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Biomarker Responses to Static Axial Trunk Loading and Unloading

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Abstract: Work-related musculoskeletal disorders (WMSDs) continue to be prevalent and costly in a wide variety of industrial settings. Biomarkers related to tissues commonly involved in WMSDs may be useful in assessing physiological damage following exposure to risk factors. Recent studies have demonstrated that exposure to dynamic spinal loading, through lifting tasks or repeated compressive forces, elicits measureable changes in these biomarkers. There is, however, little evidence examining the effect of static loads on these biomarkers, despite clear evidence linking both static and dynamic forces to an increased risk of WMSDs. Here, changes in cartilage oligomeric matrix protein (COMP), interleukin-6 (IL-6), and creatine phosphokinase (CPK) were monitored before and after 1hr of exposure to static axial trunk loading and again after 1hr of prone rest. Low to moderate loads were applied while seated, at 0 (control), 20, and 40% of individual body weight. COMP significantly decreased over time, while CPK was unaffected. Though not significant, IL-6 exhibited a delayed increase (+0.177 ng/L normalized change) in the 40% load condition. All three biomarkers exhibited low sensitivity to the current levels of static axial loading, which contrasted with previous evidence in dynamic spinal loading and a clear dose-response patterns in reported discomfort. COMP, IL-6, and CPK appeared insufficiently sensitive to reflect isolated tissue stress or damage, and suggest longer exposure times or larger sample sizes may be required. Additionally, COMP may require an unloading period to achieve baseline levels. More work is needed to explore the utility of biomarkers in conditions of low to moderate static spinal loading.

Keywords: Biomarker; Interleukin-6, Cartilage Oligomeric Matrix Protein, Creatine Phosphokinase, Static Trunk Loading

1. Introduction

Many spinal injuries, particularly in the occupational domain, appear related to and likely caused by excessive mechanical loading (da Costa & Vieira, 2010; Kumar, 2001). Among several methods for assessing injury risk, a common approach is to compare tissue loads with respective tolerances. As recently argued (Christian, 2014), however, such an approach is limited in several respects, including incomplete characterization of tissue tolerances and knowledge of specific tissue/failure mechanisms, and the fact that pain can manifest in sub-failure situations.

Tissues respond to stress with a variety of biological responses such as the production of inflammatory, supportive, and regenerative molecules (Brancaccio et al., 2010; Christian, 2015). Cartilage oligomeric matrix protein (COMP) is found ubiquitously in high collagen fibrous areas (Saxne & Heinegard, 1992), where it functions to provide mechanical strength to collagen fibers (Amanatullah et al., 2012). Leakage of COMP into the blood supply is associated with frequency and magnitude of cartilage loading and degradation (Kim et al., 2009; Niehoff et al., 2010). Interleukin-6 (IL-6) is an inflammatory cytokine produced by muscle in proportion to the volume and duration of muscle used (Christian & Nussbaum, 2015; Toft et al., 2011), with respect to frequency of muscle use (D'Ambrosia et al., 2010), and whose biological purpose is inducing regenerative collagen synthesis (Reihmane & Dela, 2013). Creatine phosphokinase (CPK) is a muscular protein used as a buffer for phosphate molecules (Brancaccio et al., 2010) which leaks into the blood supply during muscle damaging

eccentric exercise (Hirose et al., 2004; Sietsema et al., 2010). Serum level changes have been correlated with both muscle trauma and delayed onset muscle soreness (Chen et al., 2013).

These three biomarkers therefore serve as quantitative indicators of physiological change; cartilage damage, collagen synthesis, and muscle use and damage. For these reasons, biomarkers have utility in assessing WMSD risk and workers' compensation scenarios (Christian & Nussbaum, 2013). Christian and Nussbaum (2015) provided strong evidence that IL-6 and CPK are responsive to repetitive dynamic spine loads and muscle force. The goal of this study was to investigate such relationships under more controlled conditions, specifically the effect of low-moderate levels of static axial trunk loading/unloading. We hypothesized that these three biomarkers (COMP, IL-6, and CPK) would be responsive to these more isolated, static loads, respectively indicating cartilage damage, muscle use and damage, and collagen synthesis. These results may aid future occupational biomarker studies, by indicating sensitivity and specificity of these biomarkers to static spinal loading.

2. Methods

Table 1. Participant Characteristics with *t*-tests Performed by Gender.

