical evidence for increased systemic inflammation and inhibited tissue repair capacity due to the abundance and volume of adipose tissue (Nieman et al., 1999; Weisberg et al., 2003). A substantial portion of systemic IL6 is expressed in adipose tissue, and accounts for

the results that BMI had the highest correlation with IL6 in a population-based survey (Christian et al., 2011). In addition higher IL6 levels occur among individuals who are obese following exercise (Galassetti et al., 2011). Despite a lack of evidence concerning the effects of obesity on CK and COMP, the latter is expected to be positively correlated with BMI due to greater cartilage loading with obesity. As with age, determining the influence of weight status on biomarker levels is considered important, due to the wide range in the working population.

Finally, there is evidence that females are at an increased risk for developing WMSDs (Luoto et al., 1995; Silverstein et al., 1987). If biomarkers are to be used as a risk assessment tool, it can be argued that they should indicate differences in WMSD risk between genders when performing comparable tasks (at least in some circumstances). Following a maximum exercise protocol, females showed a continual increase in IL6, after 60 minutes, while levels in males began to decrease, indicating possible differences in tissue recovery (Edwards et al., 2006). CK levels appear to be primarily increased in males following concentric exercise (Stupka et al., 2000), possibly due to greater muscle mass or differences in strength. As with age and BMI, the evidence for differences in biomarker levels between genders is lacking.

Although there is physiological and physical theory and some evidence that indicates differences in COMP, IL6, and CK associated with personal factors such as age, BMI, and gender, this evidence is limited and incomplete. If biomarkers are to be used in the workplace as a WMSD risk assessment tool they should be able to account for differences between workers as well as the tasks they perform.

1.4 Current Research Needs

Most existing studies of work-related biomarkers in humans have not considered the potential confounding factors noted above, or have avoided them using exclusion criteria. But, contemporary trends involving an increase in obesity (Hertz et al., 2004) and age (Bureau of Labor Statistics, 2009) in the workplace emphasize the particular need to account for these possible influencing factors. Furthermore, not accounting for these factors results in substantial threats to internal validity (Kim et al., 2007; Toft et al., 2002; Yang et al., 2011), while excluding participants limits external validity (Miles et al., 2008b). To our knowledge, there is no reported research that specifically examined the effects of potential influential factors in isolation, yet doing so is especially important to account for such effects in future studies. The evidence presented above suggests that we are likely to observe differences in the selected biomarkers for people of different ages, BMIs, and genders, with possible interactive effects. Beyond these influences, there is also evidence that several other personal factors can influence biomarker levels, such as time of day, diabetes, general physical activity, ethnicity, and psychosocial variables. However, such influences are considered beyond the current scope of work. By studying the effect of age, BMI, and gender, which the literature suggests have the most substantial effects on biomarker levels, and which are considered the most occupationally relevant, changes due to confounding factors can be accounted for in future studies.

To assess the validity of biomarkers for predicting WMSD risk, prospective studies are, ideally, needed that correlate long-term changes in biomarker levels with incidence of WMSDs. Such evidence is lacking, and without a clear understanding of contributions of confounding factors, such as age, BMI, and gender, any prospective studies would have compromised validity. One cross-sectional study did find an association between IL6 and the severity of diagnosed upper-extremity disorders (Carp et al., 2007). While this result suggests IL6 may be used to predict WMSD risk, additional studies are needed that establish temporal biomarker changes following exposure to known WMSD risk factors. Comparing changes in biomarkers between groups with high and low exposure to WMSD risk factors would demonstrate their ability to differentiate occupations and argue for their use as a WMSD risk assessment tool.

As discussed earlier, there is evidence for an association between biomarker levels and exposure to physical exertion, but the relevant studies used exertions that involved multiple body regions (Kim et al., 2009; Splittstoesser et al., 2012; Yang et al., 2011). As such, it is unclear regarding the influences of specific tissues on biomarker changes reported in these studies. Current tools for estimating WMSD risk have made valuable contributions to our understanding of risk factors. It is generally agreed that force (both compressive and exerted) and task repetition contribute greatly to WMSD risk (Waters et al., 1993) by proportionally decreasing tissue tolerance with increased exposure. Studies have further demonstrated significant changes in rat models of WMSDs, suggesting an interaction of force and repetition on inflammatory biomarker levels (Barbe et al., 2008; Barbe et al., 2013). While existing research has established a general relationship between biomarker changes and task-related factors, few have

done so while controlling which tissues or tissue groups are utilized (Kim et al., 2007, 2009; Marklund et al., 2012; Waskiewicz et al., 2012; Yang et al., 2011). Determining a more precise relationship between biomarker changes and task related factors will both allow us to better predict expected biomarker changes following exposure to high-risk tasks and align biomarker changes with current theories of WMSD causation.

1.5 Research Goal and Contributions

The main goal of this dissertation work was to explore the effects of personal and occupationally-relevant factors on select biomarkers of physiological damage. This work was considered especially important, since biomarkers may more accurately reflect physiological damage, and thus predict risk of WMSDs better than current methods. Achieving this goal can help in future studies to account for biomarker changes due to personal factors and help assess the validity of biomarkers as a WMSD risk assessment tool. In support of this goal are three aims, each of which was achieved in a separate experiment.

The first aim was to quantify the diurnal changes in biomarkers and the modifying effects of age, gender, and obesity on these changes. Given the current trends of increasing obesity and age in the workplace, and a body of evidence suggesting biomarker differences due to these personal factors, accounting for these factors is considered particularly important. This was the first study to investigate biomarker differences due to these factors in isolation, thus allowing us to account for differences between people and with biomarker levels obtained at different times of day.

The second aim was to assess the sensitivity of biomarkers to a range of exposures to known WMSD risk factors. Studies of biomarkers of physiological damage in high WMSD risk industries have not yet been reported, though correlations between biomarkers and WMSD severity have been reported (Carp et al., 2007; Carp et al., 2008b). Here, biomarker levels will be obtained before and after work, from workers in both sedentary jobs and those with high physical demands. From this, biomarker changes due to occupational exposure will be determined, along with estimates of recovery rates outside of work and trends over a working week. Results were expected to provide support for biomarker sensitivity to occupational exposures, and associations between biomarker levels and perceived discomfort.

The third aim was to determine whether there is a dose-response relationship between biomarker changes and the level of exposure to known WMSD risk factors. Here, the magnitude and frequency of lower back loads were examined. While many studies have found associations between biomarker changes and general physical work output (Kim et al., 2009; Libardi et al., 2012; Marklund et al., 2012), and some have also found such associations for specific muscle groups (Jubeau et al., 2012; Kouda et al., 2012), none have observed changes in muscle groups typically injured in WMSDs. An association between biomarker changes and controlled levels of exposure to low back loads was expected, and would provide further support for the validity of the biomarkers.

2. An Exploratory Study of the Diurnal Variation and Reliability of Serum Levels of Cartilage Oligomeric Matrix Protein, Interleukin-6, and Creatine Kinase

2.1 Abstract

Select biomarkers have been identified that reflect biomechanical loading of cartilage and muscle tissues. Factors beyond biomechanical exposures, though, may influence these biomarkers and thus should be quantified. This study determined: 1) whether diurnal variations exist for Cartilage Oligomeric Matrix Protein (COMP), Interleukin-6 (IL6), and Creatine Kinase (CK); and 2) if these diurnal variations are influenced by certain personal factors (i.e., age, obesity status, and gender).

Twenty-seven participants were dichotomously classified by age, obesity status, and gender, and from whom blood samples drawn at six time points over a 24-hour period (22:00, 07:00, 10:00, 14:00, 18:00, 22:00). For the 48-hours prior to and during the observation period, participants restricted their physical efforts other than those required for normal daily activities.

COMP levels were significantly higher for males, had significant diurnal variation, and this diurnal variation differed between genders. IL6 did not have any significant diurnal variation or differences related to age, obesity status, or gender. CK levels exhibited significant diurnal variation, and levels were significantly influenced by age and an obesity status x gender interaction. Reliability (between two samples at 22:00) was excellent for all biomarker levels.

The observed diurnal differences in COMP and CK were likely due to differences in body composition between the groups. The present results may aid in future study design and in interpreting the levels and magnitudes of observed changes in the current biomarkers.

2.2 Introduction

Long term exposure to biomechanical stress is commonly associated with degradation of tissues such as cartilage and muscle, and may result in musculoskeletal disorders (MSDs) (Clarkson and Hubal, 2002; Kjær et al., 2009). It is estimated that approximately half of the adult population in the US will report an MSD in their lifetime, and the associated medical costs account for about 2.5% of annual gross national product (MacKay et al., 2010). Biomarkers (molecules, proteins, etc.) have long been used in the medical community to objectively assess changes in the physiological state of tissues (Jain, 2010), and some biomarkers are specific to cartilage and muscle, tissues that are commonly involved in MSDs. Recent studies have demonstrated that exposure to common MSD risk factors, such as force exertion, duration, and repetition, elicit detectable biomarker changes proportional to the extent of exposure (Kim et al., 2007, 2009; Niehoff et al., 2010; Yang et al., 2011).

Given these diagnostic properties, select biomarkers have been proposed as tools for predicting MSD risk, specifically in the occupational domain (Carp et al., 2008b). In the US workforce alone, the estimated total (direct and indirect) cost of work-related MSDs was in excess of $200 billion in 2010 (da Costa and Vieira, 2010). A number work-

related MSD risk assessment methods have been developed, but discrepancies between estimated and observed risk levels (Franzblau et al., 2005; Lavender et al., 1999; Waters et al., 2011b) have often been found, suggesting potentially limited validity of these methods. Factors beyond the specific exposure to MSD risk factors, though, may influence biomarker levels, and therefore determining the effects of these factors is important to facilitate injury risk prediction (e.g., to avoid and/or account for potential confounding). Of particular interest here was determining the diurnal changes in biomarkers during a period of restricted, but otherwise normal, physical activity, during which there were relatively low levels of exposure to MSD risk factors. In contrast, other studies have investigated diurnal changes in participants confined to bed (Sothern et al., 1995; Vgontzas et al., 2005; Vgontzas et al., 1999). Given our interest in assessing the potential utility of biomarkers in the occupational domain, and specifically using biomarkers to identify high-risk workers or tasks, we considered confinement to bed not to be a relevant referent condition (e.g., vs. those with high exposures to MSD risk factors). Diurnal changes were determined for three specific biomarkers that were selected as described below, and which were considered potentially useful for assessing MSD risk (to cartilage and muscle tissues, especially in the occupational domain).

Cartilage Oligomeric Matrix Protein (COMP) is synthesized in cartilage under mechanical compression (Saxne and Heinegard, 1992) and serves to provide additional strength to collagen molecules (Amanatullah et al., 2012). Levels of COMP were found to significantly increased following 100 drop landings (Niehoff et al., 2011) and throughout marathon running (Kim et al., 2007, 2009), indicating sensitivity to both

acute and more prolonged cartilage loading. COMP also changes with the progression of age-associated medical conditions, such as rheumatoid and osteoarthritis (Fujikawa et al., 2009; Tseng et al., 2009; Verma and Dalal, 2013), implicating age as a possible confounding factor. Since COMP levels are sensitive to the level of cartilage loading, it can be hypothesized that other factors influencing cartilage loading (e.g., diurnal changes in loading and obesity) will also need to be accounted for when interpreting COMP in terms of reflecting cartilage degradation. To the authors' knowledge, only one study has investigated diurnal changes in COMP in healthy individuals, as a control to arthritic patients, and which found that it was responsive to daily physical activity; specifically, COMP had an inverted 'U'-shaped diurnal pattern (Lottenburger et al., 2011).

Interleukin-6 (IL6) is an inflammatory cytokine that has both pro- and anti-inflammatory functions (Pedersen et al., 2004). In relation to physiological damage and biomechanical loading, IL6 levels have been demonstrated consistently to increase during exercise (Helge et al., 2011; Kouda et al., 2012; Wallberg et al., 2011; Yang et al., 2011), and often in proportion to the duration or intensity of that exercise. The majority of systemic IL6 produced during exercise is likely produced in muscle cells (Steensberg et al., 2002; Toft et al., 2011), although any dependency on fiber type remains unclear (Pedersen and Febbraio, 2008). Since IL6 acts as a general inflammatory marker, a number of other factors may alter levels. Increased age has been associated with systemic inflammation, and an increased baseline IL6 level is present among older compared to young adults (Miles et al., 2008a). Adipose cells may also contribute up to 35% of systemic IL6 levels (Mohamed-Ali et al., 1997), indicating a

possible confounding effect of obesity. In support of this, successful lifestyle interventions among individuals who are obese have resulted in decreased systemic IL6 levels, even after a five-year follow-up (Olszanecka-Glinianowicz et al., 2012). Finally, females appear to have a higher incidence of musculoskeletal injuries (Treaster and Burr, 2004; Widanarko et al., 2011), and further demonstrate a sustained IL6 increase following exercise compared to males (Edwards et al., 2006). Similar to COMP, only one study to date has investigated diurnal variation in IL6, and demonstrated a 'U'-shape diurnal pattern (Miles et al., 2008b).

Creatine Kinase (CK) is an enzyme found primarily in muscle tissue with the purpose of buffering cellular phosphate molecules with adenosine di-/tri- phosphate and creatine molecules. Following eccentrically-induced muscle cell rupture and Z-disc disruption, CK is released into the bloodstream (Brancaccio et al., 2010; Kuipers, 1994). Given this association with muscle damage, CK levels are frequently used clinically to determine whether a patient has experienced a heart attack (Jain, 2010). Several studies have shown that levels of CK following exercise are increased relative to the intensity and duration of diverse tasks, including simulated lifting (Yang et al., 2011), knee bends (Sorichter et al., 1995), and marathon running (Kim et al., 2009). An effect of gender on CK following exercise has also been demonstrated, specifically that levels in males are higher immediately following exercise and have greater peak levels (Sewright et al., 2008; Wolf et al., 2012). It is likely that age- and obesity-related differences contribute to changes in CK levels, for example through associated differences in muscle fiber composition and muscle. No studies (to the authors' knowledge) have confirmed this, however, nor have any assessed diurnal effects on CK.

Despite numerous studies describing the impact of diverse forms of physical activity on levels of COMP, IL6, and CK, few have taken into consideration the effect of potential individual differences (e.g., age, obesity, and gender), and fewer yet have determined whether diurnal changes in populations with restricted physical activity are substantial for those biomarkers. The purpose of this exploratory study was thus to determine: 1) whether diurnal variation exists for COMP, IL6, and CK during a period of restricted physical activity (with low levels of exposure to MSD risk factors), and 2) if this diurnal variation differs with age, obesity status, and gender. Due to the exploratory nature of this study, and given limited resources, analysis was limited to the three selected biomarkers as justified earlier. We hypothesized that biomarker levels will exhibit diurnal variation, and that this diurnal variation will be significantly affected by age, obesity status, and/or gender.

2.3 Materials and Methods

2.3.1 Participants

Prior to any data collection, all participants provided informed consent by reviewing and signing a consent form that described the aims and procedures of the study. The study procedures, including the consent form, were approved by the Virginia Tech Institutional Review Board. Twenty-seven participants completed the study, and were recruited as a convenience sample from the local population. Participants were specifically recruited to form eight groups, involving two levels each of age, obesity status, and gender as follows: young (18-30 years) and older (50-65 years); non-obese ($18.5 \leq BMI < 25$) and

obese (30≤BMI<40); and male and female. Due to a paucity of existing evidence regarding diurnal biomarker changes and individuals differences in these, a formal sample size calculation was not possible. Instead, the maximal sample size was used within resource constraints.

Given our interest in the potential occupational application of biomarkers, groups were chosen to represent typical extremes of the working population and current demographic trends (World Health Organization, 2011a; Lewis and Cho, 2011). The specific obesity classification used was designed to include individuals with both Class I (30≤BMI<35) and Class II (35≤BMI<40) obesity, which covers the majority of individuals who are obese in the US population (Wyatt et al., 2006). This range avoided Class III (40≤BMI) obesity, which is associated with several medical conditions that may confound biomarker levels, such as diabetes (Lazar, 2005), heart disease (Sarwar, Thompson, & Di Angelantonio, 2009), and inflammatory conditions (Posey & Hecht, 2008). Additionally, participants were excluded with the presence of self-reported: anemia, diabetes, inflammatory conditions (arthritis, Crohn's disease, etc.), heart disease, smoking, recent MSDs (an injury in the last three years resulting in a cost exceeding $1 (Rosenblum and Shankar, 2006), excessive (>81mg daily) non-steroidal anti-inflammatory drug (NSAID) use, being on lipid-lowering medication, or having any blood-borne diseases. To minimize residual effects of prior physical activity, participants were required to reduce their physical activity in the 48 hours prior to and through completion of the study, and specifically to exclude heavy lifting, playing sports, or other physical activities beyond those required to perform their normal daily activities. Participants unable or unwilling to reduce their physical activity for the required time

were excluded from the study (e.g., those whose jobs required high levels of physical effort, such as manual lifting).

2.3.2 Questionnaires

Participants completed two questionnaires prior to beginning the study. The first was a custom background questionnaire designed to determine age, gender, whether participants had any recent prior injuries or conditions that limited mobility, and to confirm prior reduction of physical activity. Next, participants completed the long-form International Physical Activity Questionnaire (IPAQ), to determine their habitual physical activity for the past seven days in several categories: occupational, transportation, household, and recreational/leisure. The long-form IPAQ was used since it has relatively good reliability (Craig et al., 2003) and validity (Hagstromer et al., 2006) for assessing total physical activity, as compared to the short-form IPAQ (Lee et al., 2011). Based on the IPAQ responses, the number of metabolic equivalent minutes/week (MET-min/week) were calculated.

2.3.3 Experimental Protocol

Six blood draws were taken over a period of 24 hours for each participant. The first meeting took place at 22:00 in the authors' laboratory on campus. Stature and body mass were first measured, and then the first blood sample was obtained (as described below). Before leaving the laboratory, participants were given explicit instructions to fast overnight (no food or drink, except water), and to not to rise from bed until 5-10 minutes before the morning blood draw so as to minimize biomarker changes due to physical activity. The next blood draw occurred at 07:00 (referred to as the "baseline"

measure in subsequent analyses), or earlier to accommodate work schedules, and was done at the participant's residence. Throughout the remainder of the day, participants returned to the laboratory for four additional blood draws at 10:00, 14:00, 18:00, and 22:00. These specific six "time points" for blood draws were used to: 1) obtain two samples at the same time of day (for assessing reliability), and 2) to have distributed samples during the waking period. Between blood draws participants performed their normal daily activities, with the requested physical restrictions noted above.

