1.0 Cover Page

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2.0 Executive Summary

Musculoskeletal disorders (MSDs) have been the single largest component of nonfatal occupational injuries and illnesses in the United States [1]. In the mining industry, MSDs account for the highest number of occupational injuries [2]. Exposure to whole-body vibration (WBV) has been identified as a leading risk factor for the development of MSDs, especially low back pain (LBP) and neck pain [3]. There is still a lack of biological and physiological evidence to explain underlying injury mechanisms and causalities related to WBV exposures [3, 4], especially for mining vehicle operators who are exposed to WBV comprised of transient shocks and multi-axial components that may cause unique physiological stress compared to on-road vehicle drivers [5, 6]. Several studies have investigated potential injury etiology of work-related musculoskeletal disorders using biological markers in order to better understand the exact injury mechanism and causal relationships between WBV and MSDs; however, these studies were based on animal models and not related to vibration exposures [7, 8]. Therefore, the objective of this study was to employ validated biological markers to quantify the physiologic consequences of exposure to WBV in order to better understand WBV-related musculoskeletal injury mechanisms and exposure-response relationships. We also investigated whether physiological differences occur between exposure to vertical-axial dominant and multi-axial dominant WBV and whether biological and physiological stress can be attenuated by engineering interventions designed to reduce exposure to WBV. Using an innovative, interdisciplinary approach by combining our expertise in biomechanics and physiology, we have tried overcome limitations of those who came before us. As a result, we were able to successfully show feasibility of the proposed innovative approaches and complete the first research study to employ validated biological (human-model) markers to quantify the physiologic consequences of exposure to WBV.

In a laboratory-based study with a repeated-measures design, we played actual field-measured floor vibration profiles into a 6-degree-of-freedom motion platform (MB-E-6DOF/24/1800KG; Moog Inc.; East Aurora; NY) to create different types of realistic WBV exposures. The four vibration conditions were: 1) vertical-dominant vibration collected from long-haul trucks on a single-axial (vertical) passive suspension seat (VA), 2) multi-axial vibration collected from mining heavy equipment vehicles (haul trucks, bulldozers and scrapers) on a single-axial (vertical) passive suspension seat (MA), 3) multi-axial WBV exposure on a multi-axial active suspension seat (MI) and 4) no vibration (control condition; CC). Circulating biomarkers of interest were cortisol and catecholamines (epinephrine and norepinephrine) to assess physiological stress, interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα) to test for inflammation, thiobarbituric acid reactive substances (TBARS) to measure oxidative stress, and myoglobin and plasma creatine kinase to assess muscle damage. We collected blood samples at pre-exposure (0 hour), during-exposure (2 and 4 hours), and two hours into recovery after the WBV exposure (6 hours) in all four exposure conditions.

Overall, our results suggest a single, 4-hour acute exposure to WBV may not be sufficient to induce skeletal muscle damage or physiologic stress measurable in the blood. No significant differences were observed between conditions for any of the biomarkers that could be attributed to the interventions. These findings further indicate known complications of mining vehicle-related WBV exposure likely arise secondary to chronic, repeated exposures. Chronic exposure is likely to occur among miners, as a typical minining-vehicle driving shift can range from 8 to 12 hours. The lack of effects in these results are in-line with previous findings that MSDs develop from prolonged exposure to WBV and not from acute exposure to physical risk factors. Therefore, future studies should evaluate extended WBV exposure to determine whether chronic exposure

significantly affects biological stress markers and muscle activity differently than acute exposures. Furthermore, limited effects of WBV on muscle demage and physiological stress measures may also indicate the response to such exposures is subclinical (i.e., below the level of an overt clinical measurement). Futher studies employing unbiased assessment of circulating metabolites may be able to capture subclinical physiologic responses to WBV that give rise, over time with repeated exposure, to the development of clinical outcomes including MSDs and LBP.

3.0 Problem Statement and Objective

Mining vehicle operators suffer from a high prevalence of various injuries including musculoskeletal disorders (MSDs). Although a total number of mining injuries has significantly decreased over the past decades, the injuries associated with mining vehicle operation has increased. Consequently, mining vehicle-related injuries account for approximately 50% of occupational injuries [2]. The incidence rates of MSDs among mining vehicle operators is substantially higher when compared to administrative workers in mining industries [9]. Furthermore, MSDs account for the single largest component among all mining injuries [1]. Due to their severity and prevalence, the average cost of mining injuries and illnesses is higher than any other industries [2]. Therefore, there has been substantial economic burden on federal and local governments, the mining industry and mining workers due to worker's compensation claims, lost productivity, and reduced personal well-being of mining workers.

Previous studies have shown that mining vehicle operators incur a high level of WBV exposures [10, 11]. Exposure to WBV is a leading risk factors for MSDs, especially in the neck and low back regions [3-6]. The high level of WBV exposure among mining vehicle operators may explain the disproportionally high prevalence of MSDs among mining vehicle operators. Among two distinct components of WBV: a continuous oscillatory component from general operating conditions and a transient shock component from travelling over rough terrain, impulsive WBV exposures are more prominent in mining vehicles compared to on-road vehicles [10, 11]. These transient shock exposures are known to contribute to the degeneration of lumbar spine more than the continuous oscillatory component [12]. As many mining equipment operators operate vehicles for approximately 90% of their 12-hour shifts with limited breaks [13], it is more likely for them to be exposed to such transient shocks.

Moreover, while WBV exposures in on-road vehicles are predominantly in the vertical (z) axis, in off-road vehicles, the predominant WBV exposure axis is not necessarily limited to the vertical (z-axis) but can be either fore-aft (x-axis) or lateral (y-axis) as shown in **Figure 1** [14, 15]. Because of the substantial mass of the torso and head, such multi-axial components of WBV exposures can substantially increase the shear and rotational forces in the spine and associated muscle loads to counterbalance the inertia of the torso and head. The long periods of operating off-road vehicles can result in the overuse and damage to the soft tissues in the low back and neck regions, which is a known precursor of musculoskeletal injuries [16-19]. ***Therefore, off-road vehicle operators may be at greater risk for musculoskeletal injuries compared to on-road drivers whose WBV exposures are prominently continuous oscillatory on the vertical axis.***