	Mean (SD)		*t*-test
	Male	Female	(*P*-value)
n	3	3	
Age (years)	24.3 (1.5)	24.7 (3.8)	0.90
Stature (cm)	176.1 (5.3)	162.6 (2.5)	0.06
Body mass (kg)	74.7 (7.9)	70.0 (10.1)	0.57

Participant characteristics are shown in Table 1. None reported any current or prior musculoskeletal disorders; due to the experimental procedures (see below), participation was limited to individuals with body mass ≤90kg and reporting no blood-borne diseases. Prior to data collection, all participants completed informed consent procedures that were approved by the Virginia Tech Institutional Review Board.

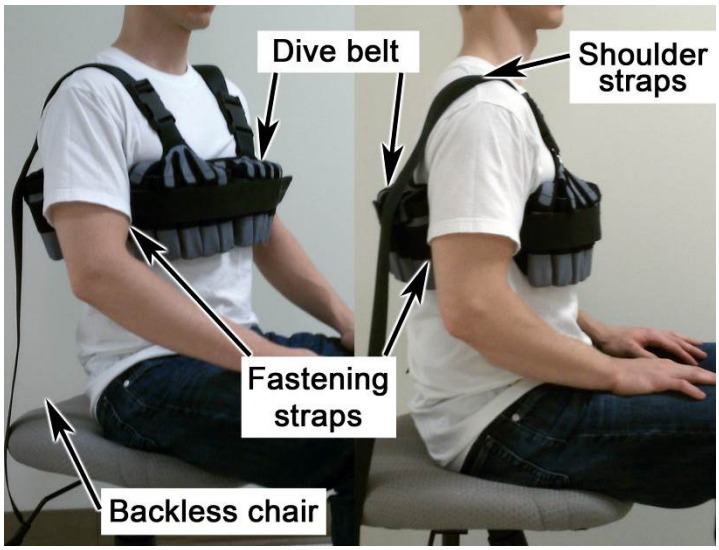

Figure 1. Experimental Setup, Demonstrating a Participant in the Seated Posture and with the 40% Body Weight Load Applied.

A repeated-measures design was employed, involving exposure to static axially-directed trunk loading at 0 (control), 20, and 40% of body mass. Presentation order was counterbalanced (Latin Squares). Each exposure occurred in sessions on separate days, at the same time of day, with at least three days between each, and participants were required to avoid any

moderate-high levels of exercise for at least 24 hours prior to each session. Static axial loading was induced using a weighted diving vest (Figure 1). Small weights were inserted into pockets such that the loads were symmetric in the antero-posterior and medio-lateral directions. Shoulder straps were used to maintain the load height, such that the inferior border of the vest was at roughly the inferior margin of the ribcage. Loadings were applied while participants sat in a backless chair, and they were instructed to sit upright (maintaining lumbar lordosis).

Immediately upon arrival a first blood sample (baseline) was obtained. Subsequently, the axial loading was applied continuously for 1 hour. After exposure, the load was removed and a second blood sample was drawn. Participants were then asked to lie prone and relaxed on a pad for 1 hour (rest period), following which a third and final blood sample was drawn. Blood draws were obtained from the antecubital fossa, by a certified phlebotomist, into serum separator tubes. Two tubes were obtained at each draw, yielding two biomarker measures per draw. Blood samples were kept on ice until being centrifuged, serum poured off, and stored at -20°C. Enzyme-linked immunosorbent assays (ELISA) were performed according to manufacturer instructions to determine the levels of three biomarkers: 1) COMP (BioVendor®, Chandler, NC); 2) IL-6 (BioVendor®, Chandler, NC); and 3) CPK (TSZ®, Waltham, MA). Beginning just before load application, and every 15 minutes thereafter, participants rated their perceived level of lower back discomfort using a 10-point scale (Borg, 1982).

As there were limited financial resources, and given the initial results obtained, COMP, IL-6, and CPK analyses were run only for the 0% and 40% exposures. For biomarker responses, dependent measures were obtained as normalized changes relative to pre-exposure levels (i.e., [post-pre]/pre). There were no significant or substantial effects of exposure order on any measures and no significant differences between pairs of biomarker measures from a given blood draw. Repeated-measures analyses of variance (ANOVA) were used to assess the effects of load and time on COMP, IL-6, CPK, and perceived discomfort. Throughout, gender was included as a blocking factor, and effects considered significant when $P<0.05$. COMP values of one participant for the 40% exposure were excluded as clear outliers (studentized residuals). Summary statistics are presented as mean (SD).

