THE INFLUENCE OF EXTERNAL LOAD DURING HIKING ON MARKERS OF JOINT STRESS AND MOVEMENT EFFICACY

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Exercise and Nutrition Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

January 2022

ACKNOWLEDGEMENTS

The author thanks Dr. David Graham, and Dr. Ron June for their input and feedback on this project to make it possible. A special thanks to Dr. Mary Miles for her support and mentorship in accomplishing this research during a very trying 2021.

The author thanks Hunter Fausset and Brian Bothner from Montana State University for their discussion and assistance with blood metabolite measurements and identification.

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ABSTRACT

PURPOSE: Complications to lower extremity joints, including injury and inflammation, are prevalent issues that arise during prolonged external load-bearing exercise. Metabolomic blood analysis can provide insight into the metabolic processes that occurs during this type of exercise. METHODS: Eight healthy, active men and women participated in a series of blood sample collections and motion capture recording before and after completing a 7.2-mile hiking protocol under two separate conditions. Blood was collected prior to hiking, 15-20 minutes after hiking, 8-hours after, 24-hours after, and 48 hours after. Movement coordination and efficacy was measured with a motion capture system while performing the y-balance test and an obstacle clearance task. Hiking conditions were randomized as backpacking with 20% of body mass external load or daypacking with minimal external load for each participant and separated by two weeks. Serum was analyzed to detect differences in metabolite upregulation between conditions. Biomechanical data were analyzed for inter- and intra-differential values relevant to fatigue between conditions. RESULTS: Analysis found clear differences between conditional metabolite upregulation at all post-hike timepoints. The upregulation of cortisol was significant in backpacking conditions at the post-hike timepoint. Glycerophospholipids were significantly upregulated in backpacking at 8-hours post-hike All significant metabolite upregulation switched to daypacking conditions at 24-hours post-hike. Significant metabolite upregulation varied between conditions at 48-hours post-hike. The only findings of significance in movement coordination and efficacy were between the y-balance lateral leg movement. CONCLUSIONS: The presence of cortisol is consistent with the physiological and mental stress of external load-carriage and alludes to exposure that can lead to decreased bone mineral content. Glycerophospholipid metabolism pathways play an important role in joint degradation, which could explain their upregulation in backpacking conditions. Ceramide, omega-3s, and fatty acid/triglyceride cycling are functions of cell proliferation and turnover which may be upregulated with more efficiency in daypacking conditions. Upregulation of anserine at 48-hours post-hike in daypacking supports the idea of more efficient exercise recovery occurring in this condition. While significant differences were not clear in motor control measures, the findings show potential reliability for future study designs.

CHAPTER ONE

INTRODUCTION

Development of the Problem

Several recreational and occupational pursuits involve the transport of external loads through the use of various forms of baggage, though this primarily involves the use of backpacks. Lower body joint degradation and discomfort is one of the leading issues that arise from these load bearing pursuits while traversing varied terrain (Gentile et. al. 1992). Such activities typically encompass backpacking, wildland firefighting, mountaineering, and military operations among others. These forms of physical exercise typically result in either acute traumatic injury during the course of the activity or gradual overuse that results in dysfunction of connective tissues, particularly knee joints (Purchio, 2017). Traumatic injuries tend to be the leading consequence of slips, trips, or falls while performing in the field (Britton et. al., 2013), and tend to result from a loss of neuromuscular control while hiking over prolonged periods (Orr et. al., 2015). This loss of control can often be attributed to central fatigue, exercise-induced influence on the central nervous system (CNS) decreasing performance (Meeusen et al., 2006), or peripheral fatigue, a reduction in force-generating capacity induced by skeletal muscle work (Ce et. al., 2020), resulting in decreased neuromuscular motor control functions. This loss can be attributed to factors associated with principles of burnout, gradual depletion from high amounts of stress (Gerber et. al., 2013) and/or insufficient physical preparation through exercise training (Chiu & Barnes, 2003). This is an important consideration due to the enormous demand required of external load bearing activities, particularly over steep and uneven terrain which could lead to injury and possibly subsequent death. Overuse injuries more commonly take place over the course of years doing these activities and typically result in chronic joint inflammation or discomfort and can exacerbate underlying conditions, such as osteoarthritis in those susceptible to it (Johnson & Hunter, 2014). While it is understood that many injuries, occurring both acutely and over time, are a result of either overuse or insufficient physical preparedness, simultaneous investigation of tissue damage and fatigue resulting from load carriage may elucidate the influence of fatigue on tissue trauma.