2.3.4 Blood Collection and Analysis

All blood samples were obtained by a licensed phlebotomist (MC) from an antecubital vein into evacuated serum separator tubes using a standard venipuncture technique. After clotting, blood samples were centrifuged (Sorvall X1R, Asheville, NC) for 13 minutes at 3300rpm to obtain the serum. Samples were stored in microcentrifuge tubes at -20°C until analysis. Levels of serum COMP (BioVendor, Ashville, NC), High Sensitivity IL6 (BioVendor, Ashville, NC), and CK (Antibodies-online.com, Atlanta, GA) were determined using commercially-available enzyme-linked immunosorbent assays (ELISAs) and according to the manufacturer's instructions. All sample concentrations were measured in duplicate and means used in subsequent analyses. Mean intra-assay coefficients of variability (σ/μ) for COMP, IL6, and CK were 2.7%, 3.8%, and 2.7% respectively.

2.3.5 Analysis

Statistical analyses were performed using SPSS for Windows (version 20.0, IBM, Armonk, NY). Differences in MET-min/week with respect to age, obesity status, and

gender were assessed initially using a three-way analysis of variance (ANOVA). For biomarkers, dependent measures included both actual levels and change scores that were derived relative to baseline levels (obtained at 07:00). Effects of age, obesity status, and gender on temporal changes in biomarker levels (both actual and change scores) were determined using separate three-way multivariate ANOVAs (MANOVAs), with *post hoc*, Bonferroni-adjusted, paired *t* tests performed between mean levels at each time point. Due to the relatively small sample size, only main and first-order interaction effects of the participant factors were included. CK data for one older, obese, female participant and IL6 data for one young, obese, male (both actual levels and change scores) were excluded due to clear technical errors. Three-way ANOVAs were conducted for each biomarker time point, using the same model noted above. Between-day reliability of biomarker levels (both actual and change scores) was assessed using the two levels obtained from each participant at 22:00. Both absolute and relative reliability of biomarker levels at 22:00 (actual and change score) were assessed, respectively using the standard error of measurement (SEM) and the intraclass correlation coefficient (ICC). Reliability scores were calculated using a repeated-measures ANOVA model (Eliasziw et al., 1994), with time point as a random effect, and correspond to the ICC(3,1) classification of Shrout and Fleiss (1979). ICCs were qualitatively interpreted using the following criteria: 0.00–0.39=poor, 0.40–0.59=fair, 0.60–0.74=good, and 0.75–1.00=excellent (Cicchetti and Sparrow, 1981; Fleiss, 1986). Consistent with the exploratory nature of this study, statistical significance was concluded when $p<0.1$. All summary statistics are reported as means (SDs).

2.4 Results

Descriptive statistics for each of the participant groups are shown in Table 2.1. MET-min/week values did not differ significantly between age ($F_{1,19}$ = 0.57, p = 0.46), obesity status ($F_{1,19}$ = 0.66, p = 0.43), or gender ($F_{1,19}$ = 1.45, p = 0.24) groups, and there were no significant interactive effects (p > 0.25). A summary of the statistical effects of gender, age, and obesity status on diurnal biomarker levels is provided in Table 2.2, and results for each biomarker are presented in more detail subsequently.

Table 2.1 Descriptive statistics for participants, separated by age, obesity status, and gender groups.

		Non-obese		Obese	
		Male	Female	Male	Female
Young	n	3	3	5	4
	Age (years)	23.0 (5.3)	23.3 (4.9)	24.4 (3.3)	27.3 (3.4)
	BMI (kg/m^2)	23.4 (2.3)	22.1 (3.2)	33.1 (2.5)	34.2 (2.1)
	MET-min/week	9330 (11070)	3200 (1810)	4090 (2290)	4490 (2760)
Older	n	3	3	3	3
	Age (years)	54.0 (2.6)	54.3 (8.1)	52.7 (1.5)	59.7 (4.5)
	BMI (kg/m^2)	24.0 (2.4)	22.9 (2.2)	34.3 (2.3)	35.2 (4.8)
	MET-min/week	6450 (6030)	4050 (1490)	1480 (1640)	2650 (1990)

Table 2.2 MANOVA results for the effects of time (T), age (A), obesity status (O), and gender (G) on COMP, IL6, and CK (actual levels and change scores). Significant tests are indicated in bold.

	COMP		IL6		CK	
	Actual	Change	Actual	Change	Actual	Change
Effect	p ($F_{5,16}$)	p ($F_{4,17}$)	p ($F_{5,16}$)	p ($F_{4,17}$)	p ($F_{5,15}$)	p ($F_{4,16}$)
T	**<0.01 (7.58)**	0.12 (2.13)	0.50 (1.01)	0.45 (1.08)	**0.05 (2.90)**	0.34 (1.22)
T x A	0.12 (2.07)	**0.07 (2.70)**	0.98 (0.17)	0.94 (0.23)	**0.07 (2.61)**	**0.04 (3.37)**
T x O	0.15 (1.93)	0.17 (1.85)	0.27 (1.81)	0.34 (1.86)	0.20 (1.68)	0.15 (1.96)
T x G	**0.05 (2.86)**	**0.05 (3.07)**	0.25 (1.95)	0.30 (2.11)	**0.07 (2.56)**	**0.03 (3.40)**
T x A x O	0.21 (1.65)	0.11 (2.19)	0.77 (0.97)	0.68 (1.24)	0.22 (1.61)	0.24 (1.54)
T x A x G	0.20 (1.68)	0.11 (2.22)	0.51 (0.95)	0.39 (1.18)	0.49 (0.92)	0.35 (1.20)
T x O x G	0.41 (1.08)	0.53 (0.82)	0.86 (0.45)	0.75 (0.58)	**0.02 (3.61)**	**0.01 (4.79)**

2.4.1 COMP

Males had greater COMP levels overall vs. females, with respective levels over all time points of 712 (336) ng/ml and 567 (142) ng/ml, and males also had greater levels at each time point (Figure 2.1A). Actual levels of COMP varied significantly over time, and temporal changes differed between genders. Actual levels at all time points were significantly higher than baseline (07:00 sample), the latter being 548 (232) ng/ml. Pairwise comparisons indicated that gender differences were significant only for the 10:00 and second 22:00 time points. COMP change scores varied significantly over time, and temporal changes differed between age groups and genders. Age differences were significant only for the first 22:00 sample (Figure 2.1B), and gender differences were significant at all time points except 18:00 (Figure 2.1C).

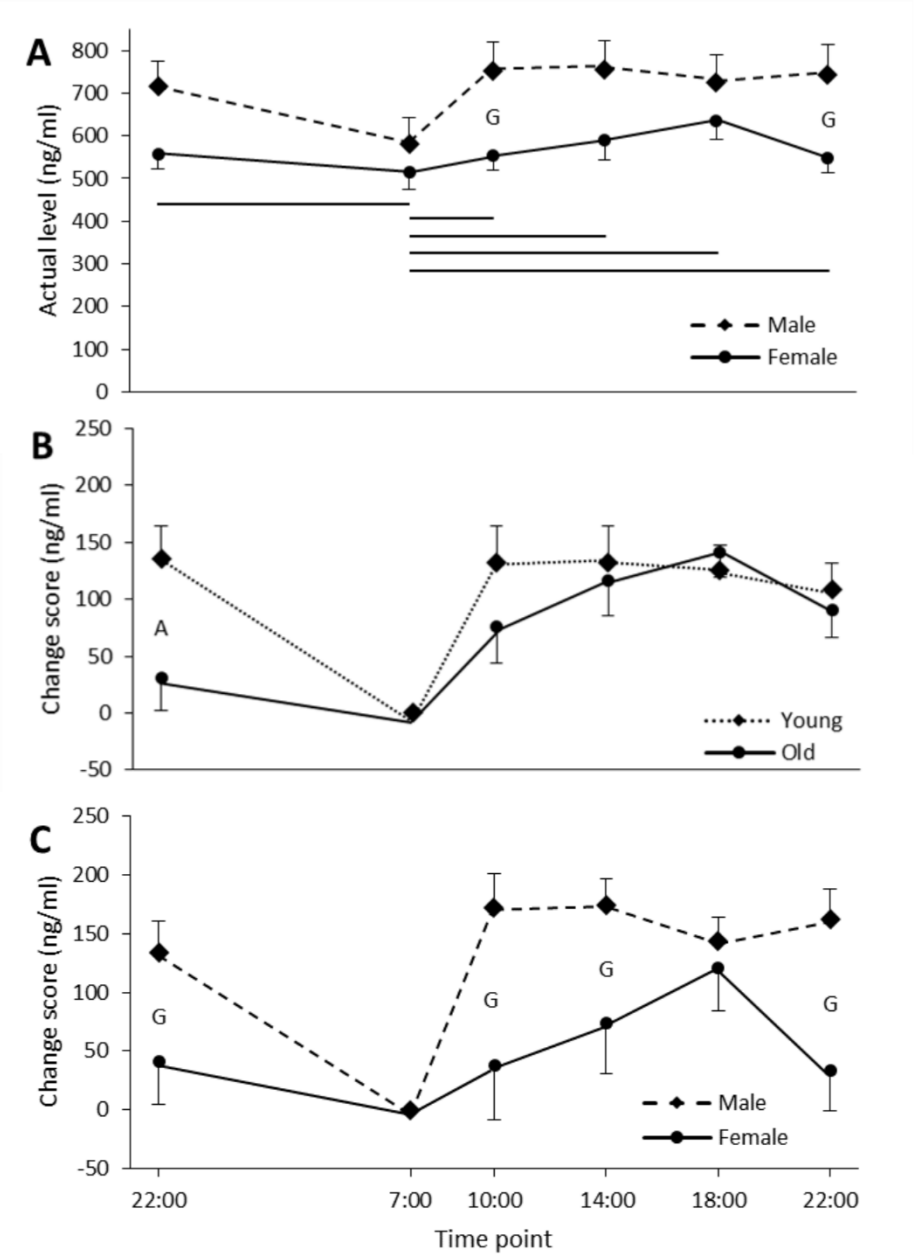

Figure 2.1 Diurnal changes in COMP actual levels (**A**) and change scores (**B** and **C**). Horizontal lines indicate significant differences between time points. Significant gender and age differences at discrete time points are indicated by "G" and "A", respectively. Error bars indicate standard errors.

2.4.2 IL6

Across all participants groups and time points, actual levels of IL6 were 2.48 (1.67) pg/ml. There were no significant temporal changes in actual levels or change scores, nor any significant main or interactive effects of age, obesity status, or gender (Table 2.2).

2.4.3 CK

Older participants had greater CK levels overall vs. young, with respective levels over all time points of 5.63 (2.97) ng/ml and 4.67 (1.49) ng/ml, and older participants had greater levels at each time point (Figure 2.2A). Further, obese males had greater CK levels overall vs. all other groups, with respective levels over all time points of 6.44 (3.35) ng/ml and 4.48 (1.02) ng/ml, and obese males had greater levels at each time point (Figure 2.2B). Actual levels of CK varied significantly over time, and these temporal effects were significantly different depending on age, gender, and the obesity status x gender interaction. *Post hoc* analyses did not reveal any significant differences in CK levels between time points. Pairwise comparisons indicated that the obesity status x gender interaction was significant at 10:00, 14:00, and 18:00 (Fig. 2a).

Temporal variations in CK change scores differed significantly depending on age, gender, and the obesity status x gender interaction. Post-hoc analyses did not reveal any significant differences in CK change scores between time points. Pairwise comparisons indicated that age differences were significant at 10:00 and 14:00 (Figure 2.2C). Additional pairwise comparisons indicated that gender differences were

significant at 10:00 and that the obesity status x gender interaction was significant at 10:00 and 14:00 (Figure 2.2D).

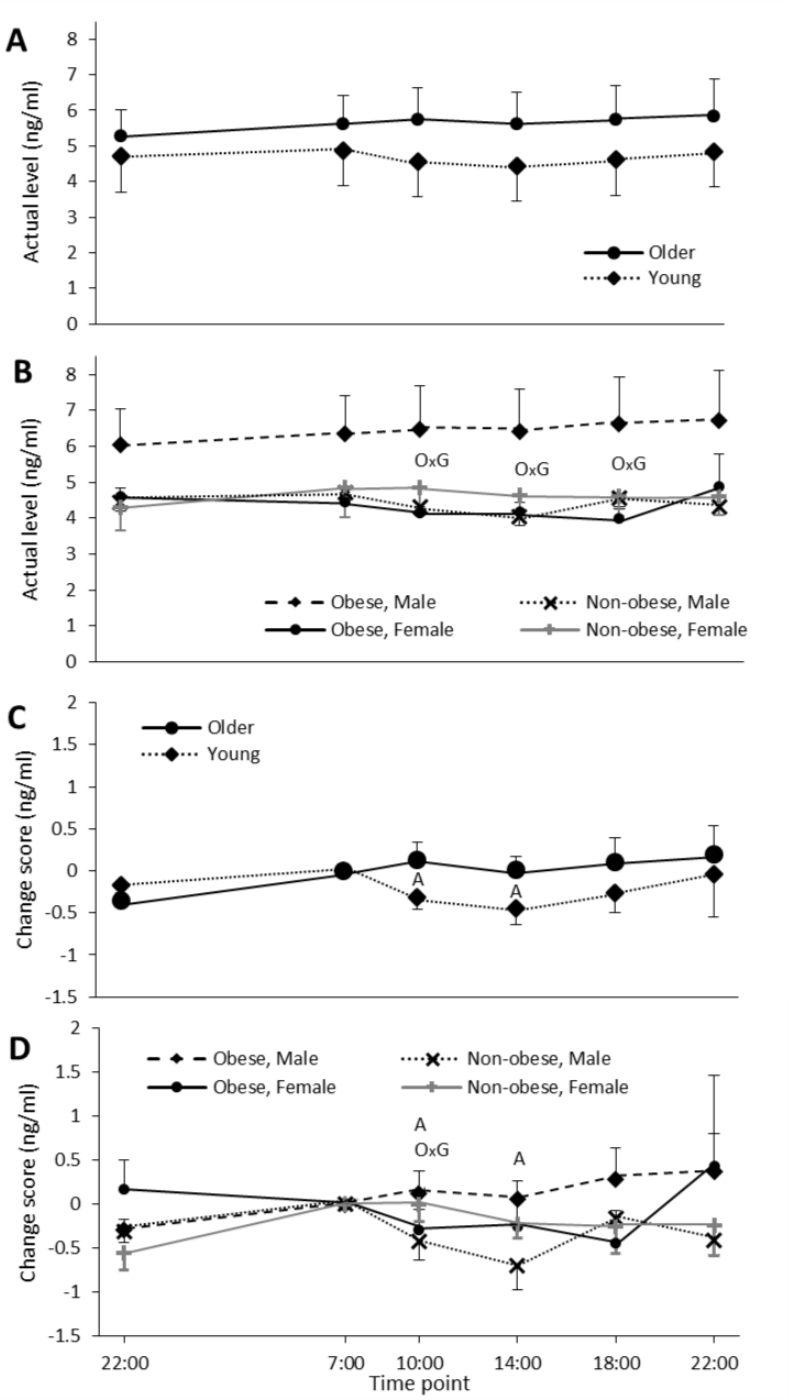

Figure 2.2 Diurnal changes in CK actual levels (**A** and **B**) and change scores (**C** and **D**). Significant age differences, and obesity status x gender interaction effects at individual time points are indicated by "A" and "OxG" respectively. Error bars indicate standard errors.

2.4.4 Reliability

ICCs of actual levels for all three biomarkers were statistically significant and were qualitatively categorized as “excellent”. Change scores ICCs were only significant for COMP and CK, and these were categorized as “fair” (Table 2.3). For both actual levels and change scores, there appeared to be a subset of participants for whom there was relatively high variance between samples obtained at the same time (Figure 2.3).

Table 2.3 Reliability results for COMP, IL6, and CK actual levels and change scores. Intraclass correlation coefficients (ICCs) are given, along with associated *F* tests (significance indicated in bold), 95% confidence intervals (CIs), and qualitative descriptions. Standard errors of measurement (SEM) are also provided (values are the same for actual levels vs. change scores).

	COMP		IL6		CK	
	Actual	Change score	Actual	Change score	Actual	Change score
ICC(3,1)	0.899	0.509	0.894	-0.11	0.863	0.41
$F_{25,25}$	**18.806**	**3.075**	**17.824**	0.979	**13.55**	**2.389**
95% CI	(0.788, 0.953)	(0.159, 0.745)	(0.778, 0.951)	(-0.390, 0.372)	(0.717, 0.936)	(0.034, 0.684)
Qualitative	Excellent	Fair	Excellent	N/A	Excellent	Fair
SEM	90.1ng/ml		1.70pg/ml		1.35ng/ml	

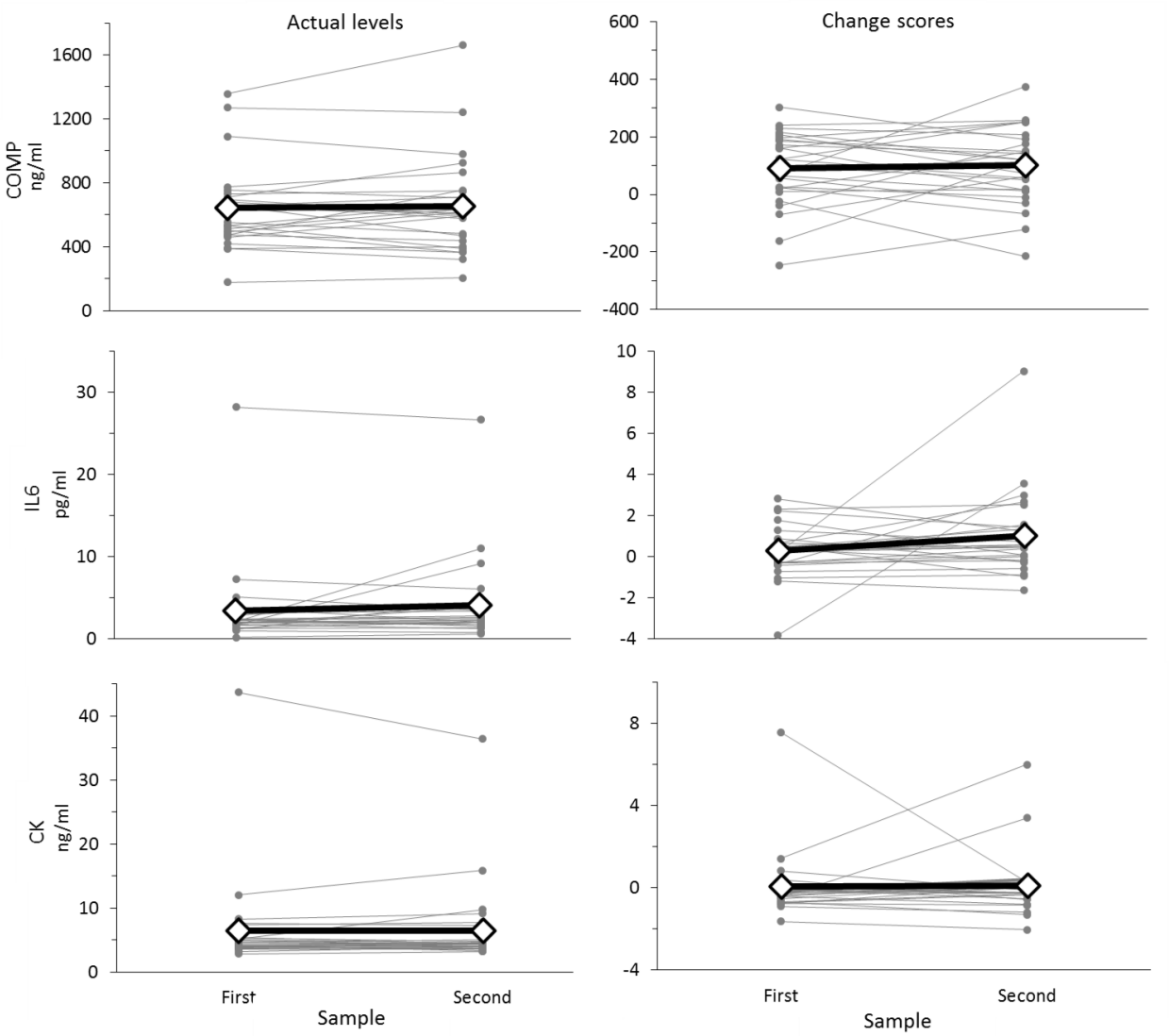

Figure 2.3 Actual levels and change scores for all participants at the first and second 22:00 samples. Mean values are denoted by the symbol ◇.