As mining vehicle operators are exposed to more severe types of WBV exposures (transient shocks and multi-axial exposures), exposure-response relationships between WBV exposures and musculoskeletal stresses in mining vehicle operators are expected to be different from those in on-roach vehicle operators. Furthermore, because most previous WBV-related studies have been cross-sectional epidemiologic research (only few longitudinal studies), underlying mechanisms

and injury causalities have not been well documented. Some previous biomechanical studies have shown that exposure to WBV may elevate spinal load [20, 21], cause muscle fatigue in the supporting musculature [22], and is linked to the degeneration of the intervertebral discs and subsequent herniation [22, 23]. However, due to the study limitations including small sample sizes, unrealistic sinusoidal unidirectional (mainly vertical) vibration, and indirect measures, ***there is still lack of biological and physiological evidence to explain underlying injury mechanisms and causalities related to WBV exposures.***

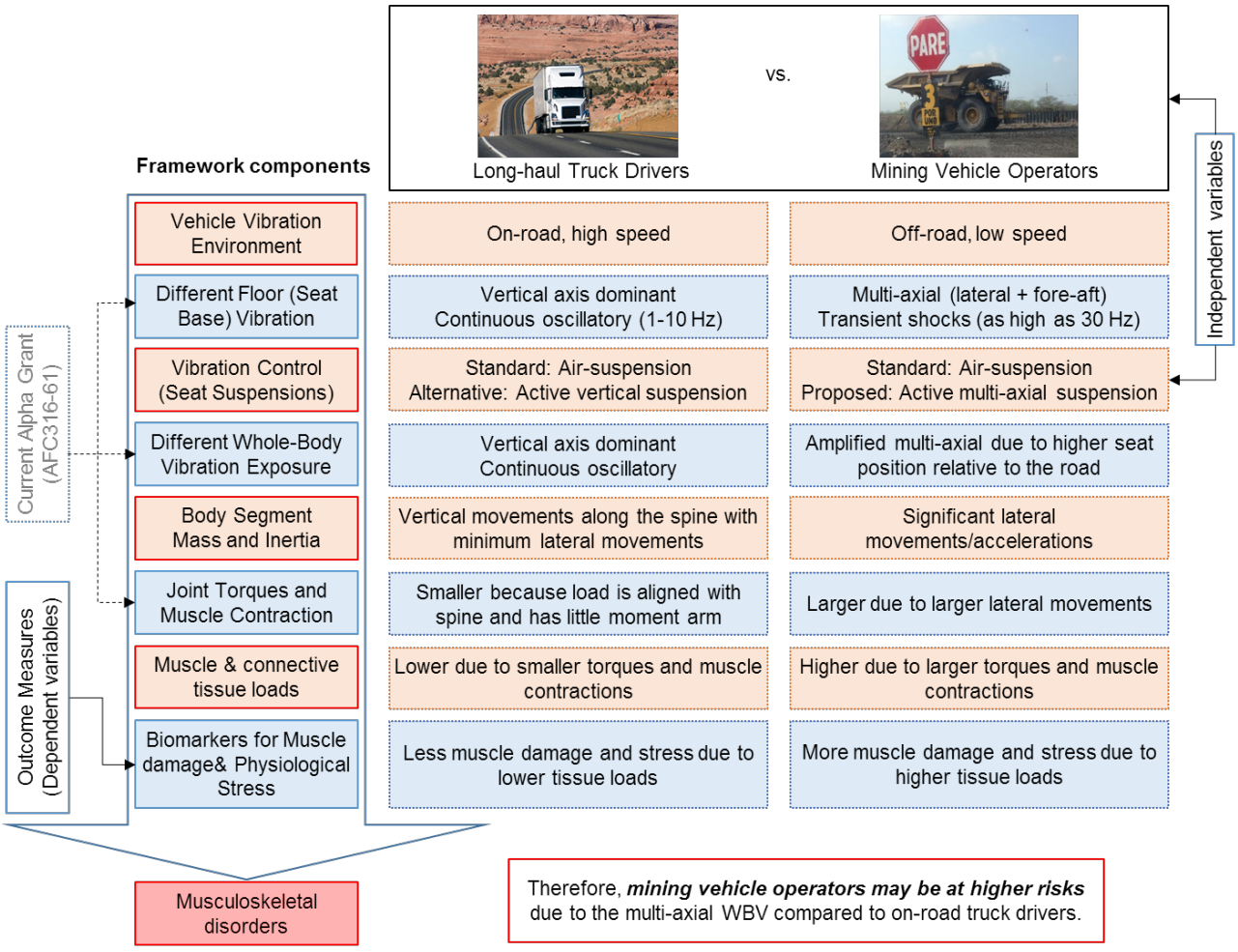

Figure 1. Conceptual Framework illustrating the effects of different vehicle vibration environment and vibration control on WBV exposures and physiological responses (adopted from on-going Alpha Grant: AFC316-61, PI: Kim). Comparisons between the two different vibration environments (long-haul trucks: vertical-axial dominant WBV vs. mining vehicles: multi-axial WBV) indicate that mining vehicle operators may be at higher injury risks.

To fill such research gap, our study objectives were 1) to quantify the relative impacts of different types of WBV exposures (vertical dominant vs. multi-axial WBV) on physiological stress, and 2) to determine whether engineering controls designed to reduce WBV lower physiological stress. Our central hypothesis was that exposure to WBV causes muscle damage,

oxidative stress, inflammation and other physiological stresses that can be detected by blood sampling. We also hypothesized that these physiologic stresses would be higher with multi-axial WBV exposure (common in mining vehicles) compared to vertical-dominant WBV exposure (common in on-road vehicles). These hypotheses were developed based on previous studies showing that repetitive muscle contraction and possible overuse increase muscle damage, oxidative stress, inflammation and physiologic stresses [24-26]. As multi-axial WBV exposure may significantly increase spinal loading and associated muscle loads to counterbalance the inertia of the torso and head, multi-axial WBV exposures can cause the overuse and damage to the soft tissues, especially in the low back and neck regions, which can be objectively measured by validated biomarkers. To achieve our research objectives, we completed a repeated-measures laboratory study using 15 subjects to quantify the effects of WBV exposures on biological and physiological stress in the following specific aims:

Specific Aim 1: Determine the relative effects of the multi-axial WBV exposure on muscle damage, oxidative stress, inflammation, and physiological stress compared to vertical-dominant WBV exposure. In a laboratory setting where actual field-measured vibration profiles were played into a motion platform, we collected blood samples to assay circulating biomarkers for muscle damage, oxidative stress, inflammation and physiological stress from three different exposure conditions with an industrial standard vertical suspension seat: (a) vertical dominant WBV exposure, (b) multi-axial WBV exposure, and (d) no WBV exposure (control) in **Figure 2**. In each condition, we collected blood samples at pre-exposure (0 hour), during-exposure (2 and 4 hours), and in recovery two hours after exposure (6 hours).