3. Results

Perceived lower back discomfort was significantly affected by load ($P<0.0001$), time ($P<0.0001$), and their interaction ($P<0.0001$). Mean values increased monotonically during all conditions over the 60 min exposures, but to a significantly greater extent when there was an external load (Figure 2). Reported discomfort following exposure (≥75min) returned quickly to pre-exposure levels.

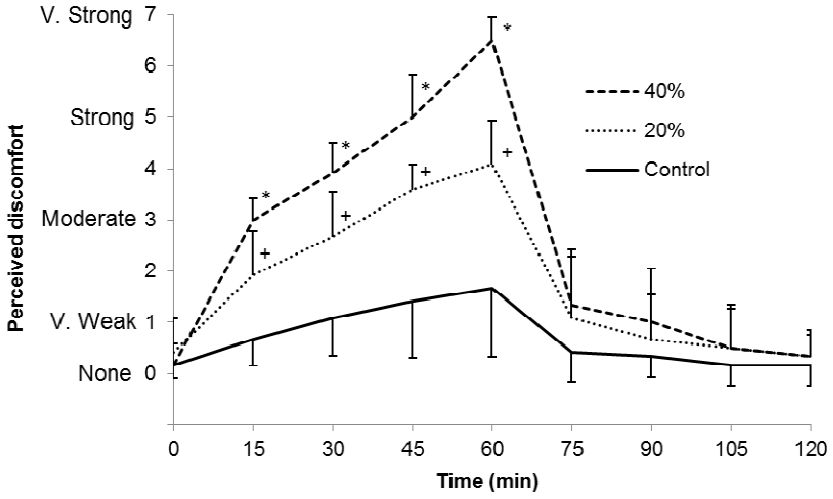

Figure 2. Perceived Discomfort Ratings for the 0% (Control), 20% and 40% Loading Conditions. Exposures Occurred between 0 and 60 Minutes, Followed by 60 Minutes of Prone Rest. Error Bars Represent Standard Deviations. The + Symbol Indicates a Significant (P<0.05) Difference between the 20% Load and Control Conditions, and the * Symbol a Significant Difference between the 40% Load and Control Conditions.

Pre-exposure values of COMP were 381 (98.3) and 354 (63.8) ng/ml in the 0 and 40%BW conditions, respectively. COMP decreased significantly (P=0.0001) after exposure and in the control, more so for males (P=0.031), and remained lower that pre-exposure values after the rest period (Figure 3). Pre-exposure values of IL-6 were 3.87 (0.87) and 5.35 (1.97) pg/ml in the 0 and 40%BW conditions, respectively. Changes in IL-6 immediately post-exposure were not significant (P=0.29). After the rest period, however, IL-6 concentrations in the 40%BW condition exhibited an upward trend (P=0.12) vs. pre-exposure (+0.177 normalized change). CPK concentrations were initially 106 (59) and 152 (49) U/L for the 0 and 40%BW conditions, respectively, and did not change significantly over time (P=0.55).