2.5 Discussion

For COMP, actual levels significantly changed over a 24 hour period, exhibiting a depression immediately after waking (07:00); values at this time point were significantly lower compared to all other samples obtained, a finding consistent with that of

Lottenburger et al. (2011). This observed morning depression is likely due to a removal of biomechanical stresses overnight, given that mechanical loading increases COMP levels (Kim et al., 2007, 2009; Niehoff et al., 2011). The noted study (Lottenburger et al., 2011), however, concluded that diurnal COMP variation did not differ between genders, in contrast with the present results. In our study, males had higher COMP levels and change scores at all time points, and gender differences in both actual levels and change scores were significant at all time points except 07:00 and 18:00. Similarly, one large cross-sectional study (Jordan et al., 2003) also found a significantly higher level of COMP for males, on the order of ~200ng/ml vs. females, which is comparable to the results of this study. Authors of that study (Jordan et al., 2003) hypothesized that differences in body composition (e.g., bone density, cartilage and tendon mass, and skeletal and joint size) accounted for these gender differences. Our results further indicate that males maintain a relatively consistent level of COMP throughout the waking day, other than the depression at 07:00, whereas levels among females increase after waking, with a peak at 18:00. These gender differences could be related to differences in levels of daily physical activity. However, acute levels of activity were controlled and recent habitual levels of physical activity were comparable between genders (cf. Table 2.1). It is possible that the gender difference in COMP diurnal changes are also due to the noted differences in body composition, since these should lead to differences in accumulated loading over time. Practically, the gender differences in COMP suggest that future work would benefit from standardized sampling protocols. For example, samples could be obtained immediately after waking, or at about 18:00 (assuming relatively low physical activity throughout the day). Alternately, samples

could be obtained at both of these times to account for the contribution of daily physical activity. It is also important to compare the magnitude of diurnal COMP changes observed here to those previously observed in response to physical activity. COMP levels after running a marathon increased by about 100% (Kim et al., 2007, 2009), and after 100 drop landings by about 30% (Niehoff et al., 2011). As mentioned above, levels in this study varied, by about 200 ng/ml over a waking day, and maximal levels were about 800 ng/ml. Hence, diurnal changes may account for substantial changes in COMP levels, relative to those elicited by physical activity, and will need to be taken into account during future studies.

There was also an effect of age on the diurnal variation of COMP change scores, consistent with the diurnal study discussed earlier (Lottenburger et al., 2011). Interestingly, the age effect in the current study was only significant (and substantial) at the first 22:00 blood sample but not at the second. This inconsistency may indicate that there are age-related differences in the reliability of 22:00 COMP levels later in a waking period, or perhaps reflects differences in participants' physical activity during day of sampling. Though not statistically significant, differing patterns of diurnal COMP changes were evident between age groups (Figure 2.1C); future studies, with larger sample sizes, are recommended to assess this effect.

No significant diurnal changes of IL6 were observed nor any differences between participant groups. As mentioned earlier, only one previous study has investigated diurnal variation in IL6, as a control condition to exercise-induced IL6 changes (Miles et al., 2008b). In that study, IL6 levels decreased after waking (at 12:00 and 16:00), but the authors acknowledge that these decreases were 'slight'. Based on the very limited

existing evidence, IL6 appears to have some level of diurnal variation, but the contributions of age, obesity, and gender (and possible other factors) clearly requires further investigation.

Diurnal variation existed in CK, and this variation was influenced by age, and by obesity status and gender (i.e., significant interaction effect). Older participants had higher CK levels at all time points (Figure 2.2A), though no pairwise differences were significant. Only few studies have investigated age-related differences in CK. One study found that young participants have a larger CK increase following exposure to eccentric exercise, but no significant difference in pre-exposure levels (Toft et al., 2002). An explanation for the observed age-related increase in CK levels here is that, with increasing age, muscles exhibit histological changes in comparison to young individuals (Andersen, 2003), possibly leading to increased injury susceptibility (Close et al., 2005). Obese males had higher CK levels at all time points (Figure 2.2B), though no pairwise differences were significant. Miles et al. (2008b) did not investigate diurnal changes in CK, but did find a gender effect at a 07:00 'baseline' measure. Males is their study had CK levels approximately 60% higher than females (Miles et al., 2008b), while the results here suggest a 20% increase at 07:00. Given the consistent association of systemic CK levels with muscle cell damage (Brancaccio et al., 2010; Hornemann et al., 2000), the observed differences in CK levels are likely due to a combination of previously-described effects; increased muscle strength with obesity and greater muscle mass in males (Cavuoto and Nussbaum, 2013; Lafortuna et al., 2005). It should be noted that all of the current age, obesity, and gender changes in CK levels are relatively small, on the order of 1-2 ng/ml, and that eccentric exercise can increase levels by 10-100 times

(Kim et al., 2009; Yang et al., 2011). As such, diurnal variations and individual differences are likely small sources of confounding effects vs. those of eccentric exercises. However, in cases where the level of muscular effort is less defined and/or largely concentric, accounting for differences related individual factors may still be important. Since CK levels were not found to differ between time points accounting for diurnal effects seems less critical.

Regarding reliability, ICCs for actual levels of COMP, IL6, and CK were significant, and reliability was qualitatively categorized as "excellent" for these (at least at 22:00, the only time point assessed). Only COMP and CK had significant ICCs for change scores, though, and for which there was "fair" reliability for the specific differences between baseline (07:00) and both 22:00 samples. Practically, these results suggest that, in the absence of substantial levels of physical activity, similar biomarker levels will be obtained at similar times of day. Future investigations, though, are needed to verify the reliability of these biomarkers at additional time points. SEM values (Table 2.3) may be of value in subsequent studies, for interpreting the magnitude of biomarkers changes (e.g., due to physical activity) and for sample size planning.

Future studies may also consider the use of allometric scaling methods to normalize biomarker levels related to specific tissues (Nevill and Holder, 1995). Logarithmic relationships have been shown between body mass and total muscle mass (Pollock and Shadwick, 1994), and cartilage thickness (Malda et al., 2013). Using these relationships to normalize biomarkers could help reduce the confounding effect of between subject variability in tissue mass (or volume) on these biomarker levels.

An important limitation of this study is the resolution of both time points and personal factors that were investigated. Obtaining additional samples over a day would enhance resolution of diurnal changes. The need for such resolution appeared most relevant for COMP, given that the magnitude of diurnal changes were most substantial for this biomarker. Quantitative personal factors here (age and obesity status) were addressed using only two categories, and as such the functional forms of any relationships with biomarkers levels or diurnal changes could not be assessed. It should be noted that the present classification of obesity using BMI has known limitations (Prentice and Jebb, 2001). Obesity was verified visually here (vs. substantial muscularity), though future work may benefit from more specific measures (Nevill et al., 2006). The current sample size was relatively small, and thus may have been underpowered with respect to some of the effects of interest. Another potential limitation was the lack of rigorous control of physical activity during the day of sampling. Although participants were asked to refrain from strenuous physical activity leading up to and during the study, only a brief questionnaire prior to the first blood sample assessed adherence to this request. Future studies may thus benefit from direct monitoring of physical activity. Finally, the current study did not account for all potential factors that may influence these biomarkers. Rather, it was designed to account for factors considered likely to be influential, based on existing evidence, and subsequent work will be needed as other influential factors are identified.

2.6 Conclusions

In summary, the current study indicates that important diurnal variations exist for COMP and CK, but likely not for IL6. Further, COMP levels are lowest immediately after waking, exhibiting an inverted 'U'-shaped pattern throughout the day, and this diurnal variation was substantially different between genders. As for CK, levels remained consistent over 24 hours, but age, and the interaction of obesity and gender, substantially influenced overall levels. Future investigations will needed to increase the resolution of both time-of-day and the influences of age and obesity, as well as investigating other influential factors. The current results here may aid in future clinical, athletic, or occupational applications of biomarkers, for example in terms of study design and interpreting the magnitudes of observed changes.

3. An Exploratory Study of the Effects of Occupational Exposure to Physical Demands on Biomarkers of Cartilage and Muscle Damage

3.1 Abstract

Biomarkers of tissue damage, derived from tissues commonly injured as a result of occupational physical demands, may be of use for future prediction of work-related musculoskeletal disorders (WMSDs). This exploratory study assessed whether selected biomarkers are likely to be sensitive to the level of occupational physical demands.

Serum levels of Cartilage Oligomeric Matrix Protein (COMP), Interleukin-6 (ILG), and Creatine Kinase (CK) which respectively indicate cartilage damage, muscle use, and muscle damage, were obtained over one working week. Twenty four participants were recruited to form two groups, with relatively high and low levels of occupational WMSD risk.

COMP levels varied significantly over time, but not between groups. IL6 levels were greater in the high-risk group at all time points and varied significantly over time and between groups. CK levels did not vary significantly over time or between groups.

IL6 successfully differentiated between the high and low risk groups, suggesting potential use in the occupational domain. Prospective studies are needed, though, to associate biomarker levels/changes with WMSD risk.

3.2 Introduction

Work-related musculoskeletal disorders (WMSDs) continue to be prevalent in a number of occupational sectors, and which lead to a substantial financial burden (da Costa and Vieira, 2010). Prospective epidemiological studies have identified an increased WMSD risk with greater exposure to several "physical" risk factors, including force exerted (Gallagher and Heberger, 2013), spinal loads (Coenen et al., 2012b), and repetition (Leclerc et al., 2004). A number of WMSD risk assessment methods or tools have also been developed, but discrepancies between estimated and observed risk levels have often been found (Franzblau et al., 2005; Lavender et al., 1999; Waters et al., 2011b), suggesting potentially limited validity of these methods. Biomarkers, particularly those derived from tissues that are commonly involved in WMSDs, may be a useful adjunct to existing risk assessment methods, since such biomarkers can objectively reflect physiological damage, especially when it is subclinical (Carp et al., 2008a; Jain, 2010). Figure 3.1 illustrates a high-level conceptual model of the potential occupational application of biomarkers for WMSD identification. Recent studies (discussed below), and the work presented here, have focused on assessing relatively acute changes in biomarker levels following exposure to known WMSD risk factors.

Figure 3.1 Conceptual model of the progression of WMSDs and the use of biomarkers in occupational settings. Current evidence supports the use of biomarkers for assessing levels of sub-clinical tissue damage. Following work to determine predictive ability, biomarkers levels may also be used to assess WMSD risk associated with specific occupations, tasks, etc.

Several studies have demonstrated changes in selected biomarkers following exposure to physical stresses that are similar to those experienced during workplace tasks. Cartilage Oligomeric Matrix Protein (COMP) provides additional mechanical strength to collagen fibers and is expressed in cartilage tissue via a mechanosensitive promoter region (Amanatullah et al., 2012; Giannoni et al., 2003). Levels of COMP have been found to increase following diverse physical activities, including walking and running tasks (Kim et al., 2009; Mündermann et al., 2005; Niehoff et al., 2010) and repeated drop landings (Niehoff et al., 2011), and to increase longitudinally among soccer players over an athletic season (Hoch et al., 2012); as a whole, such evidence indicates sensitivity to both acute and more prolonged cartilage loading. Interleukin-6 (IL6) is a systemic cytokine that has both pro- and anti-inflammatory effects in muscle, following exposure to damaging and non-damaging exercise, respectively (Reihmane and Dela,

2013). Muscle tissue is the main source of systemic IL6 following exercise (Keller et al., 2005), and consistent with this diverse forms of physical activity generate increased IL6 levels, including running (Reihmane et al., 2012) and cycling (Toft et al., 2002) in humans, and repetitive reaching in rats (Barbe et al., 2008). Creatine Kinase (CK) is found primarily in muscle, where it helps buffer cellular phosphate molecules and leaks into the bloodstream following eccentrically-induced rupture of muscle cells (Brancaccio et al., 2010). Studies have demonstrated consistent increases in CK levels proportional to the intensity of muscle exercise (Kim et al., 2009; Toft et al., 2002; Yang et al., 2011), and correlations between these increases and delayed onset muscle soreness (Chen et al., 2013). These three highlighted biomarkers, related to cartilage or muscle damage, have exhibited consistent changes following exposure to several forms of physical demands. However, with prior to concluding that biomarkers could be used for WMSD risk prediction, there is value in determining whether biomarker changes are associated exposure to occupationally-relevant levels and forms of physical demand.

A few studies have described the impact of exposure to WMSD risk factors on biomarkers related to cartilage and muscle damage. Yang et al. (2011) demonstrated significant increases in IL6 and CK levels (and other biomarkers) following exposure to a simulated repetitive lifting task with varying box weight. Interactive effects of force and repetition rate on histological changes and select biomarkers were recently demonstrated in a prospective rat model, suggesting increased WMSD risk in the high exposure group (Barbe et al., 2013). Increasing lumbar loading rate in a feline model significantly altered IL6 in an intervertebral disc (Pinski et al., 2010). In terms of the relative risks between occupational groups, construction workers were found to have a

greater collagen turnover rate at a single time-point, compared to a sedentary population (Kuiper et al., 2005). Another study followed vocational trainee nurses over a period of seven months, also finding a greater collagen turnover rate in comparison to a sedentary population (Kuiper et al., 2002). These studies demonstrate the potential use of biomarkers for differentiating between occupations with distinct physical demands, but further work is needed to determine the sensitivity of biomarkers to WMSD risk factors.

In summary, changes in biomarkers related to cartilage and muscle damage may be sensitive to common physical (force and repetition) and physiological (cartilage degradation) WMSD risk factors, further supporting their potential use in the occupational domain. Longitudinal studies, comparing biomarker levels with WMSD risk factor exposure and incidence, would be of clear value in establishing the value of these biomarkers for WMSD prediction. Since such a study imposes a substantial burden (in terms of time and financial requirements), an exploratory investigation was considered an appropriate step building on current evidence. The current exploratory study was completed to determine whether selected biomarkers are likely to be sensitive to occupational physical demands, and to provide data that can facilitate the design of future studies (e.g., in terms of biomarker selection, sample sizes, and sampling schedules). The specific purpose of this study was to compare biomarker level changes over one working week, between workers classified as having relatively high vs. low exposures to WMSD risk factors. The specific biomarkers used here (COMP, IL6, and CK) were chosen based on the noted evidence suggesting they can be used to track the effect of physical demands on short term physiological changes.

3.3 Methods

3.3.1 Participants

Twenty-four participants completed the study, and were recruited from the local community. Participants were specifically recruited to form two groups consisting of individuals exposed to relatively high and low levels of WMSD risk. Given the paucity of biomarker data in the occupational domain, sample size calculations were not feasible; instead the current sample size was the maximum given available financial resources. Target occupations were identified initially based on the prevalence of WMSDs (Bureau of Labor Statistics, 2011b), and then participants were recruited from among these. Participants in the HIGH group were employed in construction (n=8), maintenance (n=2), landscaping (n=1), and nursing (n=1), while all those in the LOW group (n=12) had occupations involving primarily sedentary (office) work. Participants were required to have a Body Mass Index (BMI) of $18.5 \leq BMI < 40$ and to be 18-65 years old; these exclusions were used to avoid potential biomarker confounds associated with Class III obesity ($BMI \geq 40$) and older age (>65 years), respectively. To further reduce potential confounding personal factors between groups, participants in the HIGH group were recruited first, and then the LOW group was formed to achieve group-level matching based on BMI, age, and gender distribution. Regarding the latter, there were 10 males and two females in each group. To avoid the influences of medical conditions and/or medications, participants were excluded based on the self-reported presence of: anemia, diabetes, inflammatory conditions (arthritis, Crohn's disease, etc.), heart

disease, smoking, recent WMSDs (an injury in the last three years resulting in a cost exceeding $1 (Rosenblum and Shankar, 2006)), high use of non-steroidal anti-inflammatory drugs (NSAIDs), being on lipid-lowering medication, or having any blood-borne diseases. To minimize residual effects of prior physical activity, participants were required to reduce their physical activity in both the 48-hours prior to beginning the study and outside of their occupation through completion of the study. They were specifically instructed to exclude heavy lifting, playing sports, or other physical activities beyond those required to perform their normal daily activities. The research protocol was approved by the Virginia Tech Institutional Review Board, and all participants provided written informed consent.