Hypothesis 1.1: Exposure to the WBV will increase the level of muscle damage, oxidative stress, inflammation, and physiological stress indicated by the biomarkers [compare (a) with (d) in Figure 2].

Hypothesis 1.2: Markers of muscle damage, oxidative stress, inflammation, and physiological stress levels will be higher with mining vehicles' multi-axial WBV exposures compared to on-road vehicles' vertical dominant WBV exposures [compare between (a) and (b) in Figure 2].

Specific Aim 2: Determine whether muscle damage, oxidative stress, inflammation, and physiological stress will be alleviated by a new engineering control (multi-axial active suspension seat). With the same research methods described in Aim 1, we collected blood samples to compare the levels of muscle damage, oxidative stress, inflammation, and physiological stress between two different engineering controls: a single-axial suspension seat (industry standard) and a multi-axial active suspension seat (newly developed intervention).

Hypothesis 2: The use of a multi-axial active suspension seat will reduce the effects of multi-axial WBV exposures on markers of muscle damage, oxidative stress, inflammation, and physiological stress [compare (b) and (c) in Figure 2].

4.0 Research Approach

This study was developed upon an existing research project (Alpha Grant: AFC316-61) that evaluates the effects of WBV exposures on biomechanical loadings on musculoskeletal systems of mining vehicle operators. In order to delineate underlying injury mechanism of WBV-related musculoskeletal disorders in mining vehicle operators, we added physiological stress measures onto our on-going study (Alpha Grant: AFC316-61) and evaluated how such biomechanical loadings (measured in our on-going research) affect muscle damage, oxidative stress, inflammation, and physiological stress.

In a repeated-measures laboratory study, we played actual field-measured floor vibration profiles onto a 6-degree-of-freedom motion platform (MB-E-6DOF/24/1800KG; Moog Inc.; East Aurora; NY) to create different types of WBV exposures (vertical-dominant and multi-axial WBV). During four different vibration exposure conditions (**Figure 2**), we collected WBV exposures per ISO 2361-1 WBV standard and blood samples to assay circulating biomarkers for muscle damage, oxidative stress, inflammation and physiologic stress. In each condition, we collected blood samples before exposure (0 hour), after 2 hours of exposure, after 4 hours of exposures, and in recovery two hours after exposure (6 hours). For the control condition (no WBV exposure), subjects were restrained on the same testing seat without WBV for 4 hours. The order of all conditions were randomized and counter-balanced across subjects to avoid any systematic bias due to the experimental order.

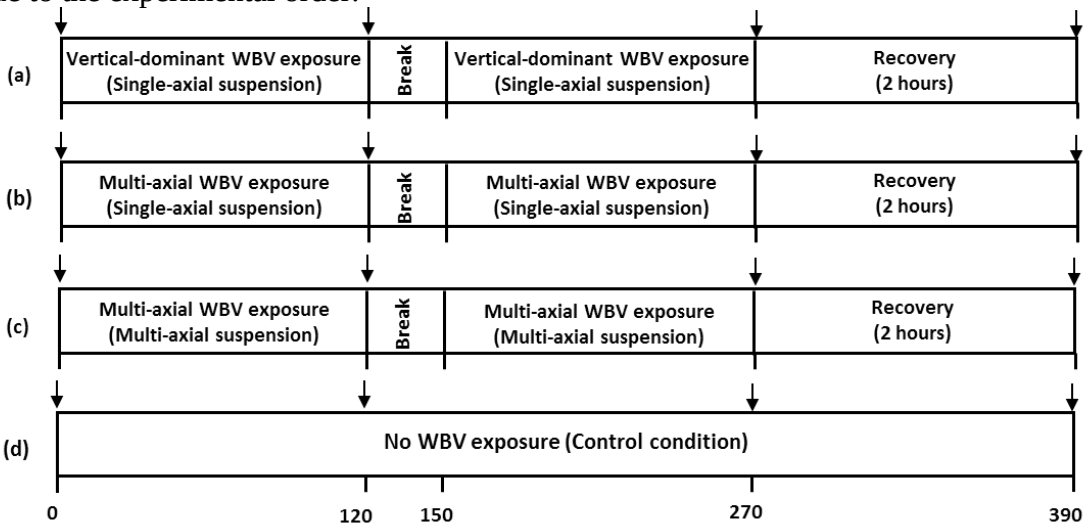

Figure 2. Four experimental conditions: (a) Vertical-dominant WBV exposure with a single-axial suspension seat, (b) Multi-axial WBV exposure with a single-axial suspension seat, (c) Multi-axial WBV exposure with a multi-axial suspension seat, and (d) No WBV exposure (control). Arrows denotes blood sampling. Each subject will participate in all four conditions and the order of conditions will be randomized and counterbalanced.

4.1 Subjects

Fifteen subjects (13 males and 2 females) were recruited via email solicitation and printed flyers. Their average (SD) age, height, and weight were 30 (8) years, 176.6 (4.4) cm, and 88.6 (26.1) kg, respectively. Eligibility criteria consisted of no pain in neck, shoulder, and back regions (over past 7 days); no history of musculoskeletal disorders; a minimum of one year of driving experience; no pregnancy. These criteria were determined to avoid any potential adverse health effects from being exposed to field-measured vibration for four hours. The experimental protocol was approved by the University's Institutional Review Board and all of the participants signed the consent form prior to the experiment.

4.2 Vibration Simulation

A 6-degree-of-freedom motion platform was used to recreate two different types of actual field-collected vibration profiles in the laboratory. This large scale 6-DOF motion platform consists of 6 electric linear servo actuators which can replicate the same vibration exposure

measured in the field. As this motion platform is based on electro linear servo actuators, it provides much greater precision and repeatability in motion control as compared to hydraulic actuator-based motion platforms.

