Biomarkers of Tissue Damage: Assessing Diurnal Variation and Sensitivity to Occupational Physical Demands

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A proposal for funding by the OSHRC

A. Significance

Background - Work-related musculoskeletal disorders (WMSDs) continue to be prevalent and costly. According to a conservative NIOSH estimate, the costs attributed to WMSDs total >$13 billion/year [1]. Overexertion remains the top cause of occupational injuries, involving nearly 25% of the total number [2]. Lifetime incidence rates of low-back pain (LBP) specifically is about 80-85% in industrialized countries [3]. These numbers vary greatly between industry and gender. Male construction workers contribute the most cases, with an overall WMSD prevalence of 17.6% [4], and the majority of cases (54%) involving the lower back pain [5]. Nursing reports the highest prevalence of LBP for females at 18.1%, with 78% of cases classified as "intense" pain [6]. These numbers present a significant burden to individual workers, the construction industry, and the medical system.

Diverse ergonomic/biomechanical approaches exist to estimate occupational LBP risks, given a measurement or estimation of task exposure. Some current methods compare estimated loads on the spine to tissue tolerances, originally determined from cadaver studies. For example, the Revised NIOSH Lifting Equation (RNLE) incorporates multiple task-related factors to estimate a maximal load and relative risk of injury, the latter using the Lifting Index (LI) [7]. A recent study suggests that the NIOSH lifting equation demonstrates good predictive power when assessing medium LI tasks [8], yet relatively poorer predictions otherwise. Furthermore, the RNLE and similar models can be limited in application (e.g., the RNLE is not relevant for one-handed tasks). More recently, models using electromyography (EMG) have incorporated lumbar muscle activation to estimate spinal loads [9, 10]. While these show promise, long term EMG measurement can be impractical and invasive, and the validity of these models has not been consistently established, especially for more complex tasks. Subjective ratings, questionnaires, and strength data are other popular methods of determining individual task capacity and risk of future LBP. Outputs of these approaches, though, can vary widely between people, and overall demonstrate a low predictive power for risk of occupational lower back injury [11]. Overall, existing methods have several limitations, the most important being poor or inconsistent success at LBP/WMSD prediction. Furthermore, these methods only infer physical damage, rather than measuring it directly. This is a major limitation, since prevailing theories on injury causation suggest an accumulation of physiological damage over time [12]. Thus, a method that can measure such damage directly, especially when it is sub-clinical, would likely be a promising method to predict future WMSDs.

Biomarkers: A Potential Tool for WMSD Risk Assessment - Biomarkers are molecules that, "can be objectively measured and evaluated as an indicator of physiological change" (pg. 1) [13]. Currently, their main use in physiological medicine centers on measuring injury recovery and tracking disease progression [14-16]. Recent reviews suggest that select biomarkers are released into the systemic blood supply following short term injury of joints, muscles, and connective tissues, due to the rupture of cells, thus providing a valid measure of physical injury [17, 18]. The origin and biological purpose of a biomarker can also indicate more specific injury characteristics [19, 20], and by measuring levels of specific biomarkers we may be able to evaluate the extent and type of injury following physically demanding tasks. However, some fundamental aspects of biomarker responses have yet to be investigated, representing a current impediment to eventual occupational application. To advance biomarkers as a tool in the occupational domain, several relationships must be first established. Time of day [21, 22], and personal factors such as age, weight, smoking, etc. [17], have been shown to influence baseline biomarker levels. Accounting for these factors in future studies is critical to minimizing confounding, and accurately describing biomarker changes. Sensitive and relative increases have been observed due to various physical exertion levels [23-25]. Establishing these differences in working populations, and assessing sensitivity to differing physical work demands, is needed before biomarkers can be related to injury risk. By investigating these issues, we can help to establish biomarkers as a valid tool for determining injury risk.