3.3.2 Experimental Protocol

Participants initially completed two questionnaires. The first was used to determine age, gender, and whether participants had any recent prior injuries or conditions that limited mobility, and to confirm the 48-hour prior reduction of physical activity. Next, participants completed the long-form International Physical Activity Questionnaire (IPAQ), to determine their habitual physical activity for the past seven days in several categories (i.e., occupational, transportation, household, and recreational/leisure). The long-form IPAQ was used since it has relatively good reliability (Craig et al., 2003) and validity (Hagstromer et al., 2006) for assessing total physical activity, as compared to the short-form IPAQ (Lee et al., 2011). Based on the IPAQ responses, the number of metabolic equivalent minutes/week (MET-min/week) were calculated (both overall and specific to occupational activities).

Six blood draws were obtained over one working week, which was defined to be four or five consecutive days of work (one participant in each group had a 4-day work week). Participants reported to the authors' lab before and after work on Monday, Wednesday, and Friday (or Monday, Wednesday, and Thursday for a 4-day work week) for each blood draw. At the first meeting (Monday morning), stature and body mass were measured initially, and then the first blood sample was obtained (as described below). Immediately after work, participants reported back to the lab for the next blood draw. Throughout the remainder of the working week, participants returned for blood draws before and after work, as noted above. Participants were instructed/reminded to perform their occupations normally, while restricting their physical activity outside of work as noted above.

At each blood draw participants completed two additional questionnaires. The Hollmann Index of physical work load was used to determine prior exposure to WMSD risk factors, based on responses regarding the frequency with which body postures are adopted and weights are handled (Hollmann et al., 1999). Hollmann Index values obtained on Monday morning were used to confirm prior reduction of physical activity, while values obtained on the two subsequent mornings were used to reflect exposures on the previous days. All responses after work were used to assess the current days' exposure.

3.3.3 Blood Collection and Analysis

All blood samples were drawn by a licensed phlebotomist (MC) from the antecubital region into 5ml evacuated serum separator tubes using a standard venipuncture

technique. Upon clotting, blood samples were centrifuged (Sorvall X1R, Waltham, MA) to obtain the serum, and samples were stored in 1.5ml microcentrifuge tubes at -20°C until analysis. Levels of serum COMP (BioVendor, Ashville, NC), High Sensitivity IL6 (BioVendor, Ashville, NC), and CK (Antibodies-online.com, Atlanta, GA) were determined using enzyme-linked immunosorbent assays (ELISAs) per the respective manufacturer's instructions. All sample concentrations were determined in duplicate, and means were used in subsequent analyses. Mean intra-assay coefficients of variability (σ/μ) for COMP, IL6, and CK were 2.0%, 3.8%, and 2.8% respectively.

3.3.4 Statistical Analysis

Statistical analyses were performed using SPSS for Windows (version 20.0, SAS, Chicago, IL). Differences in demographic aspects between the HIGH and LOW groups were assessed using unpaired, two-tailed *t* tests. For biomarkers, dependent measures included both actual levels and change scores. The latter were derived as changes from baseline levels, obtained on Monday morning, and were determined separately for each participant. Separate two-way, multivariate analyses of variance (MANOVAs) were used to assess the effects of time and group (HIGH vs. LOW) on biomarker levels (both actual and change scores), and Hollmann Index values. Effect sizes were assessed using partial eta-squared (η^2) (Cohen, 1973). *Post hoc* paired comparisons were done using Bonferroni-adjusted *t* tests. Consistent with the exploratory nature of this study, statistical significance was concluded when $p<0.1$. All summary statistics are reported as means (SDs).

3.4 Results

Descriptive statistics for the HIGH and LOW groups are shown in Table 3.1. Participants in the LOW group were significantly older, but BMI did not significantly differ between groups. MET-min/week was significantly higher in the HIGH group, for both overall and occupational categories. A summary of the effects of time and group on biomarker levels is provided in Table 3.2, and these results are presented in more detail subsequently.

Table 3.1 Participant information in the HIGH and LOW groups, reported as means (SD), along with *p* values from unpaired *t* tests (significant differences are indicated in bold).

	HIGH	LOW	*p*
Age (years)	26.4 (7.7)	32.4 (7.4)	**0.08**
Height (cm)	176.8 (3.8)	173.8 (9.9)	0.98
Body mass (kg)	92.2 (20.2)	82.9 (22.6)	0.82
BMI (kg/m^2)	28.6 (7.4)	27.9 (3.9)	0.80
MET-min/week (overall)	20,620 (13,070)	1,700 (1,370)	**<0.01**
MET-min/week (occupational)	15,760 (9,930)	130 (250)	**<0.01**

Table 3.2 MANOVA results for the effects of time (T) and group (G) on COMP, IL6, and CK actual levels (AL) and change scores (CS). Entries in each cell are *p* values, (*F* statistics), and effect sizes (*partial*-η^2), and significant effects are indicated in bold.

	COMP		IL6		CK	
Effect	AL	CS	AL	CS	AL	CS
T	**<0.01** **(4.86)** **0.57**	0.39 (1.09) 0.19	0.98 (0.16) 0.04	0.77 (0.46) 0.09	0.33 (1.25) 0.26	0.67 (0.60) 0.11
G	0.23 (1.51) 0.06	**0.09** **(3.26)** **0.13**	0.36 (0.87) 0.04	0.44 (0.63) 0.03	0.27 (1.29) 0.06	0.83 (0.34) 0.02
T x G	0.50 (0.906) 0.20	0.43 (1.01) 0.18	**0.08** **(2.41)** **0.40**	**0.07** **(2.53)** **0.35**	0.42 (1.05) 0.23	0.83 (0.36) 0.07

3.4.1 COMP, IL6, and CK

Actual levels of COMP varied significantly over time, but not between the HIGH and LOW groups. Regarding the temporal effect, there were significant changes in actual COMP levels both within and between working days (Figure 3.2A). COMP change scores differed significantly between HIGH and LOW groups, but not over time (Figure 3.2B). The HIGH group had greater actual levels of IL6, with respective levels over all time points of 3.31 (4.91) pg/ml and 1.90 (1.18) pg/ml, and the HIGH group had greater levels at each time point (Figure 3.2C). Both actual levels and change scores of IL6 were significantly affected by a time x group interaction, with divergent temporal patterns between the two groups (Figure 3.2C and 3.2D). Mean (SD) CK levels over all time points for the HIGH and LOW groups were 5.48 (2.13) ng/ml and 6.75 (3.58) ng/ml

respectively, however, neither actual levels nor change scores of CK were significantly affected by time or group.

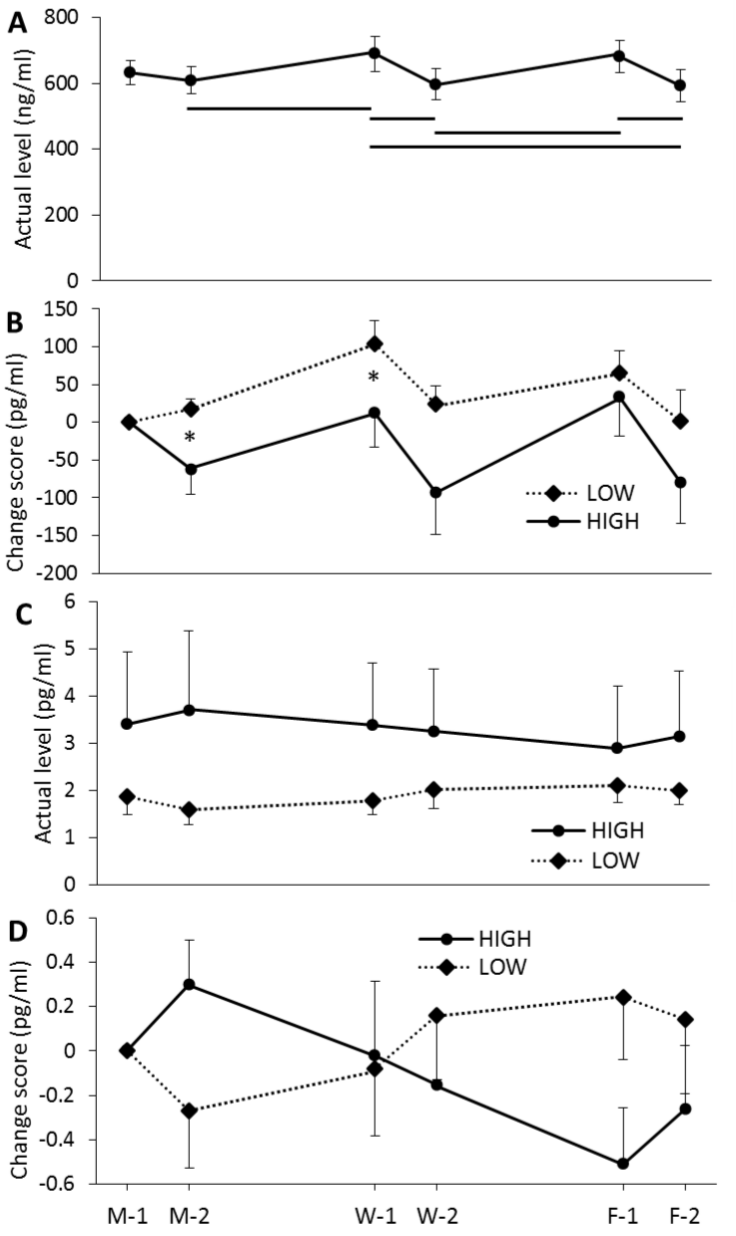

Figure 3.2 Temporal changes, over one week, in COMP actual levels (A) and change scores (B), and IL6 actual levels (C) and change scores (D). Note that samples were obtained on Monday (M), Wednesday (W), and Friday (F), both before (1) and after (2) work. Horizontal lines indicate significant differences between time points. Significant differences between groups are indicated by the symbol ✱, and error bars indicate standard errors.

3.4.2 Hollmann Index Values

Hollmann Index values were significantly affected by time (p=0.03, F=5.64), group (p<0.01, F=36.60), and a time x group interaction (p<0.01, F=10.10). Effect sizes for time, group, and time x group were 0.61, 0.63, and 0.74 respectively. All subsequent Hollmann Index values were significantly different from baseline, and group differences were significant at all time points except baseline (Figure 3.3).

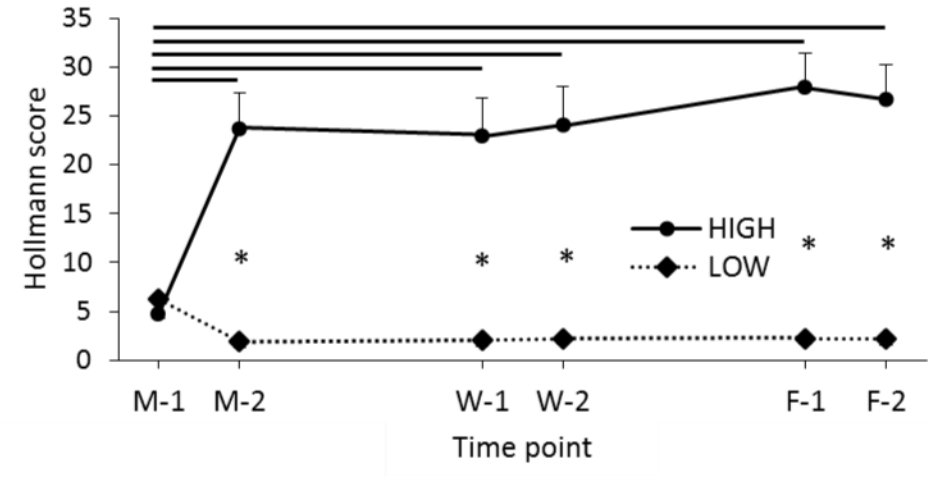

Figure 3.3 Temporal changes, over one week, in Hollmann Index values. Note that samples were obtained on Monday (M), Wednesday (W), and Friday (F), both before (1) and after (2) work. Horizontal lines indicate significant differences between time points. Significant differences between groups are indicated by the symbol ✱, and error bars indicate standard errors.

3.5 Discussion

Actual levels of COMP changed significantly over a working week, but these changes did not differ between the HIGH and LOW groups. Specifically, COMP levels decreased pre- to post-work (Figure 3.2A), though this was only statistically significant on Wednesday and Friday. For the LOW group, this decrease may reflect a decrease of cartilage tissue damage while at work, due to the sedentary nature of their occupations. These decreases are somewhat counterintuitive for the HIGH group, given that COMP levels typically increase with greater physical demands (resulting in cartilage damage) (Kim et al., 2009; Niehoff et al., 2010). Further, reducing physical demands, such as simply sitting down (or more generally, cessation of substantial physical activity) decreases COMP levels in as little as 30 minutes (Mündermann et al., 2005). However, these observed changes rely on the assumption that short term changes in COMP levels closely track the biomechanical demands on collagen molecules. If COMP levels instead change more substantially in response to alterations in physical demands, over- or under-shooting the required levels of COMP to maintain mechanical strength of collagen, this could explain several of the present results. The observed decrease in COMP levels over a working day could thereby be explained by morning COMP levels being overshot by the physical demands associated with morning activities prior to work. Then, the decline after work would reflect a recovery from any occupational physical demands, between the time of finishing work and that of obtaining the blood sample. An explanation such as this would also support the LOW group having higher change scores overall (Figure 3.2B), since the occupational and non-occupational physical demands this group experienced would be similar, and therefore

result in less over-/under-shooting of COMP levels over a working day, as observed. Unfortunately, the time/distance required for travel to the laboratory before and after work was not recorded; these may have differed substantially between participants, further contributing to the counterintuitive COMP levels observed. Given the similarity in the temporal patterns of change score between the HIGH and LOW groups, future studies using COMP will need to carefully consider the timing and resolution of samples, since systemic levels appear to respond rapidly to changes in physical demands.

IL6 actual levels and change scores differed between the HIGH and LOW groups. Participants in the HIGH group had higher IL6 actual levels at all time points (Figure 3.2C), consistent with the higher levels of physical demands required in their occupations. IL6 levels increase with greater muscle use and consequent damage (Keller et al., 2005) and, as noted earlier, increase with both repetitive reaching (Barbe et al., 2008) and lifting (Yang et al., 2011). Based on such evidence, the current results suggest that actual IL6 levels can be used to distinguish between workers who experience high and low occupational physical demands, and therefore also may be a viable candidate for assessing future risk of WMSDs. IL6 change scores also varied significantly over time and between the HIGH and LOW groups (Figure 3.2D). The temporal pattern of change scores suggests that the HIGH group experienced initial inflammation resulting from occupational muscle use early in the week, followed by recovery in the latter half of the week (Pedersen, 2012). LOW participants demonstrated the opposite pattern, suggesting that long-term work in a sedentary occupation leads to increased systemic inflammation. Future studies, though, will need

to investigate IL6 changes with respect to a greater variety of occupations, and occupational risk groups.

CK actual levels and change scores did not differ significantly between the HIGH and LOW groups or vary over time. Given that eccentrically-induced muscle cell damage is the primary reason for changes in CK levels (Brancaccio et al., 2010), the HIGH group was expected to exhibit higher levels. However, it is possible that the HIGH group did not perform sufficiently frequent or high enough levels of eccentric contractions to elicit CK changes. Alternately, previous studies have demonstrated a repeated bout/training effect, blunting increases in systemic CK levels for 2-4 days between exposures to maximal eccentric exercise (Chen et al., 2013). Most previous studies examining CK levels have exposed participants to levels of physical exercise at or near maximum capacity, such as participating in an ultra-marathon (200km) (Kim et al., 2009), performing eccentric exercise at (and greater than) aerobic capacity for more than 50 minutes without breaks (Toft et al., 2002), and lifting up to 11.3 kg continuously for 2 hours at a rate of 6 lifts/minute (Yang et al., 2011). CK increases in these studies ranged from 5- to 50-fold, and CK remained elevated up to 5 days following exposure. Since occupational exposure to eccentric exercise is doubtfully near maximal capacity, it is possible that HIGH participants may be accustomed/adapted to the eccentric muscle activity they experience at work, resulting in no significant change throughout a working week. Since extensive muscle damage (possibly indicative of future WMSDs) appears to elicit very noticeable changes, CK may still have utility in assessing exposure to WMSD risk factors and/or predicting WMSD risk. Future studies will need

to more closely examine levels of CK, eccentric muscle activity, and WMSD incidence to determine if CK can be used in the occupational domain.

As discussed earlier, only two studies (to the authors' knowledge) have investigated biomarker levels between occupational groups with distinct WMSD risk (Kuiper et al., 2005; Kuiper et al., 2002). The results presented here expand on these prior studies, both by assessing alternate biomarkers over a working week between HIGH and LOW occupational risk groups and while simultaneously assessing levels of occupational physical demands. Consistent for the use of all three biomarkers, though, future studies will need to monitor levels over a longer period of time, evaluate exposure to WMSD risk factors in greater detail, and track the incidence of WMSDs. In this way, clearer links between biomarker levels and WMSD risk could be established.

Hollmann Index values varied significantly over time and between HIGH and LOW groups. *Post hoc* analyses demonstrated that groups were significantly different at all time points except baseline (Figure 3.2A). This indicated that participants likely adhered to the reduced physical activity over the weekend leading up to the study, and that the HIGH group was exposed to higher levels of WMSD risk factors during the observation period (confirming successful group separation). The results presented here also support the use of the Hollmann Index as a tool for differentiating between occupational risk groups, consistent with earlier work (Nabe-Nielsen et al., 2008). It should be noted that the populations here were selected as likely to have differential work exposures to occupational risk factors, and the purpose of the Hollmann Index was to confirm such group differences. Although the biomarker data appears less sensitive than Hollmann Index values, the predictive validity of either tool was not assessed here, and future

work is recommended to assess the correspondence between measures of physical exposure and biomarker levels.

Despite attempts to match age, BMI, and gender distributions between groups, the LOW group was significantly older (by a mean of 6 years), leading to a potential confounding effect. In one study, older adults (mean 69 years) exhibited a greater increase in IL6 following exercise, but a diminished CK response in comparison to a younger (mean 24 years) group (Toft et al., 2002). Given that the LOW group was only six years older here, it thus seems unlikely that their responses would differ to the extent found in the noted earlier study. Further, if age was confounding the current results, IL6 and CK levels would be expected to be higher in the LOW group, in contrast to what was actually found. Therefore, the age difference between groups likely had only insubstantial influences on the current major results.

Another potential limitation of this study is the variation in occupations that were included within the HIGH group. WMSD risk varies substantially between occupational sectors (da Costa and Vieira, 2010), but even within construction, within which the majority of the current participants were employed, WMSD risk can depend on trade (e.g. electrician, plumber, etc.) (Schneider, 2001). To increase sensitivity, future studies may benefit from more specific, controlled recruitment (e.g., by occupation and/or trade). It is also important to note that physical activity during the work week was not directly assessed in the current study. The IPAQ was used to estimate physical activity during a 'normal' week, and which may not have accurately represented participants' physical activity during the week of this study. Although the IPAQ has good reliability (Craig et al., 2003) and validity (Hagstromer et al., 2006), with rising levels of actual

physical activity there is also an increasing tendency to overestimate perceived physical activity using the IPAQ (Craig et al., 2003). More direct and objective measures of physical activity (e.g. heart rate, oxygen consumption) could thus be used to supplement biomarker ratings and enhance sensitivity for differentiating between occupational risk groups.