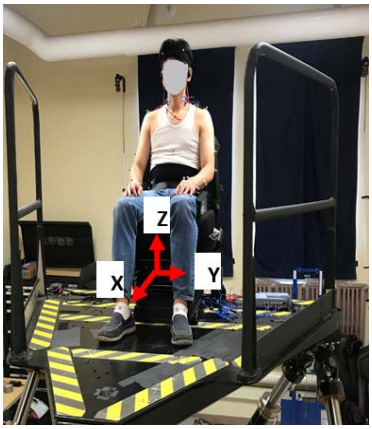

Figure 3. Motion platform

For the multi-axial WBV exposures (exposure condition (b) and (c) in Figure 2), the vibration data profiles were chosen from tri-axial vibration data collected from 38 vehicles (11 different vehicle types) with 123 mining equipment operators [17]. The multi-axial vibration profiles used in this study were selected in order to have significant lateral (Y-axis) vibration that reflects the average WBV parameters in the previous study by Marin et al. [17]. For the vertical-axial dominant vibration exposures, the most representative tri-axial acceleration data collected from long-haul trucks [10] was selected to reflect the average WBV parameters of 105 long-haul trucks. The ISO 2631-1 WBV parameters for two input vibration exposures are shown in **Figure 4**. The selected field-measured vibration profiles were iteratively brick wall filtered and converted to displacement data by integration. The displacement data were imported (Replication software; Moog Inc.; Aurora, NY) to reproduce the same accelerations on the motion platform.

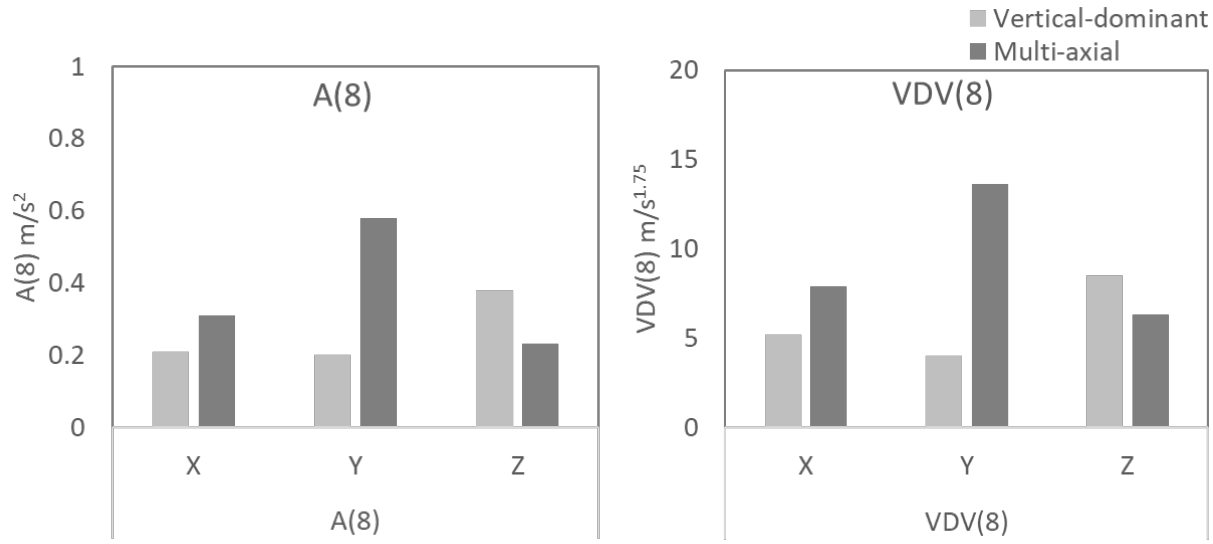

Figure 4. ISO 2631-1 WBV parameters of two input vibration profiles: vertical-dominant vibration collected from on-road semi-trucks and multi-axial vibration collected from off-road mining vehicles. A(8) is root mean square weighted average vibration normalized to 8 hours; VDV(8) is vibration dose value normalized to 8 hours.

4.3 Measures

Whole body vibration: While subjects were exposed to the actual field-measured vibration on the motion platform for 4 hours, we collected raw un-weighted acceleration data at 1,280 Hz using an eight channel data recorder (DA-40; Rion Co. LTD; Tokyo, Japan) with a tri-axial seat-pad accelerometer (Model 356B40; PCB Piezotronics; Depew, NY) mounted on the testing seats and

a tri-axial accelerometer (Model 352C33; PCB Piezotronics; Depew, NY) magnetically mounted to the floor of the motion platform per ISO 2631-1 WBV standard. The raw unweighted WBV data were processed and analyzed using a custom-build LabVIEW program (v2016; National Instruments; Austin, TX) to calculate WBV exposure parameters per ISO 2631-1 and 2631-5 standards. The ISO WBV exposure parameters included root mean square weighted average vibration (Aw); vibration dose value (VDV), which is more sensitive to impulsive vibration and reflects the total vibration, as opposed to average vibration; and static spinal compression dose (S_{ed}). These values were normalized to 8 hours: A(8), VDV(8), and $S_{ed}(8)$

Biomarkers: We collected blood samples to assay circulating biomarkers for muscle damage, oxidative stress, inflammation and physiologic stress from the four different conditions. In each condition, we collected blood samples before exposure (0 hour), after 2 hours of exposure, after 4 hours of exposures, and in recovery two hours after exposure (6 hours). Blood samples were collected from an indwelling intravenous (IV) catheter placed in the antecubital vein and kept patent with a saline lock. IV catheter placement occurred 30 minutes prior to the first sample collection. At each sampling time, 10 ml of venous blood was collected into tubes containing K_2-EDTA (for plasma), EGTA-glutathione (for catecholamine analysis), or serum clot activators. Blood collection tubes were centrifuged at 1000 x *g* at 4°C for 10 minutes to separate plasma or serum and stored at -80°C until analysis.

To reduce variability and/or bias in biomarker metrics, physical activity, diet and timing of the experimental visits were controlled. Participants arrived for study visits after an overnight fast (i.e., 10 hours with no food or drink other than water). Between the experimental conditions (study visits), a minimum of 48 hours were allocated to minimize any residual biological damage or fatigue effects from the prior experimental condition. Furthermore, subjects were asked to refrain from any moderate-to-vigorous physical activity for 48 hours preceding each study visit, and to communicate any unexpected/novel physical activities (e.g. new workplace or leisure time activities) during the course of their participation in the study. Finally, to control for any circadian influence on the biomarkers, the experimental condition trials began at the same time of day for each subject (~8am).