Conclusions and Proposed Research – While current methods for assessing WMSD risks have had numerous benefits, a number of important limitations remain. Use of biomarkers to predict such risk is a potential alternative, since it is a relatively non-invasive method that uses objective and quantitative measures that are directly related to physical damage. Recent studies have demonstrated a mechanosensitive change in biomarker levels following task exposure (see below), supporting the feasibility of using biomarkers in the occupational domain. At present, though, the occupational use of biomarkers is hindered by a lack of knowledge concerning basic properties. These include the effect of time of day, personal factors, and the level of sensitivity to differences in occupational physical exposures. Assessing these properties is critical to furthering the use of biomarkers in the workplace, and to this end we propose two preliminary studies. In **Study 1**, *we will determine the effects of age and obesity on diurnal changes in select biomarker levels.* Individually, these factors have been shown to lead to differences in biomarker levels. Understanding these changes more comprehensively, though, is necessary so that such sources of variability can be accounted for, and confounding thereby minimized. In **Study 2**, *we will quantify biomarker changes throughout a working week, and assess biomarker sensitivity by comparing results between physically active worker groups and a sedentary population*. As discussed above, short term studies have shown that physical activity leads to altered biomarker levels. By observing changes throughout the week we can determine if there are measurable differences in physiological damage between the groups, thereby supporting the applicability of biomarkers within the workplace.

NORA Priority Research Areas - The proposed research addresses three NORA Priority Research Areas: **Low Back Disorders**, **Exposure Assessment Methods**, and **Risk Assessment Methods**. Within the **National Construction Agenda**, we specifically address the topic of **Musculoskeletal Disorders**, subsections 7.3 and 7.4, which aim to develop practical methods for assessing exposure and associating exposure with development of WMSDs [26].

B. Innovation

The use of biomarkers to assess physically demanding work is quite new, with very limited existing evidence reported. Biomarkers allow for a specific and quantitative individual analysis of physiological damage due to workplace tasks, and show high potential in predicting future risk of injury. This project will investigate two fundamental properties of biomarkers, which have yet to be explored in humans (i.e., differences in diurnal variation differences between populations, and sensitivity to workplace physical demands). Results from the proposed studies will help lay a foundation for a more widespread use of biomarkers in the workplace. Using biomarkers provides several potential advantages as described above. In the future, it is likely that these can be combined with existing ergonomic/biomechanical methods to develop innovative approaches to assessing and controlling WMSD risks.

C. Approach

The purpose of these studies is to determine whether diurnal variations in selected biomarkers differ with age and/or obesity and whether these biomarkers are sensitive to occupational physical demands. We propose to examine *three specific biomarkers*: Cartilage oligomeric matrix protein (COMP), Interleukin-6 (IL6), and Creatine phosphokinase (CPK). Each of these biomarkers gives insight into a unique aspect of physiological damage, respectively to cartilage, collagen, and muscle.

With injury to collagenous tissue, changes in several biomarkers have been observed [27-29]. Cartilage oligomeric matrix protein (COMP) is a molecule that is produced ubiquitously in high-collagen tissues [30]. At a molecular level, COMP binds to collagen fibers, helping to anchor them and providing additional mechanical strength [31]. There is a mechanosensitive increase of COMP with respect to differential loading protocols [23, 32], which corresponds to an increased need for mechanical strength. Interleukin-6 (IL6) is an inflammatory protein that is up-regulated and released into the bloodstream in correspondence to the volume and duration

of muscle mass use [20]. Additionally, IL6 appears to induce production of collagen fibers [28], which will increase mechanical strength of tissues similarly to COMP. Together, these two biomarkers provide insight into the state of collagenous tissue following mechanical exposure, and thus lend themselves well to use as predictive biomarkers for repetitive and overuse injuries.

Muscles are the crucial structure of the body that provide strength and force for movement. Although controlled damage, known as 'microtrauma', has beneficial effects such as in weight training [33], debilitating injuries due to overexertion injuries are common [12]. Creatine phosphokinase (CPK) is an enzyme that buffers phosphate molecules required for force production [34]. During injury, muscle cells rupture, thus leaking CPK into the blood supply [35]. Eccentric muscle exercise is especially damaging and greatly increases CPK concentrations in the blood [36, 37]. Yang et. al [24] showed a clear dose-response relationship of IL6 and CPK with hand-held loads during a 2hr lifting task . Thus, while IL6 can be used as a measure of total muscle use, CPK indicates physical damage.