Finally, an important limitation of this study is the lack of statistical power. A liberal approach to determining significance was used here (i.e., $p<0.1$), given the exploratory nature of the work, but this clearly increases the likelihood of a Type I error. It should be noted that effect sizes for all significant results were qualitatively 'large', with *partial*-$\eta^2 \geq 0.13$ (Cohen, 1988). Despite this, the results of this study should be interpreted with appropriate caution. The purpose of this study was to explore the effect that exposure to occupational risk factors has on short-term changes in biomarkers related to physiological damage in cartilage and muscle tissue. As discussed earlier, the current results suggest that these biomarkers (at least COMP and IL6) may have utility in differentiating between occupational risk groups. The extent to which biomarkers (instead of, or in combination with, current physical tools) can be used to predict WMSD risk remains to be evaluated.

3.6 Conclusions

The purpose of this study was to monitor COMP, IL6, and CK levels over a working week in occupational groups with relatively high and low levels of exposure to physical demands. COMP levels significantly changed over time, but not between risk groups.

IL6 levels were higher in the high-risk group across all time points. The differences in IL6 levels were likely due to greater exposure to occupational physical stress and WMSD risk factors. CK levels did not change significantly over time or between risk groups. The current results overall suggest that certain biomarkers are sensitive to differences in occupational physical demands, could thus be used to differentiate between workers exposed to varying levels of WMSD risk, and therefore may have future utility in predicting such risk. Future studies are needed, though, to prospectively determine exposure to WMSD risk factors, biomarker changes, and incidents of WMSDs.

4. Responsiveness of Selected Biomarkers of Tissue Damage to External Load and Frequency during Repetitive Lumbar Flexion/Extension

4.1 Abstract

Biomarkers related to tissues commonly injured in occupational settings may be a useful tool for exposure assessment or predicting injury risk. Serum levels of Cartilage Oligomeric Matrix Protein (COMP), Interleukin-6 (IL6), and Creatine Kinase (CK) were obtained before and after participants completed a repetitive lumbar flexion/extension task. The task was done for one hour, at three levels of external load (as a %MVC) and three frequencies. COMP levels did not change over time or between exposure conditions. IL6 levels were significantly affected by time and by external load, while CK levels were significantly affected by external load by frequency. Greater external load and frequency (for CK only) resulted in greater peak values of IL6 and CK, and both biomarkers recovered by 24 hours after task completion. Since IL6 and CK levels exhibited a dose-response relationship to exposure levels, they may have potential use in the occupational domain.

4.2 Introduction

Work-related musculoskeletal disorders (WMSDs) remain prevalent and continue to impose a substantial financial burden in numerous industries (da Costa and Vieira, 2010). Lower back pain (LBP) and lower back disorders (LBDs) specifically account for

the largest proportion of direct costs (Liberty Mutual Research Institute for Safety, 2013). Understanding the etiological risk factors and consequent physiological changes underlying LBP and LBDs is crucial to developing tools and/or methods that can identify high-risk jobs and/or tasks. Biomarkers, specifically those related to physiological damage, may be useful adjunct to existing exposure and risk assessment methods, since by definition they are "[molecules] that can be objectively measured and evaluated as an indicator of a physiological…process" (Jain, 2010, pg. 1). Prior to integrating biomarkers in occupational settings, however, a clear understanding of the associations between risk factor exposure(s) and resulting biomarker levels is needed.

Early cadaver studies demonstrated that the likelihood of spinal tissue failure increased with greater load (Jäger and Luttmann, 1989), loading frequency (Yoganandan et al., 1994), and their interaction (Brinckmann et al., 1988). Similar dose-response relationships have been demonstrated in muscle tissue, where long-term exposure to high loads and repetitive tasks can result in histological changes such as cellular rupture (Brancaccio et al., 2010; Kuipers, 1994), tendon fraying (Barbe et al., 2003), and other structural abnormalities (Dennett and Fry, 1988). Epidemiological evidence has also associated exposure to high load and repetition tasks with greater risk of developing LBDs (Marras et al., 1995). Current tools used to assess LBP, LBD, and/or WMSD risk often incorporate an estimate of load and repetition rate, such as the Strain Index (Moore and Garg, 1995), Revised NIOSH Lifting Equation (NIOSH, 1994), and Rapid Entire Body Assessment (Hignett and McAtamney, 2000). While some evidence supports the use of these tools for identifying high-risk occupational tasks (Rucker and Moore, 2002; Waters et al., 2011b), other evidence suggests limitations, specifically

since there can be highly variable estimates of risk levels between assessment tools for the same task (Drinkaus et al., 2003; Lavender et al., 1999). Biomarkers derived from the tissues commonly injured in WMSDs, and more specifically LBP and LBDs, may overcome at least some limitations of current methods, since biomarkers can objectively determine physiological changes (Carp et al., 2008b; Jain, 2010). Initial work is needed, however, to verify the sensitivity, specificity, and predictive validity of biomarkers in the occupational context.

Injury to soft tissues, such as cartilage and muscle, is a common etiological factor underlying many WMSDs (Kumar, 2001), and levels of three particular biomarkers derived from cartilage and muscle have been shown to be sensitive to physical exposures. Cartilage Oligomeric Matrix Protein (COMP) is expressed in cartilage tissue via a mechanosensitive promoter region, wherein it functions to provide mechanical strength to collagen fibers (Amanatullah et al., 2012; Giannoni et al., 2003). Diverse forms of physical activities have been found to increase COMP levels, including walking and running tasks (Kim et al., 2007, 2009; Mündermann et al., 2005; Niehoff et al., 2010) and repeated drop landings (Niehoff et al., 2011), and longitudinally over the course of an athletic season in soccer players (Hoch et al., 2012). This evidence indicates COMP sensitivity to both acute and more prolonged cartilage loading. Interleukin-6 (IL6) is a systemic cytokine released from muscle tissue following exposure to damaging and non-damaging exercise, where it has both pro- and anti-inflammatory effects, respectively (Reihmane and Dela, 2013). The main source of systemic IL6 levels following exercise is contracting muscle tissue (Keller et al., 2005). Consistent with this, diverse forms of physical activity result in increased IL6 levels,

including running (Reihmane et al., 2012) and cycling (Toft et al., 2002) in humans, and repetitive reaching in rats (Barbe et al., 2008). The enzyme Creatine Kinase (CK) is found primarily in muscle cells, where it buffers cellular phosphate molecules. Consequently, CK leaks into the systemic blood supply following eccentrically-induced rupture of muscle cells (Brancaccio et al., 2010). Multiple studies have demonstrated that CK increases proportional to the intensity of muscle exercise (Kim et al., 2009; Toft et al., 2002; Yang et al., 2011), and CK levels also correlate with the intensity of delayed onset muscle soreness (Chen et al., 2013). Despite a clear relationship with several forms of physical demands, additional studies are needed to evaluate the effects of occupationally-relevant levels and types of physical exposures on biomarkers, before these biomarkers can be considered feasible for predicting LBP, LBD, and WMSD risk.

A number of studies have assessed changes in biomarkers in response to controlled exposure parameters relevant to occupational tasks, specifically load and frequency. Barbe et al. (2008, 2013) investigated the effect of repetitive reaching on biomarkers of injury in rats. The former study specifically varied repetition rate and found that higher repetition rate led to significantly greater systemic and tissue levels of inflammatory markers (Barbe et al., 2008). The latter study expanded upon this by varying the level of force. An interactive effect of repetition rate and force was found, with high force and high repetition rate yielding greater systemic and tissue levels of inflammatory markers (Barbe et al., 2013). Both studies also used histological results to verify the presence of tissue damage in connective and muscle tissue. Other work, by Splittstoesser et al. (2012) and Yang et al. (2011), examined biomarker levels following exposure to a 2-hour lifting task among humans. The lifting task elicited significant increases in IL6 and

CK from baseline levels (Splittstoesser et al., 2012). The latter study found that post-lifting IL6 and CK levels increased with box weight during a repetitive lifting task (Yang et al., 2011).

Both of these sets of studies demonstrate important relationships between occupationally-relevant task parameters (e.g., load and frequency) and biomarker levels indicative of tissue damage, however there are some limitations which drove the design of the work presented here. Studies performed in rats have several advantages (e.g., a highly controlled setting and the ability to harvest tissues specifically involved in the task), yet generalizability to humans performing occupational tasks is uncertain. The noted lifting studies using humans overcome both of these concerns. Yet, these latter studies involved physical demands in multiple body regions, and it is thus unclear whether systemic levels of biomarkers are influenced specifically by loading of tissues in the lower back region.

In summary, existing evidence suggests that changes in biomarkers derived from tissues commonly involved in LBP, LBDs, and/or WMSDs has the potential to reflect underlying physiological damage. The purpose of the current study was to examine changes in three biomarkers (COMP, IL6, and CK) in response loading of the lower back tissues at different levels of external load and frequency. A constrained lumbar flexion/extension task was employed, and was used to partially isolate physical loads to tissues in the lower back. To enhance external validity, occupationally-relevant levels of the task parameters (external load and frequency) were used. We hypothesized that task-induced changes in biomarkers levels would differ significantly with external load and repetition rate, and with possible interactive effects. Results of this study were

intended to aid in understanding the influence of occupational risk factors on tissue responses, specifically in the lower back, and to inform future work investigating the use of biomarkers in occupational settings.

4.3 Methods

4.3.1 Overview

A repeated-measures design was used, involving one preliminary session and five experimental sessions. In each experimental session, participants completed a task requiring repeated lumbar flexion/extension, and which was done in five distinct conditions of external load and task frequency. Blood samples were obtained before, after, and during recovery from the task, and these samples were assayed for levels of COMP, IL6, and CK. All sessions were separated by a minimum of one week, to allow biomarkers to subside from previous experimental exposures (Kim et al., 2009; Toft et al., 2002), and to minimize a repeated bout effect for CK (Chen et al., 2013), thereby reducing potential carryover effects. Additionally, all experimental sessions were performed at the same time of day to reduce confounding due to diurnal effects which have been previously observed for COMP (Lottenburger et al., 2011) and IL6 (Miles et al., 2008b).

4.3.2 Participants

Six participants (two females, four males) were recruited from among the local student population as a convenience sample. Mean (SD) age and body mass index (BMI) were

23.7 (2.2) years and 23.5 (1.9) kg/m^2 respectively. Participants were required to have BMI<25, and this restriction was used to accommodate constraints of the experimental apparatus (described below). To avoid the influences of pre-existing medical conditions and/or medications, participants were excluded based on the self-reported presence of: anemia, diabetes, inflammatory conditions (arthritis, Crohn's disease, etc.), heart disease, smoking, recent WMSDs (an injury in the previous three years with a cost exceeding $1; Rosenblum and Shankar, 2006), recent injuries or conditions (within the past 3 years) that limited mobility, high use of non-steroidal anti-inflammatory drugs (NSAIDs; >81mg daily), being on lipid-lowering medication, or having any blood-borne diseases. The research protocol was approved by the Virginia Tech Institutional Review Board, and all participants provided written informed consent prior to data collection.

4.3.3 Physical Activity and Lumbar Extensor Strength

In the preliminary session, participants initially completed the long-form International Physical Activity Questionnaire (IPAQ), to determine their habitual physical activity for the past seven days in several categories (i.e., occupational, transportation, household, and recreational/leisure). The long-form IPAQ was used since it has relatively good reliability (Craig et al., 2003) and validity (Hagstromer et al., 2006) for assessing total physical activity, as compared to the short-form IPAQ (Lee et al., 2011). Based on the IPAQ responses, the number of metabolic equivalent minutes/week (MET-min/week) were calculated. Mean (SD) habitual physical activity for all participants was 6650 (5290) MET-min/week.

Isokinetic lumbar strength was determined for each participant in the preliminary session, and was used subsequently to scale the magnitude of external loads used during repetitive flexion/extension in the experimental sessions (see below). Isokinetic, eccentric maximum voluntary contractions (MVCs) were performed while pelvis and lower extremity motions were restricted. This was done using a custom fixture and connecting "arm" (see Figure 4.1) attached to a commercial dynamometer (Biodex System 3 pro, Biodex Medical Systems Inc., NY, USA). Participants with a BMI≥25 were excluded to reduce contact between abdominal tissue and the fixture during maximum flexion. After initial warm-up exertions and a rest period, at least 5 trials, with 2 minutes of reset between each, were performed until consistent MVC values were obtained. These were done with a range-of-motion (ROM) of 0-80° of torso flexion, and at an angular velocity of 120°/s. This procedure has been shown to have high reliability for determining lumbar extensor MVCs (Keller et al., 2001). Mean (SD) eccentric lumbar extensor MVC was 329 (66) Nm.

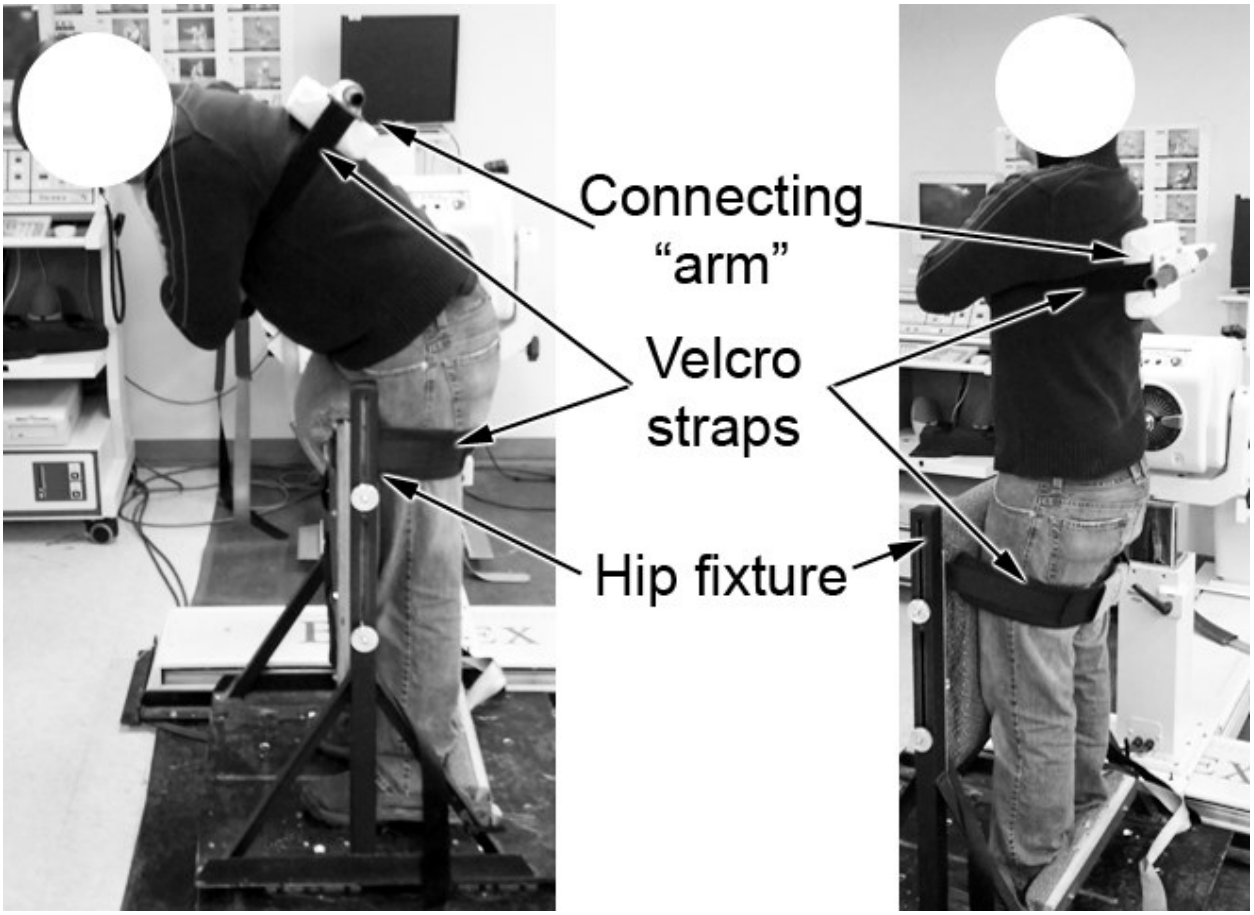

Figure 4.1 Experimental setup with participant connected to a dynamometer. Added loads were achieved using weights attached to the connecting arm.

4.3.4 Experimental Design and Independent Variables

Each participant performed a repetitive lumbar flexion/extension task under each of five distinct conditions that had a different level of external load and frequency. External load (mass) was manipulated as a percentage of MVC (described below), while frequency was controlled as the number of flexion/extension cycles per minute. The five specific conditions were (%MVC/frequency): 0%/1/min, 20%/1/min, 10%/5.5/min,

0%/10/min, and 20%/10/min. This use of equally-spaced levels of the variables was chosen to eliminate collinearity in subsequent regression analyses (described below). The presentation order of conditions was randomized across participants.

The flexion/extension task was completed with participants connected to the dynamometer (Figure 4.1), with the latter in “free” mode. Over a one-hour period, participants performed intermittent lumbar flexion/extension cycles, each with a ROM of 0-60-0° torso flexion, and at an angular velocity of 30°/s, resulting in each flexion/extension cycle lasting approximately four seconds. Throughout the flexion/extension motions, participants were instructed to keep their arms crossed over their chest and their backs “straight” (i.e., to maintain lumbar lordosis). The former was used to minimize variations in task demands. The latter was used to control (minimize) passive-tissue loading and was done to minimize within- and between-participant variability. The hip fixture was used to restrict the participants’ pelvis/legs, partially isolating the task demands to the lumbar spine and lumbar extensor muscles. The height of the hip fixture was adjusted so that the top edge was at the level of the iliac crest at maximum flexion (60°). The dynamometer center-of-rotation was set at approximately the L4/L5 level. Masses were attached to the connecting arm, such that the mean added moment applied over the ROM was at the target level (i.e., 0, 10, or 20% of MVC). This approach thereby exposed participants to the added extensor load during the lumbar flexion/extension motions, but not when resting in between each cycle.