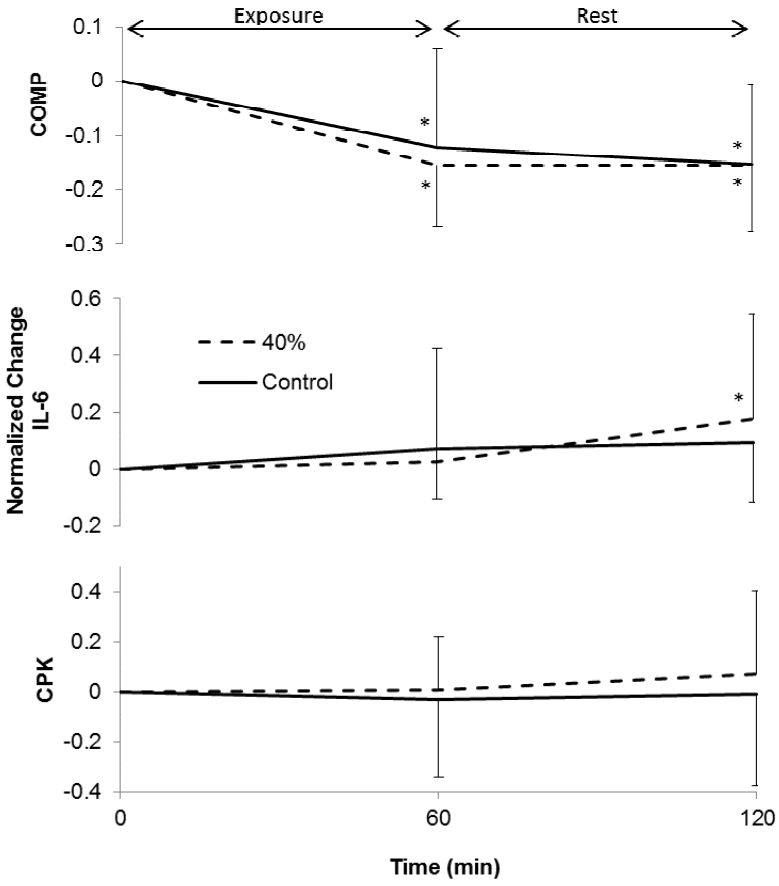

Figure 3. Normalized Changes in (A) COMP, (B) IL-6, and (C) CPK Concentrations Over Time for the 0% (Control) and 40% Loading Conditions. Error Bars Represent Standard Deviations. The * Symbol Indicates a Significant (P<0.05) Change from the Pre-Exposure Levels (Time = 0).

4. Discussion

Perceived discomfort ratings suggested that the experimental exposures were effective in terms of applying controlled low back loads. This, along with evidence summarized above, supported hypothesized increases in biomarker responses with static axial loading. Our results, however, did not support these. Decreases in COMP have been observed in previous control conditions, though increases were still observed during task exposure (Mendham et al., 2011; Niehoff et al., 2010). Our decreases are likely explained by the lack of rest prior to exposure being given. A delayed upward trend was observed for IL-6 here during the 40% loading condition as compared to the control, which could either indicate muscle utilization or collagen synthesis (Andersen et al., 2011; Brancaccio et al., 2010). Since no significant changes in CPK were observed, it may be that IL-6 increased in response to collagen synthesis resulting from the static axial load. The lack of (and in some cases counterintuitive) expected differences following different spinal loads may be explained by one of the

following possibilities: either static axial trunk loading does not cause actual physical damage (COMP) despite perceived discomfort; or dilution of the released biomarkers into the systemic blood suppressed levels below measurable effects (IL-6 and CPK).

An earlier study that found significant increases in IL-6 and CPK but involved dynamic loading, and more and larger muscle groups besides those in the lower back (Yang et al., 2011). In a study which isolated the muscles in the lower back, repetitive, dynamic, flexion/extension motions also caused changes in IL-6 and CPK (Christian & Nussbaum, 2015). Other related studies (Ishii et al., 2006; Tokunaga et al., 2010; Zivanovic et al., 2011) used biopsies from patients or animals, permitting more specificity and sensitivity in biomarker assessment. Our sampling method was likely less sensitive, though less invasive, and may further explain why our isolated exposure task did not elicit the expected biomarker increases. Sensitivity has also been an issue with COMP in previous studies (Giannoni et al., 2003). Unloaded rest appeared necessary to reach baseline biomarker levels (cf. Fig 3). To investigate this, an additional participant was given 1 hr of prone rest prior to 40% exposure for 1 hr. COMP and CPK both decreased, supporting a lack of sensitivity, and IL-6 increased, consistent with our other results.

Despite previous evidence demonstrating changes in these selected biomarkers to dynamic lower back exercises (Christian & Nussbaum, 2015; Yang et al., 2011), the static axial compression applied in this study appears not to elicit similar changes. However, it should be noted that this is in line with previous cadaver evidence that suggests that dynamic, repetitive loading is required to cause vertebral damage (Brinckmann et al., 1988). The biomarker and psychophysical data suggest contrary results which require further investigation. Prospective studies are necessary to establish cause and effect relationships between risk factor exposure, biomarker levels, psychophysical data, and future WMSD incidence.

5. Conclusion

In conclusion, all three biomarkers demonstrated relatively poor sensitivity to low to moderate levels of static axial loading, despite a clear dose-response of perceived discomfort ratings. Improved sensitivity to such static exposures may require obtaining biopsies, using longer exposures, or larger sample sizes. COMP appears to require on the order of 30 min of unloading to reach baseline levels. While biomarkers may have future utility for assessing WMSD risk, further studies are required to validate and explore their sensitivity to a range of daily/occupational loads.

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