STUDY 1 - Diurnal Variation in Biomarkers of Physiological Damage: Differences Related to Age and Obesity

The purpose of this study is to investigate whether age and/or obesity modify diurnal changes in COMP, IL6, and CPK. Age and obesity have already been shown to separately influence baseline ("resting") biomarker levels [17], respectively due to increased frailty of biomechanical structures such as collagen [38] and increased joint and muscle stresses. Diurnal changes in a "normal" population have been described for COMP and IL6, and suggest inverted-U and U-shaped relationships respectively [21, 22]. If we are to validly use these biomarkers to physiological damage at work, we must understand these diurnal changes in more detail. Subsequently, biomarker levels can be adjusted to reflect the influences of these two important personal factors. Our **hypotheses** in this Study are that **1) consistent patterns of changes in biomarker levels will be observed over a day**, and **2) these patterns will differ with age and/or obesity**. Results will support future research (e.g. accounting for diurnal effects and individual differences) and also will be used in this way in Study 2.

Design and Methods - A repeated-measures design will be used, wherein biomarker levels will be assessed from blood samples collected repeatedly throughout a day. A total of 12 participants will be included, with 3 in each of 4 groups. Specifically, we will include all combinations of *older* (50-65 yrs) and *younger* (18-30yrs) participants, and individuals who are *normal-weight* (18.5≤BMI<25) and *obese* (30≤BMI<40). The latter range is to include individuals with both Class I and Class II obesity, which will cover the majority of obese individuals in the US worker population [39]. This range will also avoid more extreme obesity, which typically also involves additional medical conditions that may confound biomarker levels. Participants will be recruited from among the VT and local communities, and those with a history of musculoskeletal disorders, having any blood-borne diseases, and smokers [40] will be excluded.

We will complete a 24hr procedure with each participant, starting on an evening and continuing through the next waking day (note, this description assumes each participant has "normal" sleep-wake timing). Participants will arrive at the test facility at 10pm, at which time informed consent will be obtained along with anthropometric measures, responses to a questionnaire (e.g., age, health status) and a blood sample. Shortly after waking (e.g., 7 am) the following morning, a fasting blood sample will be obtained at the participant's home, to minimize the amount of physical activity between waking and blood draw. Following this, samples will be obtained in the lab at (roughly) 10am, 2pm, 6pm, and 10pm, providing 6 samples in the 24hr period. Each blood draw will be 5ml of blood from the antecubital area, and performed by a certified phlebotomist (Mr. Christian). Blood samples will be kept on ice until they are centrifuged to obtain serum, which will be frozen at -20C until analysis. During the testing day, and 48 hours prior, participants will be asked to limit their physical movement, since we are

trying to observe effects due only to time of day. Sitting and walking will be allowed, but participants will be asked to refrain from more substantial exertions (e.g. heavy lifting, running); lack of such control has been a limitation of earlier studies [22]. Participants will be allowed to leave the lab between blood draws.

Analysis - Biomarker concentrations will be determined from serum samples for each time point using enzyme linked immunosorbent assays (ELISA): 1) COMP (BioVendor®, Chandler, NC); 2) IL-6 (BioVendor®, Chandler, NC); and 3) CPK (Antibodies-Online®, Atlanta, GA). As in previous studies, concentration levels will also be normalized ([baseline-timepoint]/baseline). Mixed-factor ANOVAs will be used to assess diurnal changes in biomarker levels and any differences related to age and/or obesity, with post-hoc comparisons completed as relevant. Consistent with our hypotheses above, we expect to find changes in biomarker levels throughout the day, notably a baseline at the morning draw, followed by increases over the remainder of the day (**Hypothesis 1**). This will indicate a removal of physiological stress during sleep and increases related to daily physical activity (even though such activity is minimized, it is still present as a result of regular activity). Age and obesity are expected to result in higher biomarker concentrations overall, possibly with interactive effects (**Hypothesis 2**).