A way of examining the influence of additional external loading is to measure markers of damage/inflammation in the blood, however the main focus of this approach has generally been to examine cytokines released due to chronic conditions such as osteoarthritis (Miller et. al., 2014). More recently, metabolomic analysis of blood samples has been extended to study indicators of other chemical processes within the body as a whole and range of different tissues. This has provided an important development allowing conceptual frameworks from studies of osteoarthritis to extend to the role metabolites play on joint degradation and inflammation. Laboratories have been able to study and analyze synovial fluid in animal models in order to map out morphological changes associated with arthrodegenerative conditions (Ma et. al., 2017). Using established techniques evaluating metabolomic biomarkers presents an opportunity to develop a deeper understanding joint health and metabolism, particularly during periods of high joint stress such as carrying large external loads. The outcome of this approach could lead to greater understanding of the mechanisms related to joint degeneration and joint disease.

Hiking with external loads is known to cause an increased release of inflammatory blood markers, particularly an array of cytokines associated with joint health and stress. This response is especially exaggerated in individuals susceptible to chronic arthro-degenerative conditions such as osteoarthritis (Daghestani & Kraus, 2015). However, there is little research associated with blood metabolomics and what specific markers could be associated with not only general joint inflammation, damage, or discomfort, but also which of these markers increase levels during this particular form of exercise. While the initial post-hike response may include release of these markers, the time course changes are unknown. Specifically, the inclusion of several blood draw time points allows the detection of metabolites that may circulate at separate times, otherwise missed if performing simple pre-/post-testing draws.

In addition to this biochemical approach to evaluating the role joint stress induced by carrying external loads, it is also possible to evaluate the consequences of carrying external loads by evaluating movement coordination and efficacy. The fields of biomechanics and motor control both seek to evaluate movements such as balance and decreased motor function and can determine the loss of movement efficacy related neuromuscular fatigue (Johnston et. al., 2019). In particular the Y-balance test, provides a means to evaluate lower limb stability and coordination, importantly tis metric is also capable of predicting risk of injury. The use of the Y-Balance test is reported in populations regularly carrying large external loads for potentially long durations, for example, Ritland et. al. (2012) demonstrated the relative performance of special warfare operatives before, during, and after participating in load carriage marches. Importantly, evaluation both the movement capacity and the biochemical markers of joint stress may develop a more comprehensive understanding of heavy or extended carriage of external loads. Therefore, the use of these measures in conjunction with blood metabolites could help explain the increased or maintained levels of inflammatory metabolites post load carriage hike. The use of biomechanical and motor-control testing protocols allows researchers to measure compensations and indicators of fatigue to confirm that participants are physically and metabolically taxed. The use of joint-health history questionnaires and anthropometric measures should allow for an explanation for any readings that may show abnormal metabolite counts when compared to the rest of the population. Previous studies have observed fatigue through motor control testing by itself as well as metabolomic measures in chronic conditions, but this study will utilize both techniques and protocols to determine differences between load bearing and non-load bearing exercise.

Purpose

The aim of this study is to examine the differences in biomechanical and blood metabolite measures between external load bearing and non-load bearing conditions while hiking over varied terrain to identify potential biomarkers specific to bone and joint stress. While the use of metabolomic analysis has shown promise in measuring metabolic processes that occur systemically and within joints in recent years, the identities of such markers are lacking. Furthermore, an understanding of changes in neuromuscular control may give correspondence to biochemical changes and provide a practical method of testing individuals on their susceptibility to joint damage, inflammation, or general pain.

Implications

The outcome of this work is intended to provide a better understanding of blood metabolites and time course of change after load bearing exercise performed in the field. The combination of determining risk due to family history, exercise history, level of fatigue post-protocol, and greater presence of inflammatory metabolites may be used to determine an individual’s risk of injury during these activities. The discovery of new biomarkers, specifically metabolites, will allow for future research into metabolic processes that affect joint health and degradation.

Hypothesis

It is hypothesized that higher joint stress caused by added external load compared to non-load-bearing conditions will elicit increases in metabolites relevant to joint stress and joint degeneration over time. It is also hypothesized that biomechanical/motor control measures will positively correlate with presence of inflammatory metabolites, indicating level of fatigue.

Delimitations

1. The study is restricted to healthy, active males and females aged 18-55 years old.
2. Participants were put through only a single bout of load carriage per visit.

3. Blood markers were sampled four times over the course of 48 hours to gather all possible metabolites released post-exercise.

Limitations

1. The results of the study cannot be generalized to unhealthy persons under the age of 18 or over the age of 55.
2. The results of the study cannot be generalized to unhealthy and/or inactive persons with chronic diseases or high-risk factors of chronic diseases.
3. The results of the study cannot be generalized to exercise over unvaried terrain, with load bearing weight over 20% bodyweight, or over distances greater than 8 miles.
4. There are no direct measures of joint degradation measured in this protocol.
5. The relationship between a single bout of load carriage and the accumulated stress of repeated load carriage over time cannot be assessed.