The mean added mass for the 20 %MVC sessions was 21.6 (5.1) kg. To facilitate interpretation of the current task demands, these demands can be considered roughly

comparable to a manual lifting task. Since the load was applied approximately at the shoulders, the resulting external moments on the lumbar spine were comparable to holding the weight with the arms hanging vertically (this ignores the relatively small changes related to differences in upper extremity posture). The mean added mass and the highest flexion/extension frequency (10/min) correspond roughly to the 60th and 95th percentiles of object weight and task frequency, respectively, found in a survey of industrial lifting tasks (Ciriello et al., 1999). To further assess the experimental task, added mass and locations/distances were employed to calculate a lift index (LI) using the Revised NIOSH Lifting Equation (NIOSH, 1994). In this, only the actual added mass (kg), horizontal location (cm), vertical location (cm), vertical distance travelled (cm), and frequency multipliers were calculated, with the remaining multipliers set equal to 1. Mean (SD) LIs for the experimental conditions of 0%/1/min, 20%/1/min, 10%/5.5/min, 0%/10/min, and 20%/10/min were 0 (0), 1.3 (0.3), 0.6 (0.2), 0 (0), and 2.7 (0.6), respectively.

4.3.5 Experimental Procedures

To minimize residual effects of prior physical activity on biomarker levels, participants were required to reduce their physical activity during the 48-hours prior to and through completion of each experimental session, and specifically to exclude heavy lifting, playing sports, or other physical activities beyond those required to perform their normal daily activities (see Figure 4.2 for an overview of each session). Upon arrival at the laboratory, participants were secured in the dynamometer and stood in a relaxed posture for 30 minutes. This initial standing period was included since previous work indicated that even small changes in biomechanical stresses (e.g., standing vs. sitting)

can substantially influence levels of COMP (Mündermann et al., 2005). As the experimental task was performed while standing, this initial period allowed biomarkers to equilibrate to the biomechanical stresses of standing. After this initial standing period, a baseline blood sample was obtained, and the participant then completed the repetitive flexion/extension task for one hour. Flexion/extension frequency was controlled using visual and auditory cues generated by a custom Labview™ program (National Instruments, Austin, TX, USA). The words 'DOWN', 'UP', and 'REST' were displayed according to the desired frequency, and coincided with short tones to alert the participant. Immediately after completing the task another blood sample was obtained. Participants then remained standing for an additional hour, after which another blood sample was obtained (one hour post-task). Participants were then allowed to leave the laboratory, but were required to maintain their reduced physical activity (described above) and to return twice for follow-up blood draws at 4 and 24 hours post-task.

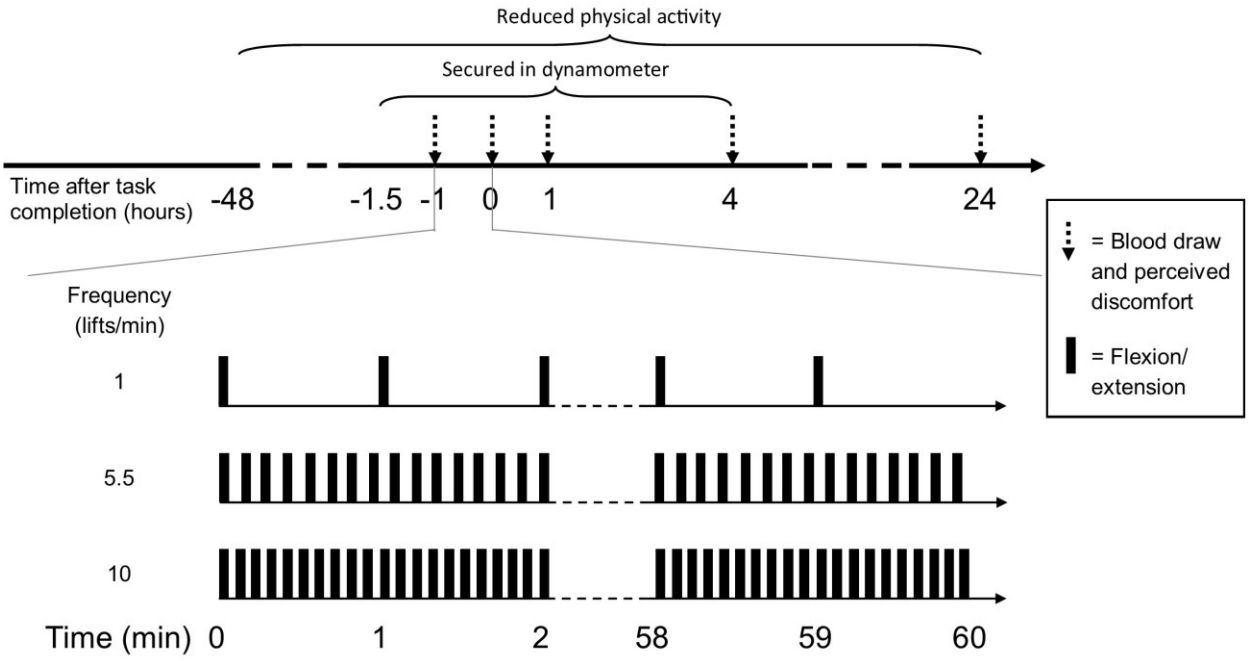

Figure 4.2 Overview of the timing of events in each experimental session (top). The timing of flexion/extension cycles at different lifting frequencies is also shown (bottom).

4.3.6 Dependent measures

Blood samples were obtained at each of the time points noted above (i.e. immediately before and after the task, and 1, 4, and 24 hours post-task). All blood samples were obtained from the antecubital area by a certified phlebotomist (MC) into 5ml evacuated serum separator tubes using standard venipuncture procedure. After clotting, blood samples were centrifuged (Sorvall X1R, Waltham, MA) to obtain the serum, which was stored in 1.5ml microcentrifuge tubes at -20°C until analysis. Levels of serum COMP (BioVendor, Ashville, NC), High Sensitivity IL6 (BioVendor, Ashville, NC), and CK (Antibodies-online.com, Atlanta, GA) were determined using enzyme-linked

immunosorbent assays (ELISAs) observing the manufacturer's instructions. All biomarker concentrations were determined in duplicate, and means used in subsequent analyses. Mean intra-assay coefficients of variability (σ/μ) for COMP, IL6, and CK were 1.3%, 3.2%, and 2.6% respectively. Immediately before each blood draw, a custom questionnaire was used to obtain current ratings of perceived discomfort (RPDs), unilaterally in six major body areas (neck, shoulders, upper back, lower back, hips/thighs, and knees), and using a figure adapted from Kuorinka et al. (1987). These ratings were done using the Borg CR-10 scale (Borg, 1982), during which both the figure and scale were visible to the participants.

4.3.7 Statistical Analysis

Statistical analyses were performed using SPSS for Windows (version 20.0, SAS, Chicago, IL). For biomarkers, dependent measures included both actual levels and change scores, with the latter derived relative to baseline levels for each participant (i.e., pre-task values in each session). For each biomarker (both actual and change scores) and RPDs, separate multivariate analyses of variance (MANOVAs) were used to assess the effects of time, external load, and frequency. *Post hoc* paired comparisons between time points were done using Bonferroni-adjusted *t* tests. The current experimental design facilitates regression analyses, as there is no collinearity between the independent variables. As such, separate regression models were fit at each time point, with biomarker actual levels and change scores as dependent measures. Predictors included the effects of external load (%MVC), frequency, and their interaction as predictors. Main effects of the three levels of predictor variables

were coded as -1, 0, and 1. Statistical significance was concluded when $p<0.05$, and all summary statistics are reported as means (SDs).

4.4 Results

4.4.1 Biomarkers

A summary of the statistical effects of time, external load, and frequency on biomarker levels is provided in Table 4.1. There were no significant effects of time, external load, or frequency on either COMP actual levels or change scores. IL6 actual levels were significantly affected by time, but not by external load, frequency, or any interaction. IL6 actual levels exhibited an inverted "U"-shaped pattern over time (Figure 4.3A), with baseline levels of 0.42 (0.39) pg/ml, maximal levels of 0.98 (1.30) pg/ml at 4 hours post-task, and 0.37 (0.36) pg/ml at 24 hours post-task. IL6 change scores were significantly affected by time and by external load (Figure 4.3B). *Post-hoc* analyses revealed that change scores at 1 hour after task completion were significantly greater than change scores 24 hour after task completion. The most pronounced effects of external load on IL6 were observed 4 hours after task completion. At this time point, IL6 levels in the 20% load condition increased ~ 1.2 (1.66) pg/ml from baseline, in contrast to the 0% and 10% conditions in which IL6 had returned to baseline.

Table 4.1 MANOVA results for the effects of time (T), external load (L), and frequency (F) on COMP, IL6, and CK actual levels (AL) and change scores (CS). Entries are *p* values (*F* statistics), and significant effects are indicated in bold.

Effect	COMP		IL6		CK	
	AL	CS	AL	CS	AL	CS
T	0.28 (1.37)	0.17 (1.85)	**0.02 (3.76)**	**0.01 (5.23)**	0.17 (1.78)	0.09 (2.41)
L	0.91 (0.01)	0.56 (0.35)	0.12 (2.58)	**0.03 (5.30)**	0.97 (<0.01)	**<0.01 (25.81)**
F	0.56 (0.35)	0.29 (1.18)	0.78 (0.08)	0.52 (0.43)	0.69 (0.16)	0.16 (2.13)
T x L	0.16 (1.84)	0.18 (1.80)	0.28 (1.37)	0.20 (1.70)	**<0.01 (6.37)**	0.25 (1.45)
T x F	0.49 (0.88)	0.33 (1.20)	0.51 (0.85)	0.51 (0.80)	**<0.01 (5.74)**	**0.01 (5.68)**
L x F	0.96 (<0.01)	0.14 (2.33)	0.60 (0.29)	0.94 (0.01)	0.48 (0.51)	0.28 (1.24)
T x L x F	0.46 (0.94)	0.56 (0.71)	0.33 (1.23)	0.33 (1.21)	0.58 (0.73)	0.60 (0.64)

CK actual levels were significantly affected by the time x external load interaction (Figure 4.3C) and the time x frequency interaction (Figure 4.3E). *Post hoc* analyses indicated that levels at 24 hours after task completion were significantly lower than levels immediately after task completion. CK levels exhibited the characteristic inverted "U"-shaped pattern over time with 20% added load, reaching a peak increase of 0.83 (1.33) ng/ml at 4 hours after task completion, and returning to baseline by 24 hours after task completion. Effects of 10% added load on CK levels were much less substantial. Finally, 0% added load resulted in a consistent decrease in CK levels throughout the 24-hour observation period, to a level 1.35 (1.07) ng/ml below baseline at the final blood draw. CK change scores were significantly affected by load (Figure 4.3D) and the time

x frequency interaction (Figure 4.3F). *Post hoc* analyses indicated that CK change scores at 24 hours after task completion were significantly lower than levels immediately before, immediately after, and 1 hour after task completion. The 1 and 5.5 lifts/min lifting frequencies led to no significant changes throughout the observation period. At 10 lifts/min, peak levels were achieved immediately after task completion, reaching 1.09 (0.91) ng/ml, and decreased continually afterward, reaching a level 0.73 (1.33) ng/ml below baseline at 24 hours after task completion.

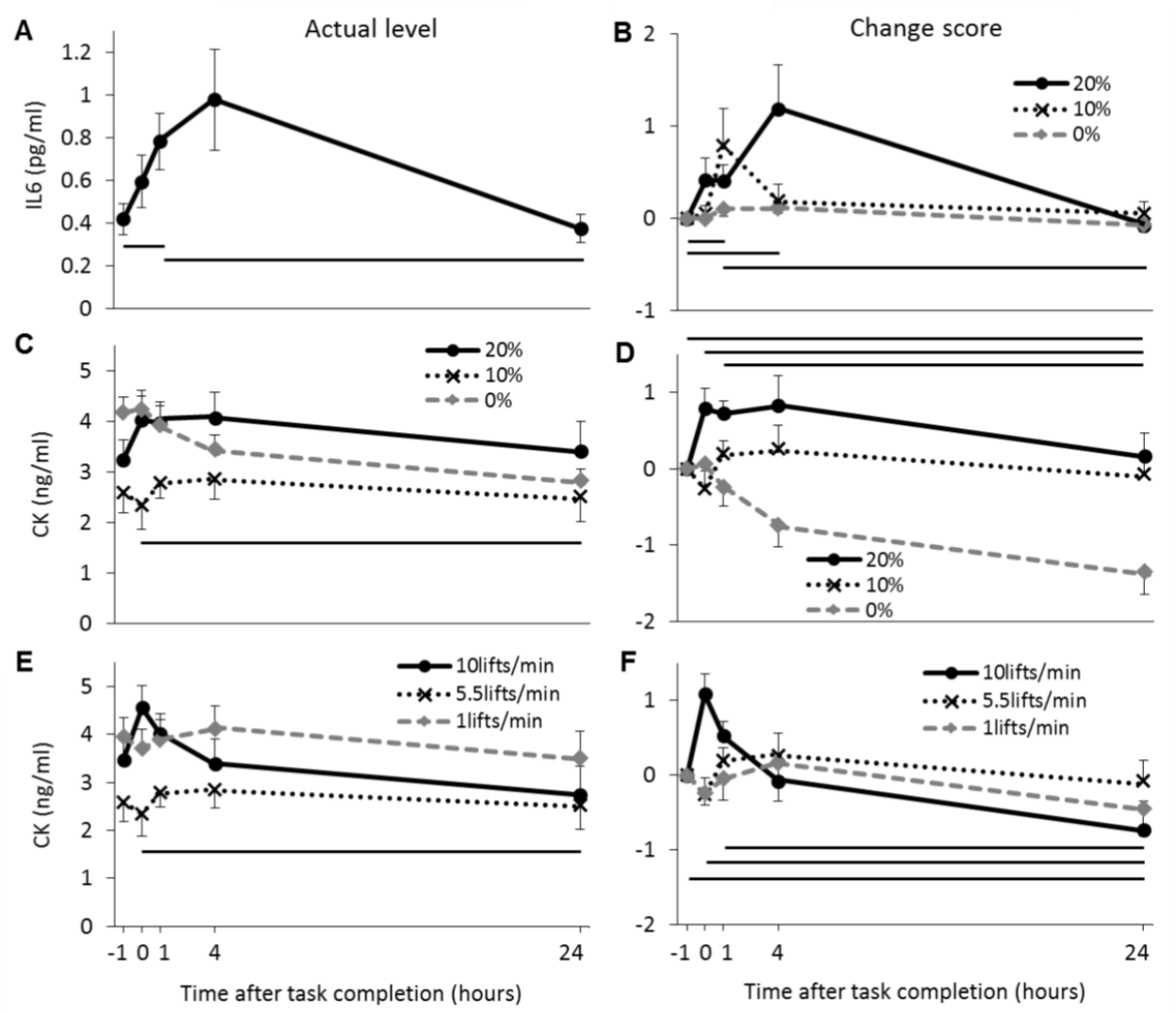

Figure 4.3 Temporal changes in IL6 actual levels (A) and change scores (B), and CK actual levels (C and E) and change scores (D and F). Note that times of -1 and 0 respectively indicate measures obtained before task initiation and immediately after task completion. Lines connecting time points were added to help visualize changes in biomarkers, however, actual temporal patterns may differ. Horizontal lines indicate significant differences between time points, and error bars indicate standard errors.

4.4.2 RPDs

A summary of the statistical effects of time, external load, and frequency on RPDs is provided in Table 4.2. Upper and lower back RPDs were significantly affected by the external load x frequency interaction (Figures 4.4A and 4.4B). *Post hoc* analyses indicated that upper back RPDs immediately after the task were significantly greater than 4 hours after task completion. Lower back RPDs reached peak levels immediately after task completion, and returned slowly to baseline levels. RPDs in the hips and knees were significantly affected by time (Figure 4.4C and 4.4D), with similar patterns of increases immediately post-task and a gradual recovery subsequently. Of note, the largest levels of RPD were evident in the lower back in all conditions. RPDs for the neck and shoulders did not significantly vary over time or with task exposure, and mean levels over all time points were 0.26 (0.53) and 0.30 (0.59) respectively.

Table 4.2 MANOVA results for the effects of time (T), external load (L), and frequency (F) on ratings of perceived discomfort (RPDs) in several body regions. Entries are *p* values (*F* statistics), and significant effects are indicated in bold.