STUDY 2 – Sensitivity of Biomarkers to Occupational Physical Demands

The purpose of this study is to determine if biomarker levels are sensitive to occupational physical demands, by comparing these levels among two groups. Specifically, we will assess levels among sedentary workers and two populations with high levels of routine physical demands and risks of LBP (i.e., construction workers and nurses). Existing evidence is somewhat limited. One study positively associated IL6, along with other biomarkers of physiological damage, with increased upper-body musculoskeletal use [41], while another demonstrated that markers of collagen degradation were associated with lower-limb disorders [42]. In addition, a recent study showed dose-response relationships with load weight in the context of simulated manual material handling [24]. Our proposed work expands upon this evidence, by quantifying trends in biomarker levels across a working week (e.g., biomarker accumulation, and the effect of nightly rest). Future longitudinal studies can then investigate biomarker levels and incidence of WMSDs. Our **hypotheses** in this Study are that **1) different changes in biomarker levels will be observed between sedentary and active population, both within days and across a working week**, **2) these differences will be associated with the amount of physical work performed and perceived discomfort ratings in isolated body areas**.

Design and Methods – A total of 15 participants will complete a 5-day procedure. Participants will include five individuals recruited from each of the three job sectors: construction, nursing, and sedentary. Before beginning the procedure, participants will provide informed consent and complete the validated International Physical Activity Questionnaire (long form). This questionnaire will be used to classify individuals as physically active (3 days/week with a total of 1500 MET-min/week, *or* 7 days/week with a total of 3000 MET-min/week) or sedentary (anything below physically active criteria) [43, 44]. A questionnaire will be used to determine individual characteristics, such as age, gender, BMI, and history of musculoskeletal disorders.

Monday, Wednesday, and Friday, both before and after work, a blood sample will be obtained (this schedule assumes a “normal” work week). After each work shift, isolated discomfort ratings [45] will be assessed, and another questionnaire used to estimate the amount and type of physical work performed prior to the current meeting [46]. Between meetings, participants will be allowed to perform their jobs normally. Over a working week, 6 blood samples and sets of questionnaires will thus be obtained.

Participants will be asked to restrict physical activity outside of their job during the weekend prior to and the week of the study. The Monday morning draw will act as a baseline level. Participants in the physically active populations will be recruited from the local region, including Montgomery Regional Hospital (female nurses) and Skansa Construction (male

construction workers). We expect the former and latter to be primarily females and males, respectively, but will seek to obtain a balanced sample. Workers with more sedentary jobs will be recruited from VT and the local region, and, to the extent possible will be age-matched and BMI-matched with the other groups. Exclusion criteria include having any current or recent (within 3 years) history of musculoskeletal disorders, any blood-borne diseases, smoking, or a BMI >25.

Analysis - Blood samples will be obtained and biomarker concentrations determined using the same procedures as in Study 1. Mixed-factor ANOVAs will be employed to assess the effects of time and group on the *dependent variables* (biomarker concentrations, normalized change values, and perceived discomfort ratings). We will also explore, using regression, the associations between biomarker responses and perceived discomfort rating. Significant effects will be concluded when $p<0.05$. Results from Study 1 will allow us to correct for diurnal variations.

Based on earlier evidence, and consistent with **hypothesis 1**, we expect to find consistent patterns in biomarkers throughout the working week. Specifically, increases in biomarkers levels are expected over the course of the week, with (incomplete) reductions found in the morning samples. We also expect to find larger increases and less complete reductions in biomarkers among the high physical workload groups (**hypothesis 2**). Finally, we expect that patterns in biomarker changes will be directly associated with similar patterns of perceived discomfort.

Limitations (both Studies) - A potential limitation of this work is that there are many other factors that could influence biomarker levels but that are not controlled (e.g. caloric intake, amount of prior sleep). However, we have selected biomarkers that are specific to cartilage damage, collagen synthesis, and muscle use/damage, which should minimize these potential confounding effects. Physical activity outside of work could also influence biomarker levels, though we are attempting to minimize this by asking participants to restrict their activity outside of work.

Expected Results and Contribution

The main purpose of these studies is to contribute to the future use of biomarkers as an occupational tool to measure and control with risk of WMSDs. In industrialized countries, low WMSDs in general and back disorders specifically are prevalent and costly problems, and are strongly associated with strenuous lifting activities [47] and more generally physically demanding occupations. Current methods do not allow for a direct and objective quantification of physiological injury. By using biomarkers, perhaps in concert with current methods, we may gain more insight into the extent and source of physical damage related to occupational physical demands and develop better methods to control these adverse outcomes. The two proposed studies will provide fundamental new information (about diurnal changes and sensitivity to occupational demands), which can then be used to better interpret and/or design future studies.