Circulating biomarkers included myoglobin and creatine kinase (muscle damage), thiobarbituric acid reactive substances (oxidative stress), interleukin-6 and tumor necrosis factor-α (inflammation), catecholamines and cortisol (physiological stress). The selected biomarkers were chosen to provide comprehensive analysis of muscle damage and other relevant physiological stresses. Due to limited existing knowledge regarding the effect of WBV exposures on circulating stress markers, the justification for the selected markers was largely derived from previous evidence in studies of muscle contraction and exercise-induced stress. Myoglobin and creatine kinase were measured because they are released from muscle in response to damaging contractile activity and impaired muscle cell integrity (indicating significant muscle damage) [29]. Interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα) were measured because they are proinflammatory cytokines that are released from muscle during contractile activity [29, 30]. Thiobarbituric acid reactive substances (TBARS) were measured to indicate systemic redox balance (i.e., oxidative stress), which is influenced, in part, by the increased production of reactive oxygen species (ROS) during muscle contraction [31]. Catecholamines (i.e., epinephrine and norepinephrine) and cortisol were measured to assess general physiological stress. Together, these measures provided a comprehensive analysis of physiological stress induced by WBV exposures and the ability of engineering controls to attenuate the stress response. All assays were performed

using commercially available kits according to manufacturer recommendations. Myoglobin, IL-6, TNFα and cortisol were assayed via ELISA (Abcam, Cambridge, MA). Creatine kinase activity (Abcam) and TBARS (Cayman Chemical, Ann Arbor, MI) were measured via colorimetric and fluorometric assay, respectively. Catecholamines were measured by the Vanderbilt Hormone Assay & Analytical Services Core (Nashville, TN) via high performance liquid chromatography.

4.4 Data Analysis

The primary dependent variables were myoglobin and creatine kinase; interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα); thiobarbituric acid reactive substances (TBARS); and catecholamines (epinephrine and norepinephrine) and cortisol. As our on-going study (Alpha Grant: AFC316-61) analyzes WBV exposure thoroughly, the WBV exposure measures in this study (8-hour equivalent average vibration [A(8)]; vibration dose value [VDV(8)], and static spinal compression dose [$S_{ed}(8)$] were considered secondary dependent variables. Normality of the raw and log-transformed primary outcome variables were carefully assessed through a combination of evidence from graphical methods, statistical tests for normality, and evaluation of shape parameters such as skewness and kurtosis coefficients. For graphical methods, frequency distributions (histograms) and quintile-quintile plots were used for evaluating normality visually. Shapiro-Wilk test was used for normal normality tests. Lack of symmetry (skewness) and pointiness (kurtosis) was inspected by transforming the two coefficients to z-scores [14]. Based on the normality test and large number of observations (4 condition x 4 repeated measures x 14 subjects = 224 observations), generalized linear mixed models (GLMM) were used to determine the effects of exposure conditions, measurement time, and interaction of these two fixed effects on the corresponding outcome variables to test our hypotheses. Random intercepts were introduced to account for within-subject correlations. Alpha level of 0.05 was used as a significance threshold in all hypothesis tests.

5.0 Results, Summary of Accomplishments, Conclusions and Impact Assessment

5.1. Results and Discussion

The aim of this research was to determine the relative effects of WBV exposures on physiological stress in order to identify causal relationships and clarify existing exposure-response relationships between WBV and MSDs. The results indicate that acute exposure to both vertical-dominant and multi-axial WBV (at the amplitudes and durations tested here) did not result in significant changes in bloodborne markers of muscle damage or physiologic stress (p's > 0.33).

Skeletal muscle damage was evaluated by testing for systemic circulation of creatine kinase and myoglobin as they are widely used as clinical measures of muscle damage [29]. The presence of these proteins in the blood can be used to indicate a response to significant challenges to skeletal muscle, such as repeated eccentric muscle contractions, or even myocardial infarction [27, 28]. As shown in Figure 5, creatine kinase showed little change in all four exposure conditions over time ($p = 0.33$), whereas myoglobin was significantly lower after 4-hour exposure to WBV in all conditions ($p = 0.02$). Despite achieving statistical significance, neither biomarker indicates clinically meaningful changes (e.g., no difference compared with control condition [CC]), thereby ruling out the possibility of overt muscle damage in response to the exposure conditions. One important consideration is that the rise in circulating concentration of these enzymes can be delayed following skeletal muscle damage, often peaking 24 to 72 hours following the damaging event [28]. However, it is unlikely that such delayed onset of these markers was present in our study given the relatively modest stress of the WBV exposure, the absence of any change in these

markers during each trial, and the lack of any increase in baseline measures in subsequent trials (0 hour). This suggests that that 2-4 hours of WBV exposures tested here may not be long or severe enough to cause measurable level of gross skeletal muscle damage; however, our data do not rule out the possibility of less severe muscle damage that would not be captured by these biomarkers.