Effect	Neck	Shoulder	Upper Back	Lower Back	Hip	Knee
T	0.20 (1.63)	0.06 (2.65)	0.09 (2.32)	**<0.01 (21.45)**	**0.03 (3.40)**	**0.01 (4.31)**
L	0.55 (0.37)	0.80 (0.07)	0.40 (0.73)	0.48 (0.52)	0.78 (0.08)	1.00 (0.00)
F	0.47 (0.54)	0.51 (0.45)	0.30 (1.11)	0.32 (1.05)	0.64 (0.23)	0.68 (0.17)
T x L	0.48 (0.90)	0.28 (1.36)	0.35 (1.18)	0.54 (0.80)	0.60 (0.70)	0.39 (1.08)
T x F	0.49 (0.89)	0.08 (2.40)	0.06 (2.62)	0.23 (1.54)	0.59 (0.71)	0.32 (1.26)
L x F	0.41 (0.72)	0.10 (2.94)	**0.03 (5.11)**	**0.02 (6.16)**	0.18 (1.90)	0.07 (3.63)
T x L x F	0.40 (1.06)	0.11 (2.17)	0.78 (0.44)	0.20 (1.65)	0.49 (0.88)	0.48 (0.90)

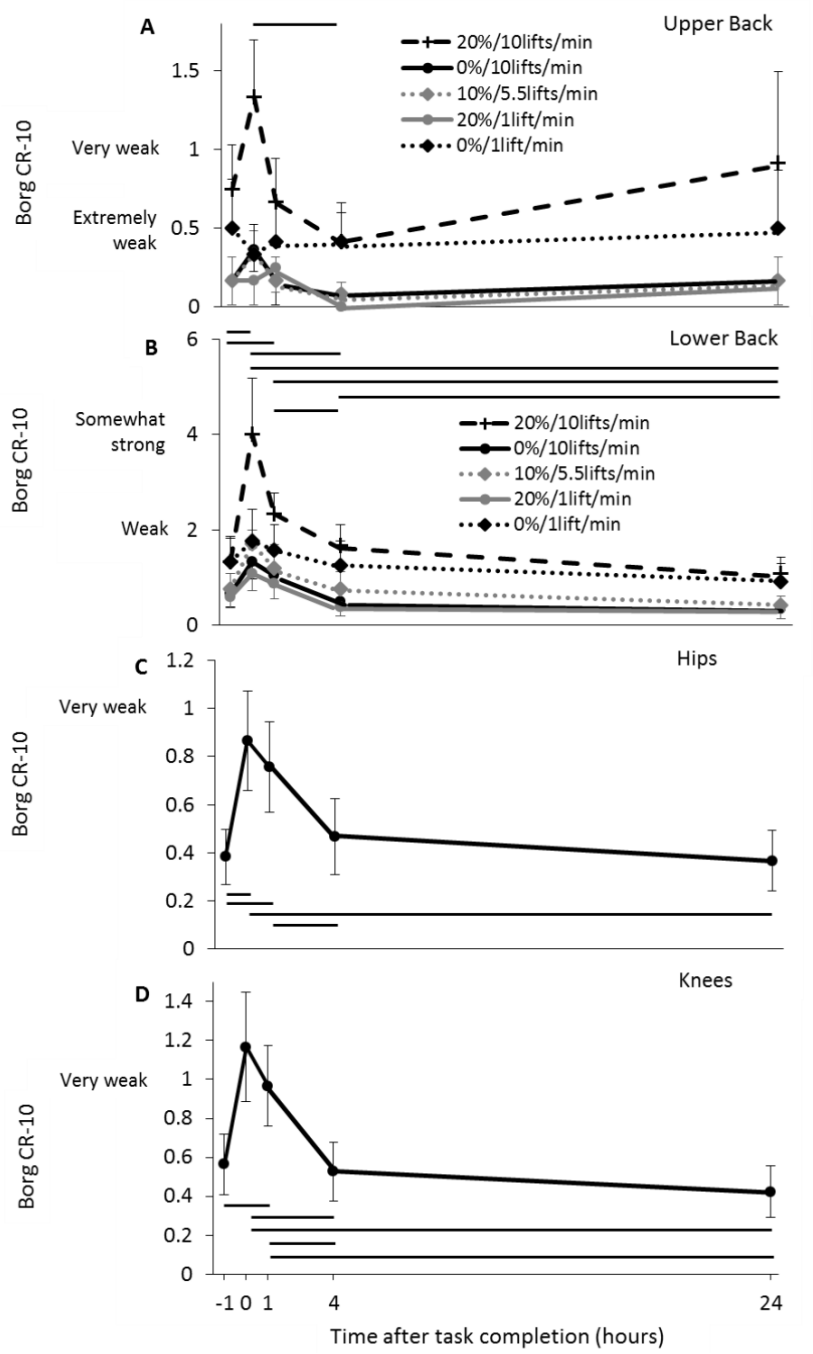

Figure 4.4 Temporal changes in ratings of perceived discomfort (RPDs) for the upper back (A), lower back (B), hips (C), and knees (D). Horizontal lines indicate significant differences between time points, and error bars indicate standard errors. Verbal anchors used by participants are noted at appropriate levels.

4.4.3 Regression Models

Table 4.3 provides a summary of regression coefficients for biomarker levels (only significant models are presented). For IL6 actual levels and change scores, significant models were obtained only at 4-hours post-tasks, and in these only the main effect of external load was significant. For CK, significant models were obtained for change scores at all time points post-task. In these, the main effects of external load and frequency were significant immediately and 1 hour post-task, whereas only the main effect of external load was significant 4 and 24 hours post-task.

Table 4.3 Regression results for significant models containing external load (L) and frequency (F) as predictors. Entries are regression coefficients (95% Confidence Intervals) and R^2 values, and significant coefficients are indicated in bold. Subscripts for biomarkers indicate the time after task completion (in hours).

Model$_{(\text{time after task completion [hours]})}$	Regression coefficients (95% CI)				R^2
	Intercept	L	F	L x F	
Actual level					
$IL6_4$	**0.98 (0.50-1.46)**	**0.54 (0.00-1.08)**	-0.2 (-0.73-0.34)	-0.05 (-0.59-0.49)	0.16
Change score					
$IL6_4$	**0.56 (0.12-1.00)**	**0.54 (0.05-1.02)**	-0.11 (-0.60-0.38)	-0.12 (-0.60-0.37)	0.18
CK_0	**0.29 (0.01-0.57)**	**0.36 (0.05-0.68)**	**0.66 (0.35-0.98)**	-0.05 (-0.37-0.26)	0.48
CK_1	0.24 (-0.01-0.48)	**0.48 (0.20-0.76)**	**0.29 (0.01-0.57)**	-0.21 (-0.49-0.06)	0.43
CK_4	0.09 (-0.35-0.53)	**0.78 (0.29-1.27)**	-0.12 (-0.61-0.37)	-0.23 (-0.71-0.26)	0.32
CK_{24}	**-0.49 (-0.89- -0.08)**	**0.76 (0.30-1.21)**	-0.15 (-0.60-0.31)	-0.03 (-0.49-0.43)	0.32

4.5 Discussion

COMP levels and change scores were not significantly affected by time or the current task parameters (load and frequency). As discussed earlier, levels of COMP have been shown to increase with exposure to diverse forms of physical exercise (Kim et al., 2007, 2009; Niehoff et al., 2010; Niehoff et al., 2011). Recall that participants stood in the experimental apparatus for 30 minutes prior to obtaining the baseline sample since prior work demonstrated significant decreases in COMP levels when participants went from standing to sitting (Mündermann et al., 2005). Given this evidence, COMP levels were

expected to increase with exposure to the lower back lifting task experienced here. Prior studies using COMP did not implement high levels of control over the areas of cartilage being stressed, e.g. marathon and other running tasks (Kim et al., 2007, 2009; Niehoff et al., 2010), and drop landings (Niehoff et al., 2011). Therefore multiple cartilaginous areas likely contributed to the observed increases in COMP levels in those reports. The current experiment was specifically designed to (at least partially) isolate the task to the lower back region. It is therefore possible that any COMP increases resulting from the lower back region contributed only negligibly to systemic levels. Future studies may still consider using COMP as a biomarker for assessing occupationally-related cartilage stress, but will need to judge whether the exposure levels are substantial enough to elicit systemic changes in COMP levels.

IL6 actual levels were significantly affected by time, but not by external load or frequency. A distinct inverted-'U' shape can be seen over time (Figure 4.3A), and which is consistent with previous evidence suggesting that IL6 levels increase with exercise (Reihmane et al., 2012; Toft et al., 2002), and specifically from contracting muscle tissue (Keller et al., 2005). IL6 levels at 1 hour after task completion here were significantly higher than at pre-task and 24 hours after task completion, and the highest mean levels of IL6 were observed at 4 hours after task completion. Additionally, IL6 levels returned to pre-task levels by 24 hours after task completion, consistent with previous findings (Toft et al., 2002; Yang et al., 2011). While the results here imply that actual levels of IL6 were not responsive to acute differences in external load and frequency of repetitive lumbar flexion/extension, the observed changes over time support the inherit risk for LBP and LBDs associated with lifting tasks (Marras et al.,

1995), suggesting that even small levels of external load or lifting frequency may cause physiological damage.

IL6 change scores were significantly affected by external load, and temporal changes demonstrated an inverted-'U' pattern in response to the 10% and 20% external load (Figure 4.3B). Responses to 0% external load trial were substantially smaller. Greater IL6 change scores with higher external load is consistent evidence, with greater muscle forces being required with larger loads and resulting in more IL6 expression and release from muscle tissue (Reihmane and Dela, 2013). Further, change scores were significantly greater than pre-task levels at 1 and 4 hours after task completion, and levels 24 hours post-task were significantly lower than 1 hour after task completion. Yang et al. (2011) also demonstrated that weight significantly affected IL6 change scores in an actual lifting task. The controls implemented in the current study, however, partially isolate the musculature used during the task to the lumbar extensors. In contrast, Yang et al. (2011) used a free-style lifting task, in which participants lifted a box, and found IL6 levels increased up to ~2.5 pg/ml above pre-task levels after lifting 11.3kg for 2 hours. At the 20% external load level here (mean = 21.6 kg), IL6 change scores peaked at 4 hours after task completion, at 1.2 pg/ml above pre-task levels. Although load levels and task duration differed between these studies, thus limiting comparability, the results here suggest that muscle activity required to perform lifting tasks in the lower back contributes substantially to changes in systemic IL6 levels. Finally, the external load regression coefficient was significant at 4 hours after task completion (Table 4.3), suggesting that IL6 change scores after lower back lifting tasks follow approximately linear relationships with an estimate of %MVC. Since IL6 levels

appear to exhibit a dose-response relationship to the level of external load, but not with frequency, for repetitive lower back demands, future studies will need to more closely investigate the functional and temporal relationships of IL6 to load levels. Given the current small sample size, future work may still consider including frequency of lifting tasks as a factor when investigating IL6 levels.

Actual levels of CK were significantly affected by the interactions of time and external load, and time and frequency, but not the three-way interaction of time, external load, and frequency. These findings are partially consistent with previous studies (Kim et al., 2009; Splittstoesser et al., 2012; Yang et al., 2011), which respectively demonstrated that duration of running and lifting tasks increased CK levels. In these studies, CK levels continually increased immediately to 24 hours after exposure, by 3-5 fold, whereas the results presented here indicate a recovery to pre-task levels by 24 hours after task exposure. The primary source of systemic CK levels are structurally damaged muscle cells (Brancaccio et al., 2010), occurring more frequently during eccentric vs. concentric contractions. Participants in the current study performed a task that was eccentric in flexion, and concentric in extension. Therefore, CK levels were expected to be influenced by the greater and more frequent eccentric contractions resulting from increases in external load and frequency, respectively. The results, however, suggest that the contributions of lower back musculature to systemic levels of CK may be minimal for a lifting task in conditions similar to those examined. It is also important to note that pre-task levels appeared to vary widely between experimental conditions (Figures 4.3C and 4.3E). Since presentation order was counterbalanced, randomized, and separated by a minimum of one week, these differences are not likely

due to a repeated bout effect (Chen et al., 2013). Despite this variability in pre-task levels, CK levels were significantly lower 24 hours vs. immediately after task completion. These results suggest that pre-task levels may be necessary to calculate change scores and to differentiate between CK increases following lifting tasks of varying intensities.

CK change scores were significantly affected by external load, and by the interaction of time and frequency. In comparison to actual CK levels, a clear pattern emerged between levels of external load and frequency. At 20% MVC external load, an inverted-'U' shape, similar to IL6 change scores, was found (Figure 4.3D), with a 0.8 ng/ml increase after exposure and a return to pre-task levels by 24 hours after task completion. In contrast, the 0% and 10% MVC loads appeared to elicit no substantial increases in CK levels over the observation period, and the 0% level even led to decrease in CK levels (1.3 ng/ml) by 24 hours after task completion. Interestingly, linear regression models were significant at all time points following pre-task, suggesting that CK change scores elicited from lifting tasks may be predicted by the %MVC required for the task. These results agree with previous work by Yang et al. (2011), which demonstrated CK change scores proportional to the weight of a lifted box. As discussed earlier, though, when muscle groups utilized in a lifting task are not controlled, CK levels continue to rise at 24 hours after task completion (Splittstoesser et al., 2012; Yang et al., 2011), while the results here suggest a recovery within that time period.

The current finding that the frequency of a repetitive low-back task also influences systemic CK levels in humans is novel to the literature. Previous studies in rats had

demonstrated that frequency of repetitive reaching tasks influences a number of other biomarkers (Barbe et al., 2008; Barbe et al., 2013). Inverted-'U' shapes over time can be seen for the 10 lifts/min level, except that levels appear to recover completely by 4 hours after task completion, and then continue to decrease to 0.7 ng/ml below pre-task levels by 24 hours after task completion (Figure 4.3F). Peak CK change scores occurred immediately after task completion for the 10 lifts/min level, increasing by 1.1 ng/ml above pre-task levels. In comparison, minimal changes were found over the observation period at frequencies of 5.5 and 1 lifts/min. Linear regression models predicting CK change scores from the frequency of a lifting task were significant immediately and 1 hour after task completion (Table 4.3).

In summary, CK levels (primarily change scores) appear to exhibit a dose-response relationship to the level of external load and frequency. This is consistent with earlier studies in cadavers (Brinckmann et al., 1988; Dennett and Fry, 1988) and using occupationally-relevant lifting tasks (Splittstoesser et al., 2012; Yang et al., 2011), and also epidemiological evidence that external load and frequency are parameters of a lifting task that are risk factors for LBDs and other WMSDs (da Costa and Vieira, 2010; Kumar, 2001). It is likely, however, that the contribution from the lower back during lifting tasks to systemic levels of CK is somewhat negligible. A number of significant regression models suggest that CK change scores following exposure can be predicted from estimates of %MVC and number of lifts/minute. Future work will need to carefully balance the level of control exhibited over lifting tasks so the source systemic CK levels can be traced to tissues of interest, while maintaining occupationally-relevant levels of task parameters.

RPDs for the upper back, and lower back were significantly affected by the external load by frequency interaction, and additionally in the lower back, hips, and knees over time. As expected, for the upper and lower back, greater levels of external load and frequency resulted in greater RPDs following exposure. Characteristic inverted-'U' shapes were seen, as RPDs tended to peak immediately after and recover by 24 hours after task completion across all experimental conditions. Since previous work has correlated RPDs with changes in other physiological measures of fatigue (Iridiastadi and Nussbaum, 2006), it is likely that some level of fatigue was achieved while performing the task. Interestingly, peak RPDs for the upper back, lower back, hips, and knees had respective means of 1.3, 4, 0.9, and 1.2. Peak RPDs in the lower back were more than three time greater than any other body region, and an RPD of 4 is qualitatively described as 'somewhat strong', vs. ratings of 1 and 2 which are respectively 'very weak' and 'weak'. These results indicate at least moderate success in partially isolating tissue loading to the lower back region during the current experimental task. For example, under the assumption that RPDs reflect the contributions of body areas to biomarker levels, the lower back would contribute about 50% of overall biomarker levels. Such an assumption has clear limitations, and therefore future work may benefit from including additional physiological measures for assessing fatigue and tissue loading in specific body areas.

As just discussed, the lack of supplemental physiological measures was a limitation of this study. Previous studies using occupationally-relevant lifting tasks had utilized electromyography of the lumbar extensor muscles to estimate specific spinal tissue loading (Splittstoesser et al., 2012; Yang et al., 2011), and such loading is an important

risk factor for injury as determined by the cadaver studies described in the Introduction. However, as described in the methods of these studies, determining subject specific parameters, to achieve model-based estimates of spinal tissue loading, requires a calibration procedure involving a series of maximum isometric contractions for ten individual lumbar muscles (Yang et al., 2011). Such a procedure was not done by the control group in this earlier work, and this difference could have contributing a confounding effect (i.e., overestimating the observed effects of lifting on biomarker responses). The MVC procedure performed here allowed for scaling of the external load to individual strengths, yet participants waited a minimum of one week prior to subsequently measurements to allow any biomarker levels to subside. As such, the experimental sessions involved little to no physical activity other than the repetitive flexion/extension task, and thereby more strongly supports a cause-and-effect relationship between lifting task parameters and the source of systemic biomarkers. Future studies considering the use of more realistic occupational tasks should also utilize physiological and physical measures which do not confound biomarkers, but which can objectively determine specific tissue loading.

The biomarkers selected in this study may also be limited. A review of literature revealed three biomarkers that appeared to represent the physiological status of tissues commonly involved in WMSDs, specifically cartilage and muscle. However, despite clear evidence suggesting COMP levels represent the intensity of cartilage loading (Kim et al., 2007, 2009; Mündermann et al., 2005; Niehoff et al., 2010), COMP levels were not statistically different between loading conditions. Financial restrictions limited the number of biomarkers (and time points) that could be analyzed. Future studies will

need to carefully review the literature for the intended biomarkers, to determine if levels are likely to change with the examined intensity of exposure and whether these changes will be on a systemic (vs. local) scale. While gathering data on a large panel of biomarkers may yield valuable information, allocating financial resources to fewer, carefully selected, biomarkers may yield more detailed information.

Sample size was limited to six participants in this study. Estimates of sample size were not possible, since few studies to date employed similar methodology. Instead, the number of participants was maximized given the available financial resources, with consideration to the number of experimental sessions (5) and blood samples (5/session) desired. Further note that participants were recruited from a student population. As a consequence, the results presented here may have limited external validity to occupational settings. However, as discussed above, the results are in agreement with prior research in several respects, and the controls implemented suggest relatively high internal validity. Future studies will need to expand sample sizes and carefully consider the desired study population.

Another limitation of this study may be the timing of blood samples. Although the exact timing for blood draws was chosen somewhat arbitrarily, the purpose was to obtain a baseline measure, to observe changes immediately and shortly after, and to follow up 24 hours later to assess whether recovery had occurred. This approach is consistent with previous work (Splittstoesser et al., 2012; Yang et al., 2011), and the timing appears to have highlighted important biomarker changes following a lower back lifting task. It should be noted, though, that a number of participants commented that, throughout the one-hour long task (specifically the 20%/10lifts/min session), RPDs

reached near maximal discomfort after about 15 minutes and subsequently decreased for the remainder of the task to tolerable levels. Ideally, a greater number of time points should be included and may help to identify other patterns, peak levels, etc. Understanding the temporal effects of task parameters on biomarker levels is important to determine optimal timing for sample collection.

A final limitation of this study may be the occupational risk factors selected and the specific levels chosen. Utilizing the specific design enabled an evaluation of the functional relationship of risk factors to biomarkers, but did not allow for a true control condition. Preferably, such a condition would involve participants being secured in the dynamometer, not performing any lifting, and obtaining blood samples at appropriate timings. The cost/benefit ratio of such a control session was not considered substantial, since pre-task (baseline) levels were obtained prior to each experimental sessions and subsequent levels compared to them. Finally, the selection of external load and frequency as independent variables was based on existing evidence that implicates these physical factors in LBP, LBDs, and WMSDs. However, many other factors have been shown to affect biomarker levels. Several of these were accounted for in the exclusion criteria for participants, but not all, such as genetics (Funghetto et al., 2013), psychosocial factors (Groer et al., 2010), etc. Future work will need to select independent variables and controls/exclusion factors judiciously to minimize confounding.