Deliverables

As described in the RFP, we will provide updates as requested, a summary upon completion, and profession poster. We plan to use these preliminary results as the basis for two proposals, one to the Jeffress Memorial Trust and one to NIOSH (R21).

D. Prior OSHRC Funding

N/A

E. References

1. NIOSH, *Musculoskeletal Disorders and Workplace Factors*, B.P. Bernard, Editor 1997: Cincinnati, OH.
2. Pleis, J.R., J.W. Lucas, and B.W. Ward, *Summary health statistics for U.S. adults: National Health Interview Survey, 2008*, 2009, National Center for Health Statistics: Washington, DC.
3. Hoy, D., et al., *Measuring the global burden of low back pain.* Best Practice & Research Clinical Rheumatology, 2010. **24**(2): p. 155-165.
4. Guo, H.R., et al., *Back pain prevalence in US industry and estimates of lost workdays.* Am J Public Health, 1999. **89**(7): p. 1029-1035.
5. Widanarko, B., et al., *Prevalence of musculoskeletal symptoms in relation to gender, age, and occupational/industrial group.* International Journal of Industrial Ergonomics, 2011. **41**(5): p. 561-572.
6. Eriksen, W., D. Bruusgaard, and S. Knardahl, *Work factors as predictors of intense or disabling low back pain; a prospective study of nurses' aides.* Occupational and Environmental Medicine, 2004. **61**(5): p. 398-404.
7. NIOSH, *Applications Manual for the Revised NIOSH Lifting Equation*, 1994: Cincinnati, OH.
8. Waters, T.R., et al., *Efficacy of the Revised NIOSH Lifting Equation to Predict Risk of Low Back Pain Due to Manual Lifting Expanded Cross-Sectional Analysis.* Journal of Occupational and Environmental Medicine, 2011. **53**(9): p. 1061-1067.
9. Jia, B., S. Kim, and M.A. Nussbaum, *An EMG-based model to estimate lumbar muscle forces and spinal loads during complex, high-effort tasks: Development and application to residential construction using prefabricated walls.* International Journal of Industrial Ergonomics, 2011. **41**(5): p. 437-446.
10. Granata, K.P. and W.S. Marras, *AN EMG-ASSISTED MODEL OF TRUNK LOADING DURING FREE-DYNAMIC LIFTING.* Journal of Biomechanics, 1995. **28**(11): p. 1309-1317.
11. Dawes, H.N., et al., *Borg's rating of perceived exertion scales: Do the verbal anchors mean the same for different clinical groups?* Archives of Physical Medicine and Rehabilitation, 2005. **86**(5): p. 912-916.
12. Kumar, S., *Theories of musculoskeletal injury causation.* Ergonomics, 2001. **44**(1): p. 17-47.
13. Jain, K.K., *The Handbook of Biomarkers*2010, New York: Springer.
14. Tseng, S., A.H. Reddi, and P.E. Di Cesare, *Cartilage Oligomeric Matrix Protein (COMP): A Biomarker of Arthritis.* Biomark Insights, 2009. **4**: p. 33-44.
15. Cuellar, J.M., et al., *Diagnostic Utility of Cytokine Biomarkers in the Evaluation of Acute Knee Pain.* Journal of Bone and Joint Surgery-American Volume, 2009. **91A**(10): p. 2313-2320.
16. Patra, D. and L.J. Sandell, *Recent advances in biomarkers in osteoarthritis.* Current Opinion in Rheumatology, 2011. **23**(5): p. 465-470.

17. Carp, S., A.E. Barr, and M.F. Barbe, *Serum biomarkers as signals for risk and severity of work-related musculoskeletal injury.* Biomarkers in Medicine, 2008. **2**(1): p. 67-79.

18. Brancaccio, P., G. Lippi, and N. Maffulli, *Biochemical markers of muscular damage.* Clinical Chemistry and Laboratory Medicine, 2010. **48**(6): p. 757-767.

19. Garvican, E.R., et al., *Biomarkers of cartilage turnover. Part 1: Markers of collagen degradation and synthesis.* The Veterinary Journal, 2010. **185**(1): p. 36-42.