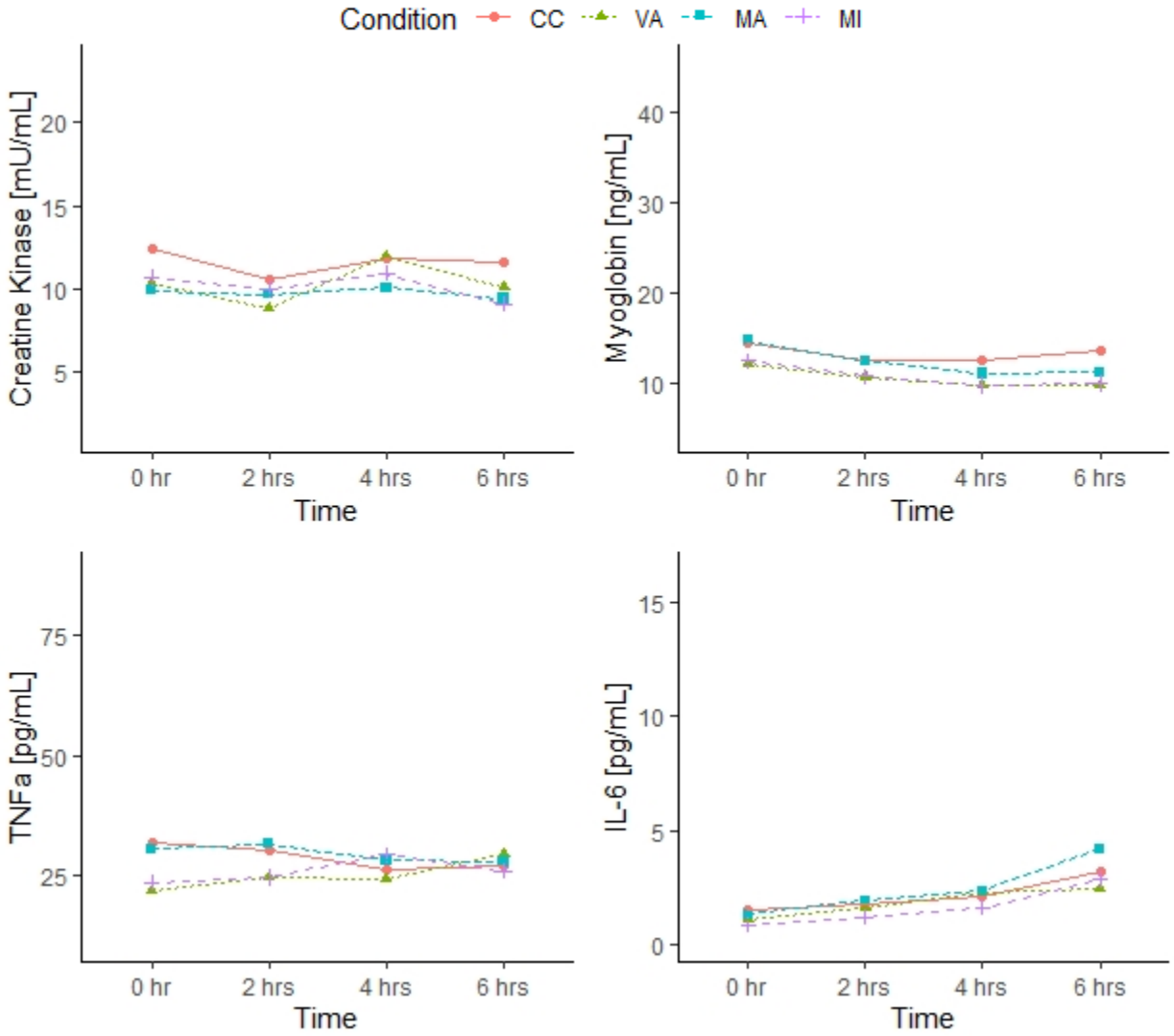

Figure 5. Comparisons of biological markers indicating muscle damage (Creatine Kinase and Myoglobin) and inflammatory response (tumor necrosis factor alpha: TNFα and interleukin-6: IL-6) among four different WBV exposure conditions over time: No-WBV control condition (CC), Vertical-dominant WBV exposure with a single-axial suspension seat (VA), Multi-axial WBV exposure with a single-axial passive suspension seat (MA), and Multi-axial WBV exposure with a multi-axial active suspension seat (MI). Error bars are omitted for clarity.

Stress-induced cytokines were also examined, including tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6). Both TNFα and IL-6 have significant immunomodulatory actions, with both increasing in response to infection and chronic disease [29-31]. Such chronic elevation in IL-6 and TNFα are generally considered adverse (i.e., chronic low-grade inflammation) [32]. However, both can be released by skeletal muscle in response to contractile activity [25]. Our

results showed that there were significant two-way interactions (Condition x Time) in TNFα measures ($p = 0.007$). That is, the time-dependent changes in TNFα varied between the exposure conditions. After the first 2-hour exposure to WBV (VA, MA, and MI), TNFα level slightly increasesd, while it decreased in no-WBV conntrol condition (CC). Also, TNFα decreased during 2 hours of recovery period (between 4 and 6 hours) in the MI condition (multi-axial WBV exposure with a multi-axial active suspension seat whereas an opposite trend was observed in the VA condition (vertical-dominant WBV exposure with a single-axial suspension seat). Nevertheless, all measures of TNFα were near the limits of detection of the assay and such changes would not be considered clinically significant. Serum IL-6 content was increased in all trials as a function of time. Because the increase in IL-6 also occurred in the control condition (without WBV exposure), it is likely that this reflects known circadian release of IL-6 rather than an effect of the intervention [33]. Previous studies have also found release of IL-6 from muscle is dependent upon on intensity of contractile activity, with little to no increase during lower-intensity exercise [34].