4.6 Conclusions

The purpose of this study was to quantify the responsiveness of COMP, IL6, and CK to the levels of external load (weight) and frequency during a repetitive lumbar flexion/extension task. COMP levels were not significantly affected by time or between task parameters. Actual levels of IL6 were significantly affected by time, and change scores by external load groups. These differences were likely due to greater muscle forces required during the different task conditions. CK actual levels and change scores were significantly affected by levels of external load and frequency, but not their interaction. Since CK levels are primarily affected by the extent of muscle cell rupture associated with eccentric contractions, the observed increases were consistent with existing literature. Overall, these results suggest a dose-response relationship between some biomarkers and external load (weight) and frequency, and may help explain why such task parameters are a risk factor for LBP, LBDs, and WMSDs. Future studies will need to carefully account for the source of systemic biomarkers using tissue-specific physiological tools, and possibly account for the effects of other occupational risk factors.

5. Conclusions

Recent work has demonstrated that biomarkers derived from tissue commonly injured in WMSDs may be sensitive to a number of occupational risk factors, indicating that biomarkers may be of use for assessing exposure to risk factors or even predicting WMSD risk. The aim of this dissertation was to investigate several properties of biomarkers deemed critical in context of future occupational applications. Three studies were designed to determine the levels/responses of COMP, IL6, and CK in/to a variety of settings. The specific purposes of these studies were to: 1) determine the effect of personal factors (age, obesity, and gender) on diurnal changes in biomarkers, 2) monitor biomarkers over one working week among workers exposed to high and low levels of WMSD risk factors, and 3) determine the responsiveness of biomarker levels to “classic” risk factors (load and frequency).

5.1 Effects of confounding factors on biomarkers

Consistent with much of the previous literature, we found significant effects of personal confounding factors and diurnal changes in COMP and CK. Specifically, COMP levels were significantly influenced by time of day, having lowest levels immediately after waking, and gender, with males having higher levels throughout the entire day. These results are likely due, respectively, to the removal of significant biomechanical stresses while sleeping overnight, and differences in body composition between genders. Practically, these results indicate that measuring COMP levels requires a standardized sampling protocol, to minimize the influence of time of day and gender. Previous literature had suggested that IL6 may have some diurnal and age related changes

(Miles et al., 2008b), but none were observed here. For CK, older participants and obese males had greater levels and fold changes throughout the entire day. This is consistent with prevailing theories suggesting both that older individuals are more susceptible to histological muscle tissue frailty and that obese males have a greater muscle strength and volume. Since diurnal changes were not significant, simple adjustments could be made to account for personal factors when using CK. Reliability between biomarker levels was excellent, suggesting that levels obtained from the same person on different days should be comparable when performing minimal physical exercise, as in this study. These results indicate the importance of accounting for confounding factors when using biomarkers, however substantially more work is required before a comprehensive list of confounding factors and their effects on biomarker levels is available. Although extensive exclusion criteria were implemented to limit the influence of other confounding factors, the methods (and in particular sample size) may not have been sufficient to detect the influence of some factors.

5.2 Influence of occupational risk factor exposure on biomarkers

Decades of work have illustrated the influence of occupational physical loads on WMSD risk, but also helped identify risk factors. The results presented here indicate that levels of IL6 successfully differentiated between occupational risk groups over one working week. These findings are supported by previous work implicating IL6 as a measure of physiological inflammation (Reihmane and Dela, 2013) and that specific WMSD risk factors cause physiological damage associated with a greater number of injuries (Kumar, 2001). The observed increased IL6 levels in a population exposed to greater levels of WMSD risk factors is consistent with earlier literature which demonstrated that

construction workers and vocational trainee nurses had greater levels of collagen turnover than a sedentary control group (Kuiper et al., 2005; Kuiper et al., 2002).

Contrary to expectations, COMP, a biomarker specifically chosen since it responds quickly to changes in levels of cartilage loading, exhibited unexpected patterns here over the observation period. Further, levels of CK did not differ between groups or over time. To the author's knowledge, this was the first study to examine changes in COMP, IL6, and CK levels among participants exposed to occupational levels of physical loads (e.g., instead of performing physical activity specifically designed to elicit biomarker changes). The results here highlight the need for further research to validate biomarker changes with occupational levels of physical load and risk factors. Results from Chapter 2 have some important implications on the results from Chapter 3, especially considering the significantly greater age in the LOW group. Older participants had higher levels of CK, meaning that if the HIGH group truly had greater levels of CK then observable changes may have been obfuscated. However, it is argued that the influence of age should be negligible, since ages differed by a minimum of 20 years in Chapter 2, and only 6 years in Chapter 3. Due to the exploratory nature of this study, sample sizes may not have been sufficient to detect differences in COMP and CK or include additional covariates in the model. Also, since occupational physical loads (both energy expenditure and exposure to risk factors) were self-reported, the extent of separation between the HIGH and LOW groups may not have been accuracy estimated.

5.3 Effects of lifting task parameters on biomarkers

A substantial body of literature had been developed linking occupational task parameters, notably weight and frequency, to risk of LBP, LBDs, and WMSDs. Prior work investigating the influence of such parameters on biomarkers had demonstrated effects of force and frequency in a rat model of repetitive reaching (Barbe et al., 2008; Barbe et al., 2013), and box weight in lifting (Yang et al., 2011). The results found here suggest that added load (as a %MVC) significantly influences levels of IL6 and CK. Novel to the literature, task frequency also influenced CK levels. As expected, greater exposure to risk factors resulted in greater biomarker levels. Such dose-response relationships support the use of biomarkers for assessing tissue damage associated with WMSDs in occupational lifting tasks. Both IL6 and CK are markers of muscle use and damage, and increased levels were likely due to greater exposure to task parameters associated with tissue damage.

Chapter 4 implemented a unique protocol, designed to partially isolate the musculature and tissues stressed during the task to the lumbar region. Success of this protocol was supported by perceived discomfort ratings, which were highest in the lower back and differed significantly with manipulation of added load and frequency. This level of control was considered important, since prior research had allowed for the involvement of other major muscle and tissue groups, not supporting specificity of systemic IL6 and CK levels to the tissues of interest, a limitation which must be considered in future work. The results here demonstrate that task parameters influence biomarkers derived from tissues previously associated with risk of and commonly injured in LBP, LDBs, and WMSDs. As discussed previously, sample size was maximized given the total number

of sessions required for each participant. However, it may not have been sufficient to detect changes in COMP levels and interactive effects of task parameters on biomarker levels.

5.4 Research Contributions

The purpose of this dissertation was to expand on previous research by investigating the influence of confounding and occupational risk factors on levels on select biomarkers. This work provides a better understanding of how biomarkers change between individuals following exposure to common risk factors. By formulating the research objectives in terms of occupational risk factors, studies were designed to be applicable to working populations. These studies reveal important information concerning the relevance of the selected biomarkers, favorable time points for biomarker collection, and approximate biomarker levels expected between occupations and exposure to common risk factors. To address the continued prevalence of WMSDs, this work supports the use of biomarkers as a tool for assessing levels of exposure to occupational risk factors, and eventual risk prediction.

5.5 Future Directions

In future work it is important to further explore the effects of confounding variables. As discussed earlier, existing evidence suggests that there are a large number of potential influential or confounding factors for biomarker levels, including several psychological variables. Additional variables should be explored, and even those investigated in Chapter 2 (age, obesity, and gender) require a more detailed analysis. Even if these

factors also contribute to WMSD risk, it is important to differentiate between risk contributions from multiple sources (e.g. personal factors vs. physical loading).

Chapters 3 and 4 focused on the response of biomarkers to various occupational physical loading scenarios. These studies demonstrated that biomarkers can be used to differentiate between high and low risk populations, and that biomarkers are sensitive to task parameters when performing lifting tasks. While these results indicate that biomarkers can be used to assess exposure, determining predictive validity is an important subsequent step. Such work would involve extensive prospective studies tracking biomarker levels, applying other tools using physiological and physical measures, and tracking the incidence of WMSDs. Epidemiological analyses of this data could establish risk ratios associated with biomarker levels, and compare these measures to current tools. Although the work presented here is partially motivated by inconsistencies of current tools, one must consider using biomarkers together with current tools to enhance risk prediction. Finally, it is important to note that other biomarkers related to commonly injured tissues should be explored and considered.

5.6 Overall Conclusions

This dissertation explored the potential for using biomarkers in occupational settings. Biomarkers may offer advantages over risk assessment current tools, since they are objective measures and often derived directly from the tissues commonly injured in WMSDs. The findings here suggest that select biomarkers may find occupational applications, but will require substantial addition research to determine predictive validity for WMSDs. Nonetheless, results of this dissertation suggest that select

biomarkers can be used to differentiate between risk factor exposure levels and may have future value as WMSD risk prediction tools.

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Appendix A: Informed Consent Form #12-259

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY
Informed consent form for participants of Research Project Involving Human Subjects

Title of the Project:
Individual Differences and the Effect of Occupational Physical Demands on Biomarkers of Tissue Load

Principal Investigator:
Dr. Maury Nussbaum, ISE faculty, Department of Industrial and Systems Engineering, nussbaum@vt.edu, (540) 231-6053
Co-Investigator:
Marc Christian, PhD student, Department of Industrial and Systems Engineering, marcc88@vt.edu, (234) 788-3939

I. The Purpose of this Research/Project
The purpose of this study is to investigate changes in biomarkers between different groups of people. These biomarkers measure cartilage damage, muscle damage, and the amount of muscle use. Biomarker changes will be compared between individuals different in age and body size, as well as the effects of physical demands at work.

II. Procedures
If you choose to participate in the evaluation activities, we will ask you to sign one informed consent document (this document). You will keep a copy for yourself.

On the test day you will first be given a brief questionnaire to gather basic information, such as, age, gender, weight, height, etc. Following this, you will be introduced to the test procedures, so you understand what each test session will entail.

The procedure for this experiment will take up to 5 days. After you have signed the consent form and been familiarized, your arm will be prepared and an initial sample of blood drawn. This blood draws will involve 5 ml, similar to what would occur from a routine blood sample at a doctors' office. Blood draws will be performed by Mr. Marc Christian, who is a licensed phlebotomist. Between 5 and 10 samples of blood will be drawn in the test period, at regular intervals, while your type of physical work is determined and perceived discomfort assessed. You will be allowed to perform your regular daily and occupational activities between blood draws. Throughout the test period, and 48hrs prior to that, we will ask you to minimize your physical activity, and avoid strenuous tasks outside of your daily activities and occupational requirements.

III. Risks
There are no more than minimal risks for participating in this study, other than you would encounter during a normal day. Neither our research team nor Virginia Tech have funds set aside for medical treatment should that be required during the experiment. Participants in a study are considered volunteers, regardless of whether

they receive payment for their participation. Under Commonwealth of Virginia law, workers compensation does not apply to volunteers. Appropriate health insurance is recommended for yourself. Our research team will give you enough rest time after a blood draw until you feel comfortable to leave. As noted, all blood draws will be performed by a licensed phlebotomist and thus pose minimal to no risk.

IV. Benefits of the Project
You will likely not gain any direct benefits as a result of your participation in this study. The general goal here is to examine how biomarkers change over the course of a day and how they are influenced by occupational physical demands. The results of this study may help in future research to prevent occupational injuries and to identify individuals at increased risk of injury.

V. Extent of Anonymity and Confidentiality
We assure confidentiality to all participants. However, anonymity cannot be guaranteed, because we will need to have your signatures on the Informed Consent document. We will also have to keep your name and your assigned ID number so we can continue to track your progress. At the end of the study any documents with identifying information will be destroyed. Your name will not be associated with the content of this study, but you will be assigned a three- digit number to protect your privacy. Your name will not be recorded in combination with your data; these two pieces of information will be stored separately in locked cabinets and within databases. Your number is ____, and this number is also on your folder.

All data will be collected by the researchers only. No one other than the researchers will have access to the data, unless it is aggregated first. All responses will be coded so as not to include the name of the participant. The information you provide will have your name removed and only a three- digit participant number will identify you during analyses and any written reports of the research.

This study is being conducted solely for educational and research purposes. Consistent with these academic purposes, any results would be freely publishable. However, to protect your identity, neither personal nor institutional names nor site names or distinguishing information will be used in any published works.

VI. Compensation
There is a $15 per blood draw compensation for participation in the evaluation activities. An additional $10 will be paid to those who complete the full set of procedures.

VII. Freedom to Withdraw
Participation in the evaluation is voluntary and the decision about whether you wish to participate is strictly your own. You may discontinue participation at any time without penalty or loss of benefits to which you are otherwise entitled. Withdrawal from the evaluation activities will not result in any adverse effects, and you will be compensated for any participation prior to withdrawing.

VIII. Approval of Research
This research project has been approved by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute and State University.

_____ _____
IRB Approval Date IRB Expiration Date

IX. Participant's Responsibilities

Upon signing this form below, I voluntarily agree to participate in this study. I have no restrictions to my participation in the study.

X. Participant's Permission

I have read and understand the Informed Consent and conditions of this study. I understand that all organizations participating in this study, including my employer, are not requiring my participation in the study. I understand that any complications that may arise from my participation in this study are not covered by any of the organizations participating in this study, including my employer. All of my questions have been answered. I give my consent to participate.

_____ _____
Participant's Signature Date

Should I have any questions about the evaluation or its conduct, I may contact:

Marc Christian	Email: marcc88@vt.edu	Phone: (234) 788-3939
Dr. Maury Nussbaum	Email: nussbaum@vt.edu	Phone: (540) 231-6053
Dr. David M. Moore, Chair, IRB	Email: moored@vt.edu	Phone: (540) 231-4991

Appendix B: Informed Consent Form #13-059

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY
Informed consent form for participants of Research Project Involving Human Subjects

Title of the Project: The Effect of Lower Back Loads on Biomarker Levels

Principal Investigator:
Dr. Maury Nussbaum, ISE faculty, Department of Industrial and Systems Engineering, nussbaum@vt.edu, (540) 231-6053
Co-Investigator:
Marc Christian, PhD student, Department of Industrial and Systems Engineering, marcc88@vt.edu, (234) 788-3939

I. The Purpose of this Research/Project
The purpose of this study is to investigate changes in biomarkers due to loads placed on the lower back. These biomarkers are chemicals that are measured from the blood, and that indicate potential damage to cartilage and muscle as well as the the amount of muscle use. Biomarker changes will be compared before, after, and during recovery from exposure to tasks that impose different levels of physical loads on the low back.

II. Procedures
If you choose to participate in the evaluation activities, we will ask you to sign one informed consent document (this document). You will keep a copy for yourself.

On the test day you will first be given a brief questionnaire to gather basic information, such as your age, gender, weight, height, etc. Another questionnaire will be used to determine your typical daily level of physical activity. Following this, you will be introduced to the test procedures, so you understand what each test session will involve. Then we will determine the capacity of your low-back muscles, using a strength test.

The procedure for this experiment will consist of up to 10 experimental sessions, each one lasting approximately 3 hours, and requiring you to return after the session a number of times over the following 48 hours. At the beginning of each experimental session an initial blood sample will be obtained. You will then be exposed to a 1 hour simulated lifting task, lifting up to 50% of your maximum capacity at a rate up to 20 cycles/minute. Immediately afterward another blood sample will be obtained. Following this, up to four more blood samples will be obtained at evenly spaced intervals, up to 48 hours after the lifting task. At each blood draw you will also be asked to report your perceived discomfort in various body areas. Each blood draw will be 5 ml, similar to what would occur from a routine blood sample at a doctor's office. Blood draws will be performed by Mr. Marc Christian, who is a licensed phlebotomist. You will be allowed to perform your regular daily and occupational activities between blood draws. Throughout the experimental sessions, and 48hrs prior to them, we will ask you to

minimize your physical activity, and avoid strenuous tasks outside of your daily activities and occupational requirements.

III. Risks

There are no more than minimal risks for participating in this study, other than you would while performing a lifting task or other types of moderate physical exercise. For example, you may feel some muscle soreness after the experiment, or for up to 2-3 days following a session. Neither our research team nor Virginia Tech have funds set aside for medical treatment should that be required during the experiment. Participants in a study are considered volunteers, regardless of whether they receive payment for their participation. Under Commonwealth of Virginia law, workers compensation does not apply to volunteers. Appropriate health insurance is recommended for yourself. Our research team will give you enough rest time after a blood draw until you feel comfortable to leave. As noted, all blood draws will be performed by a licensed phlebotomist and thus pose minimal to no risk.

IV. Benefits of the Project

You will not gain any direct benefits as a result of your participation in this study. Our goal is to examine how biomarkers change over the course of a day and how they are influenced by occupational physical demands. The results of this study may help in future research to prevent occupational injuries and to identify individuals at increased risk of injury.

V. Extent of Anonymity and Confidentiality

We assure confidentiality to all participants. However, anonymity cannot be guaranteed, because we will need to have your signatures on the Informed Consent document. We will also have to keep your name and your assigned ID number so we can continue to track your progress. At the end of the study any documents with identifying information will be destroyed. Your name will not be associated with the content of this study, but you will be assigned a three-digit number to protect your privacy. Your name will not be recorded in combination with your data; these two pieces of information will be stored separately in locked cabinets and within databases. Your number is ____, and this number is also on your folder.

All data will be collected by the researchers only. No one other than the researchers will have access to the data, unless it is aggregated first. All responses will be coded so as not to include the name of the participant. The information you provide will have your name removed and only the three-digit participant number will identify you during analyses and any written reports of the research.

This study is being conducted solely for educational and research purposes. Consistent with these academic purposes, any results would be freely publishable. However, to protect your identity, neither personal nor institutional names nor site names or distinguishing information will be used in any published works.

VI. Compensation

There is a compensation of $10 per hour of participation in the evaluation activities. An additional $10 will be paid to those who complete the full set of procedures.

VII. Freedom to Withdraw

Participation in the evaluation is voluntary and the decision about whether you wish to participate is strictly your own. You may discontinue participation at any time without penalty or loss of benefits to which you are otherwise entitled. Withdrawal from the evaluation activities will not result in any adverse effects, and you will be compensated for any participation prior to withdrawing.

VIII. Approval of Research

This research project has been approved by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute and State University.

IX. Participant's Responsibilities

Upon signing this form below, I voluntarily agree to participate in this study. I have no restrictions to my participation in the study.

X. Participant's Permission

I have read and understand the Informed Consent and conditions of this study. I understand that all organizations participating in this study, including my employer, are not requiring my participation in the study. I understand that any complications that may arise from my participation in this study are not covered by any of the organizations participating in this study, including my employer. All of my questions have been answered. I give my consent to participate.

_____ _____

Participant's Signature Date

Should I have any questions about the evaluation or its conduct, I may contact:

Marc Christian	Email: marcc88@vt.edu	Phone: (234) 788-3939
Dr. Maury Nussbaum	Email: nussbaum@vt.edu	Phone: (540) 231-6053
Dr. David M. Moore, Chair, IRB	Email: moored@vt.edu	Phone: (540) 231-4991