20. Pedersen, B.K. and M.A. Febbraio, *Muscle as an endocrine organ: Focus on muscle-derived interleukin-6.* Physiological Reviews, 2008. **88**(4): p. 1379-1406.

21. Miles, M.P., et al., *Diurnal variation, response to eccentric exercise, and association of inflammatory mediators with muscle damage variables.* Journal of Applied Physiology, 2008. **104**(2): p. 451-458.

22. Lottenburger, T., P. Junker, and K. Horslev-Petersen, *Diurnal variation of connective tissue metabolites in early and long-standing rheumatoid arthritis and in healthy individuals.* Scandinavian Journal of Rheumatology, 2011. **40**(2): p. 88-94.

23. Niehoff, A., et al., *Different mechanical loading protocols influence serum cartilage oligomeric matrix protein levels in young healthy humans.* European Journal of Applied Physiology, 2010. **110**(3): p. 651-657.

24. Yang, G., W.S. Marras, and T.M. Best, *The biochemical response to biomechanical tissue loading on the low back during physical work exposure.* Clinical Biomechanics, 2011. **26**(5): p. 431-437.

25. Magal, M., et al., *Relationship between serum creatine kinase activity following exercise-induced muscle damage and muscle fibre composition.* Journal of Sports Sciences, 2010. **28**(3): p. 257-266.

26. NORA, *National Construction Agenda*, 2008, Construction Sector Council: Cincinnati, OH.

27. Neidhart, M., et al., *Small fragments of cartilage oligomeric matrix protein in synovial fluid and serum as markers for cartilage degradation.* British Journal of Rheumatology, 1997. **36**(11): p. 1151-1160.

28. Andersen, M.B., et al., *Interleukin-6: a growth factor stimulating collagen synthesis in human tendon.* Journal of Applied Physiology, 2011. **110**(6): p. 1549-1554.

29. Kuiper, J.I., et al., *Serum markers of collagen metabolism: construction workers compared to sedentary workers.* Occupational and Environmental Medicine, 2005. **62**(6): p. 363-367.

30. Saxne, T. and D. Heinegard, *Cartilage Oligomeric Matrix Protein - A Novel Marker of Cartilage Turnover Detectable in Synovial-fluid and Blood.* British Journal of Rheumatology, 1992. **31**(9): p. 583-591.

31. Posey, K.L. and J.T. Hecht, *The Role of Cartilage Oligomeric Matrix Protein (COMP) in Skeletal Disease.* Current Drug Targets, 2008. **9**(10): p. 869-877.

32. Giannoni, P., et al., *The mechanosensitivity of cartilage oligomeric matrix protein (COMP).* Biorheology, 2003. **40**(1-3): p. 101-109.

33. Segesser, B., E. Morscher, and A. Goesele, *DISTURBANCES IN THE GROWTH PLATES AS A RESULT OF SPORTS STRESS.* Orthopade, 1995. **24**(5): p. 446-456.

34. Sietsema, K.E., et al., *Potential biomarkers of muscle injury after eccentric exercise.* Biomarkers, 2010. **15**(3): p. 249-258.

35. Lee, J.H. and P.M. Clarkson, *Plasma creatine kinase activity and glutathione after eccentric exercise.* Medicine and Science in Sports and Exercise, 2003. **35**(6): p. 930-936.

36. Newham, D.J., D.A. Jones, and R.H.T. Edwards, *Plasma creatine kinase changes after eccentric and concentric contractions.* Muscle & Nerve, 1986. **9**(1): p. 59-63.

37. Su, Q.S., et al., *Comparison of changes in markers of muscle damage induced by eccentric exercise and ischemia/reperfusion.* Scandinavian Journal of Medicine & Science in Sports, 2010. **20**(5): p. 748-756.

38. Chen, J.S., et al., *Effect of age-related chronic immobility on markers of bone turnover.* Journal of Bone and Mineral Research, 2006. **21**(2): p. 324-331.

39. NIH, *Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults*, 1998, National Heart, Lung, and Blood Institute: Bethesda, MD.