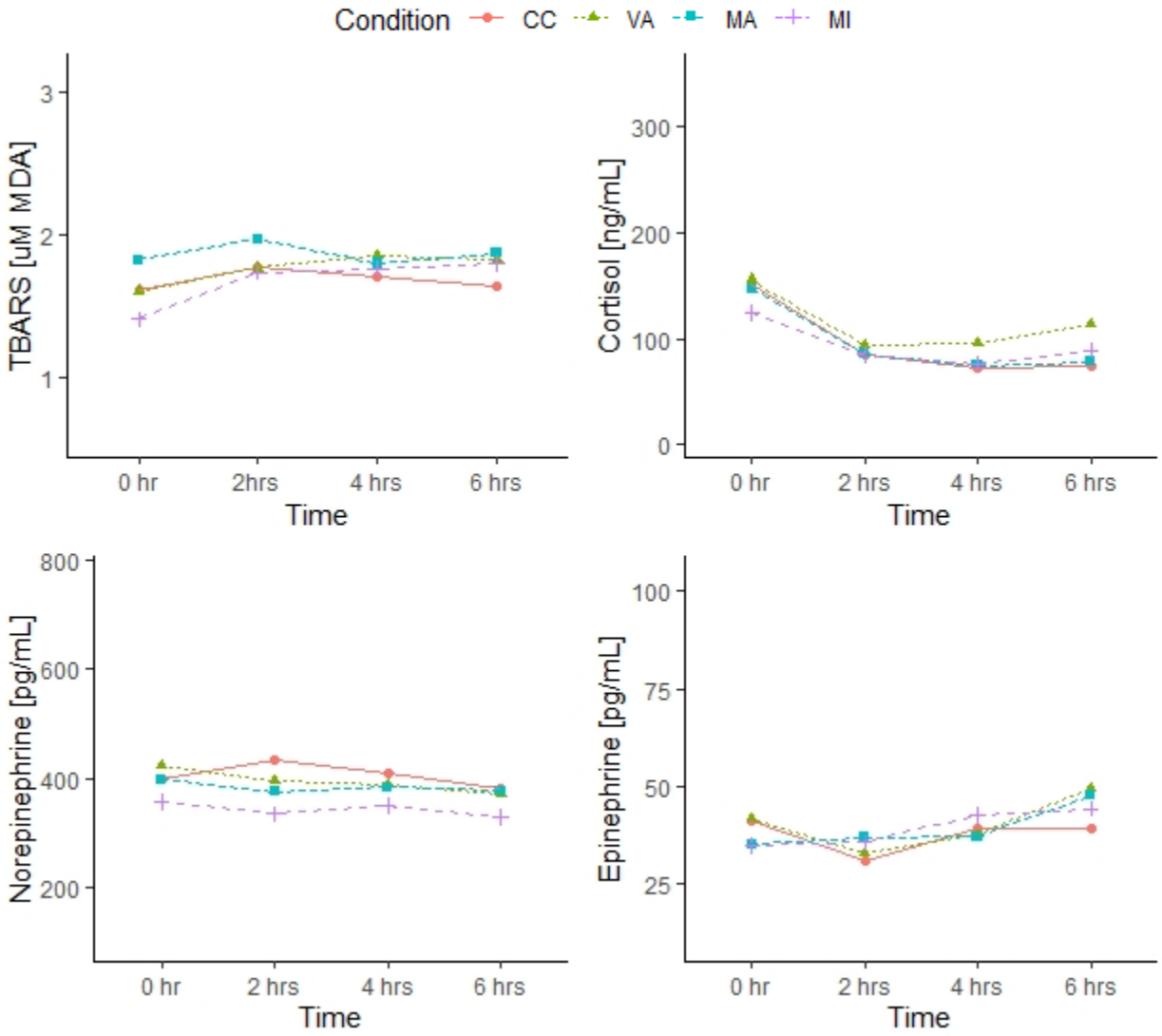

Figure 6. Comparisons of biological markers indicating oxidative stress (thiobarbituric acid reactive substances: TBARS) and physiological stress (cortisol, norepinephrine, and epinephrine) among four different WBV exposure conditions over time: No-WBV control condition (CC),

Vertical-dominant WBV exposure with a single-axial suspension seat (VA), Multi-axial WBV exposure with a single-axial passive suspension seat (MA), and Multi-axial WBV exposure with a multi-axial active suspension seat (MI). Error bars are omitted for clarity.

Lipid peroxidation was examined via measurement of thiobarbituric acid reactive substances (TBARS), as an indicator of oxidative stress. Muscle contraction is acutely associated with increased production of reactive oxygen species (ROS) and thereby potential for oxidative damage [35]. Similar to chronic low-grade inflammation, chronic oxidative stress is generally considered an adverse event in the disease state [36]. An increase in TBARS can be used to indicate increased oxidative stress and thereby potential for oxidative damage to other molecules, such as cell membranes, proteins, and DNA [35]. However, our data showed that there were no changes in TBARS measured in response to WBV ($p = 0.46$) (Figure 6), suggesting no marked changes in oxidative stress during WBV.

Cortisol and Catecholamines were measured as indicators of overall physiologic stress. Cortisol is a steroid hormone which is released in response to stress. Although cortisol is increased during intense or prolonged exercise, it is also increased in response to numerous stressors and is often considered a clinical indicator of overall stress [37]. Cortisol exhibits a well-described pattern of circadian release, peaking shortly after waking in the morning and decreasing thereafter, as reflected in our data [38, 39]. As shown in Figure 6, cortisol decreased during all trials including the no-WBV control condition ($p = 0.009$), reflecting known circadian lowering of cortisol following peak early morning release [38, 39].

Both epinephrine and norepinephrine increase in response to exercise in an intensity-dependent manner, with increasing intensity or duration of exercise resulting in increased concentrations of both biomarkers [40]. These responses are necessary for physiologic regulation in response to muscle contraction and exercise (e.g., heart rate, blood flow and pressure, nutrient availability) and are thus good markers for the general physiologic stress induced by the WBV exposures. We observed a modest trend for increased epinephrine concentrations in response to WBV exposures (p's > 0.05), possibly indicating a mild stress response. However, overall our data do not indicate any significant stress induction in response to the acute WBV exposures.

This study is limited in that it does not represent the long driving hours (8-12 hours a day) typical of professional drivers; only 4 hours of WBV exposure was tested here. Moreover, the level of WBV exposures used in this study does not reflect peak exposures (i.e., the most severe exposures) as only the 25^{th} to 75^{th} percentile of the actual exposure levels that mining vehicle operators experienced in the field were tested here. Hence, it will be merited to evalaute the effects of more realistic exposures (e.g., longer and higher exposures) in biological markers for muscle damage, oxidative stress, and phyiological stress. Additionally, the biolocial markers used in this study are largely clinical indicators, used to identify significant muscle damage and physiological stress. This may have resulted in the lack of clinically significant effects of a single bout of WBV exposure. Hence, it remains likely and important to characterize subclinical responses to (more robust and representative) acute WBV exposures that give rise, over time, to chronic MSDs.

Overall, our results do not support our original hypotheses and, instead, suggest that a single, acute exposure to WBV may not induce significant skeletal muscle damage or physiologic stress measurable in the blood (with biomarkers tested here). These findings can be interepreted to indicate known complications of mining vehicle-related WBV exposure may arise secondary to chronic, repeated exposures. The lack of acute effects on clinical markers demonstrated here are in line with previous findings that MSDs develop from prolonged exposure to WBV and not from

acute exposure to physical risk factors [23]. Previous studies note that accumulation of muscular stress during long drives may lead to an increased risk of musculoskeletal disorders [3]. Nevertheless, an equally important consideration is that repeated, subclinical responses to acute WBV were not captured here and the levels of WBV exposure were relatively modest. Taken together, future studies should evaluate extended WBV exposure duration and severity to determine whether more significant and representative WBV exposures give rise to measurable changes in subclinical physiological stress that may contribute to risk and progression of chronic MSDs.

5.2 Summary of Accomplishments and Conclusions

A main accomplishment is that this study was the first to employ measurement of validated biological markers in human subjects to quantify the physiologic consequences of exposure to WBV in order to better understand WBV-related musculoskeletal injury mechanisms and exposure-response relationships. This study has demonstrated feasibility of measuring biological markers associated with a physical exposure (i.e., WBV), which will provide a strong and unique research capacity that allows objective quantification of physiologic responses to various physical exposures in future studies.