CHAPTER TWO

LITERATURE REVIEW

Introduction

There are several common scenarios in daily life, vocation, and recreation where it is necessary to carry external loads of varying magnitude. Examples of these activities can range from enjoying a leisurely weekend backpacking trip to spending months in the field as a wildland firefighter. A consequence of engaging in these activities for prolonged periods is adding burden to joint tissues that stabilize the lower extremities. These injuries have been specifically noted in epidemiological reports that study instances and prevalence of injuries of both recreational and occupational endeavors. While injury and discomfort are difficult to study in vivo within a controlled environment, novel techniques have been utilized with laboratory blood sampling analyses to measure joint degradation through biomarkers. The field of metabolomics is a blossoming sector of research that allows researchers to study the metabolic byproducts of exercise. Metabolomic analysis has recently been utilized in the study of joint inflammation/damage in humans, with its use in external load-carriage research being quite novel. Furthermore, the relationship between these physiological changes and movement coordination due to fatigue induced by external load-bearing exercise is unknown. The use of neuromuscular control testing, like the y-balance test, can provide insight into movement coordination and efficacy. These data sets, in conjunction with one another, can create an integrated understanding of how external load carriage might affect individuals acutely and provide a path to future research in this area.

Prevalence of Injury/Discomfort during Load-Carriage

Across physical analyses that investigate external load injury epidemiology, the most common instances of bodily injury result from either musculoskeletal (MSK) trauma or gradual connective tissue damage (Birrell et. al., 2007). Meta-analyses based off medical supplies carried by mountain climbers in the state of Colorado showed that MSK trauma, sprains, and strains were the most common medical issues reported (Brandenburg & Locke, 2016). Reports from the Northwest Outward Bound School database of wilderness expeditions showed that strains, sprains, and trauma were the most common injuries (Wells et. al., 2018). These specific instances are recorded in civilian populations, which tend to carry average loads within 20-30% of the subjects' bodyweight. Occupational load-carriage tends to increase this value to around 40-60%, as well as cover distances, environments, and terrain much harsher than average recreational hikers/backpackers (Taylor et. al., 2016). Reports in Australian soldiers saw 20% of nearly 2,000 soldiers had experienced injuries sustained during load carriage, a majority of which involved the lower limbs and back and most of those involving bones and joints (Orr et. al., 2015). Furthermore, knee-specific joints tend to have high rates of overuse injuries, particularly in the iliotibial (IT) band (Stacy, 2019). The epidemiological research above has cited lower back injuries and pain to be the number one site of injury, with lower extremities following at second. This prevalence in the knee joint requires further investigation into metabolomics and movement coordination contribute to injury.

Prevalence by Demographic

Although lower extremity joint injuries and reports of discomfort are present among all demographics, further analysis has shown that certain groups are more prone to complications than others. The categories that are the most relevant predictors of injury/discomfort predisposition tend to be occupation, age (>30 years of age), and level of physical preparedness.

Studies of self-reported injury showed that there is no statistical significance between instances of injury in men versus women (Twombly & Schussman, 1995). The increasing number of females entering occupations that require heavier load carriage has brought more attention to effects that may be placed on their unique physiologies and mechanics (Orr et. al., 2020). However, in civilian populations, men and women above the age of 30 that live with sedentary lifestyles tend to have higher instances of reported injury to the lower extremities (Lobb, 2004). Furthermore, Wills et. al. (2021) has recently found that previously unknown sex-specific responses to external load-carriage in the military.

Occupational load carriage tends to present a higher risk for overuse in the lower extremities, especially in the knee joints for wildland firefighters (Semmens et. al., 2016). Instances of injury due to slips trips and falls and/or/because of overuse is a trend that specifically plagues individuals who work as wildland firefighters and military occupations, often special operations warfighters (Orr et. al., 2011). These higher instances indicate a link between prolonged external load-carriage in stressful environments may contribute to injury in the long term.

Sedentary lifestyles tend to result in a lack of muscular strength, power, and control (Buford et. al., 2010) which can bring about risk of its own to injury in the lower limbs associated with trips, slips, and falls, a risk brought about due to fatigue (Angelini et. al., 2018) which can be exacerbated by lack of physical training. This lack of physical training not only highlights deficiency in muscular control and function, but it affects the structural integrity of connective tissues at the joints (Hicks et. al., 1990). The more physically prepared through training an individual is, the more efficient they become in their movements (Zoladz et. al., 2016) and the more adaptations occur in joint tissues to reinforce their pursuits in external load-bearing exercise.