40. Anbarasi, K., et al., *Creatine kinase isoenzyme patterns upon chronic exposure to cigarette smoke: Protective effect of Bacoside A.* Vascular Pharmacology, 2005. **42**(2): p. 57-61.

41. Carp, S.J., et al., *Inflammatory biomarkers increase with severity of upper-extremity overuse disorders.* Clinical Science, 2007. **112**(5): p. 305-314.

42. Mason, H.J., G. Evans, and A. Moore, *Urinary biomarkers and occupational musculoskeletal disorders in the lower limbs.* Occupational Medicine-Oxford, 2011. **61**(5): p. 341-348.

43. Booth, M., *Assessment of physical activity: An international perspective.* Research Quarterly for Exercise and Sport, 2000. **71**(2): p. S114-S120.

44. Boon, R.M., et al., *Validation of the New Zealand Physical Activity Questionnaire (NZPAQ-LF) and the International Physical Activity Questionnaire (IPAQ-LF) with accelerometry.* British Journal of Sports Medicine, 2010. **44**(10): p. 741-746.

45. Kuorinka, I., et al., *Standardised Nordic questionnaires for the analysis of musculoskeletal symptoms.* Applied Ergonomics, 1987. **18**(3): p. 233-237.

46. Hollmann, S., et al., *Validation of a questionnaire for assessing physical work load.* Scandinavian Journal of Work Environment & Health, 1999. **25**(2): p. 105-114.

47. Heneweer, H., et al., *Physical activity and low back pain: a systematic review of recent literature.* European Spine Journal, 2011. **20**(6): p. 826-845.

F. Budget

Itemized budget:

-12 ELISA kits:

4 x COMP ($555, http://www.biovendor.com/country30/product/immunoassays/cartilage-oligomeric-matrix-protein-human-elisa) $2,220.00

4 x IL6 ($595, http://www.biovendor.com/country30/product/immunoassays/interleukin-6-human-elisa) $2,380.00

4 x CPK ($757.14, http://www.antibodies-online.com/kit/626365/Creatine+Kinase+CK+ELISA/) $3,028.56

TOTAL: **$7,628.56**

-Shipping for ELISA kits:

12 x one-day, on ice shipping.....**~$371.44**

Total funding requested from OSHRC.....$8,000.00

Budget justification:

ELISA kits:

-Each ELISA kit can run 40 samples, which means we have (4kits/biomarker * 40samples/kit =) **160 samples/biomarker**.

-Study 1 has 12 participants, at 6 samples each, i.e. (12 x 6 =) **72 samples**
-Study 2 has 14 participants, at 6 samples each, i.e. (14 x 7 =) **84 samples**
-Total samples required: 156 samples/biomarker

-ELISA kits are currently the cheapest method for determining levels of specific enzymes, proteins, and molecules. The detection limits for COMP, IL6, and CPK are 0.4ng/ml, 0.92 pg/ml, and 0.1ng/ml respectively, which are excellent for our application. We minimize the cost by performing all the analyses in house. Dr. Kevin Edgar has generously lent us a lab bench to use in the ICTAS 1 building. Even the graduate student in charge of performing the data collection has been certified as a

phlebotomist.

-Shipping for the ELISA kits is generally expensive. Since each kit is only produced on request/order, they are shipped out as soon as they become available. Thus each kit is usually shipped separately. In addition, the kits are shipped via one-day express, and require special cooling packaging. Contents of the kits can degrade in high temperatures. This usually adds on substantial costs.

****Note on the budget:**

Substantially more resources are required to complete the work proposed here, including funds for participant compensation, phlebotomy supplies, lab supplies, conference travel, etc. We are requesting that just the ELISA kits be funded, and the remaining costs will be covered by the PI (Nussbaum).

G. Timeline and Project Management

	2012									
	M	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**
Study 1										
Recruitment										
Testing										
Analysis										
Reporting										
Study 2										
Recruitment										
Testing										
Analysis										
Reporting										

Dr. Maury Nussbaum will serve as the PI for this project. He is the primary advisory to Marc Christian, a graduate student in ISE, whose dissertation work will be addressing the use and application of biomarkers in the occupational setting. Mr. Christian will have a primary role in collecting, processing, and analyzing the study results, as well as in report and paper generation.