Second, by quantifying the relative impact of different types of WBV exposures (vertical dominant vs. multi-axial) on physiological stress, we were able to fulfill the first objective of this study. Overall, our results do not support our original hypotheses and, instead, suggest an acute (2-4 hours) exposure to WBV may not be sufficient to induce significant skeletal muscle damage or physiologic stress measurable in the blood. These findings further indicate known complications of mining vehicle-related WBV exposure likely arise secondary to chronic, repeated exposures. The lack of effects in these results are in line with previous findings that MSDs develop from prolonged exposure to WBV and not from acute exposure to physical risk factors [15]. Previous studies note that accumulation of muscular stress during long drives may lead to an increased risk of musculoskeletal disorders [3]. However, as mentioned earlier, the limited effects of WBV exposures on the meausred biological markers may be due to the study limitations in exposure (level and duration) and selected biological markers; hence, it does not factor out the possibility of subclinical changes in response to acute WBV exposures contribute to development of chronic MSDs. Therefore, future studies should evaluate subclinical responses to more robust and representative) WBV exposures that likely contribute to development of chronic MSDs.

To fulfill the second objective of this study, we determined whether engineering controls designed to reduce WBV lower physiological stress were successful by comparing the MI and MA conditions. Our results showed that no significant differences were present between the MA and MI exposure conditions for any of the circulating biomarkers of interest. Therefore, the multi-axial active suspension seat was no more effective in lowering physiological stress caused by multi-axial vibrations compared to the standard passive suspension seat. This is in part due to the lack of significant changes in circulating biomarkers across all four exposure conditions.

In conclusion, this study was the first to use the human-based biological stress markers to objectively quantify physiological stress, muscle damage, and inflammatory responses associated with WBV exposures. The results indicate that an acute (2-4 hours) exposure to WBV may not be sufficient to induce skeletal muscle damage, inflammation, or physiologic stress as measurable by the bloodborne markers tested here. In future studies, it will be meried to evaluate subclinical changes that may be more important for the development of chronic MSDs using WBV exposure

which leveles and duration reflects more realistic occupational exposure (e.g., equivalaent to 8-12 hour WBV with peak exposures).

5.3 Impact Assessment and Future plans

Although previous studies have demonstrated the association between WBV and MSDs (especially, LBP), the exact injury mechanism and causal relationships are not fully understood. Hence, the main objective of this study was to identify causal relationships and clarify existing exposure-response relationships between WBV and LBP by evaluating how the exposure to occupational WBV affects muscle damage and biological stress measured by biological markers. The results did not show any clear causal relationship or exposure-response relationships. Although the results did not support our hypotheses, they are important physiological evidence that support a well-known premise that MSDs develop from prolonged exposure to physical risk factors (e.g., WBV) and not from acute exposure [15]. Moreover, a major scientific contribution of this study was demonstrated feasibility of measuring biological markers associated with a physical exposure (i.e., WBV), which will provivde strong reaerch capacity that allows us objectively quantify validated biological markers with various physcial exposures in future studies. Based on our study findings and limitations, as well as demonstrated feasibility of the overall approach, we plan to develop subsequent studies that evalaute subclinical physiological responses to WBV of increased duration and intensity.

Increased duration and intensity of WBV are needed to accurately model field-based exposures

The principal limitation of the current study is that the duration and intensity of WBV exposures tested were less than that which represent the typical daily exposure of mining vehicle operators. Though we employed actual field-based WBV measures in our model, the duration of exposure (4-hours) was markedly less than the typical 8-12-hour daily exposure common among mining vehicle operators. Furthermore, the level of WBV exposures used in this study does not reflect peak exposures (i.e., the most severe) as the exposure ranged from 25^{th} to 75^{th} percentile of the actual exposure levels that mining vehicle operators experienced in the field. Taken together, the acute exposures employed in the present study may not have been sufficient to accurately model field-based WBV exposures and thereby characterize the respective physiologic stress.

Assessment of subclinical physiological responses to WBV are needed

The current study employed measurement of validated biomarkers commonly used to identify clinically significant changes in muscle damage and physiologic stress. We demonstrated that the WBV exposures tested here did not result in overt muscle damage or significant physiological stress. This finding is a strong first-step toward achieving our aim of identifying exposure-response relationships that contribute to increased risk for MSDs in mining vehicle operators. Importantly, however, it remains likely that such WBV exposures are inducing subclinical physiologic stress that simply was not captured by changes in the selected biomarkers (i.e., clinical markers). Indeed, most chronic health conditions, including MSDs and LBP, likely arise as a function of repeated, acute subclinical exposures that accumulate to ultimately achieve clinical outcomes. An important next step will be to identify such subclinical physiologic responses to WBV exposures, as they can be expected provide critical insight into possible mechanisms of progression contributing to LBP.

Future plans to meet such needs

WBV exposure: Because this study was the first study that employed the actual field-measured vibration for relatively long exposure duration (4 hours), we chose the interquartile ranges of the field-measured WBV exposures in order to avoid any potential injuries from the exposures.

However, given the limited physiological responses to the WBV exposures used in this study, our subsequent studies will use more robust and representative WBV exposure that reflects 8-12 hour exposures and real-word intenstity by including peak exposures (i.e., the 90-95th percentile values of real WBV exposure that mining vehicle operators experience).

Subclinical measures: Advances in mass spectrometry have led to development of powerful metabolic screening platforms capable of quantifying thousands of metabolites in blood and tissue samples – often referred to as "metabolomics". Metabolomic analysis is increasingly being used for unbiased assessment of the consequences of a given condition to provide insight to possible mechanism of disease. For example, investigations using metabolomics have helped to identify numerous molecules released by skeletal muscle in response to muscle contraction and exercise. This includes amino acids [41, 42], which have been implicated as critical regulators of immune function [43, 44]. We propose to use metabolomic analysis to provide new and much needed insight into the subclinical consequences of WBV exposures in humans. Such analysis could be expected to identify many small yet important changes in circulating metabolites in response to acute WBV exposures (e.g., immunomodulatory amino acids) that with repeated exposure may contribute to the risk for LBP and other chronic MSDs.

6.0 References

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7.0 Appendices

8.0 Acknowledgment/Disclaimer

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