Causes of Injury

The main mechanisms of injury and discomfort in lower extremity joints are often categorized as acute SKM trauma or gradual overuse. While overuse injuries are difficult to quantify without clinical diagnosis (e.g. osteoarthritis), injury pathology tends to encompass both categories. Cardiorespiratory and biomechanical efficiency are paramount to load carriage performance, as there are significant increases in energy expenditure with loaded packs, exacerbated by environment (Knapik et. al., 2012). This increased expenditure can contribute to faster rates of exhaustion, which can in turn result in diminished motor control, potentially resulting in injury. Furthermore, an individual's aerobic fitness and the interaction between intensity and duration of the task in question will have determining effects on time to exhaustion (Boffey et. al., 2019). These effects experienced at a physiological level can in turn influence the neurological and muscular fatigue of an individual engaging in such physical tasks.

Crossing over varied terrain can require modulation of stride and power required to clear obstacles and navigate hazards. Lenton et. al. (2018) found that lower-limb joint work and power are modulated during load carriage, resulting in higher muscular engagement, leading to higher energy expenditure, which contributes to energy depletion and potential injury. Muscular deficiencies can lead to increased injury risk because of compensations needed and lack of strength and/or power to move joints sufficiently (Heebner et. al., 2017). Step and stride length and contributors to potential injury, particularly over varied terrain, as efficiency may be created with larger lengths (Fox et. al., 2020). Peripheral muscle fatigue has been shown to be a major contributor to lost efficiency in load carrying gait, leading to physical exhaustion and leaving an individual vulnerable to slips, trips, or falls (Grenier et. al., 2012).

Beyond acute traumatic injury, repetitive overuse of lower extremity joints seems to have an effect on damage and inflammation, especially in the knees. Epidemiological studies have shown that several wildland and structural firefighters, as well as special operations warfighters, suffer from overuse damage in later years of occupation (Stockwell, 1991). While the physiology behind this is not clear, much research regarding these reference physiological processes in osteoarthritic patients.

Blood Markers Associated with Joint Damage/Inflammation

Synovial fluid sampling provides the most direct method in gathering pertinent data on the joint environment (Vangsness et. al., 2011). Despite this, it is difficult to draw such samples from human participants, therefore a majority of these studies are conducted on animal subjects with some comparative results (van Vulpen et. al., 2015). The most effective methods of associating markers with knee joint inflammation have been systemic biochemical markers in conjunction with radiographic data (Lotz et. al., 2014), however, this methodology takes time and costs more than a simple blood draw and lab analysis. Furthermore, Vlad and colleagues (2011) have suggested that the use of blood cytokines with radiographic data is an unreliable method in finding systemic associations with chronic joint issues. However, other research has shown that grouping appropriate blood markers along with other degradation products yield more statistically powerful clinical measurement tools (Mabey & Honsawek, 2015).

Recent years have shown a growth in the field of metabolomics, which involves the identification and quantification of metabolic byproducts (metabolites) in an organism (Idle & Gonzales, 2007). The most relevant research to date involving metabolomics and joint inflammation involves measures of structural deterioration , oxidative stress, and inflammation are known to occur in joints more prone to damage versus healthy joints (Carlson et. al., 2019). While metabolomics is not a particularly novel form of analysis, the technology is being used more and more in order to understand physiological processes of an event, diet, environment, and more (Weckwerth & Fiehn, 2002). With the ability to study untargeted metabolites, novel biomarkers have the potential to be identified in the physiological processes that occur during joint inflammation as a result of external load-bearing exercise.

Functional Stability Assessments

Biomechanical and motor control assessments can enable the measurement of functional stability in individuals to evaluate movement coordination and efficacy. The y-balance test has been described as a moderate to highly reliable test of dynamic neuromuscular control in individuals, particularly regarding risk factors to injury (Pilsky et. al., 2021). Deficiencies in the scores of this test can shed light on performance differences (Schwiertz et. al., 2020), which can be vital to tactical and recreational athletes who engage in high energy expenditure activities while carrying significant loads. A lack of functional stability in these individuals could potentially act as a predictor to any traumatic or compensatory injury endured during a hike over varied terrain. The ability to achieve sufficient foot clearance when stepping over an obstacle has been observed with increased loads in individuals who were asked to achieve a simple step-over test with hurdles (Perry et. al., 2010). These observations have since been adapted to study sufficient foot clearance and its correlation to injury risk in soldiers as a result of exhaustion, which has the potential to lead to slips, trips, and/or falls. Two separate height clearances were tested on subjects while weighed down with load and found that there was a minimized risk of tripping by creating a safety margin through increased foot clearance over the obstacle (Brown et. al., 2015). This clearance height after a hike compared to before a hike, may allow researchers to understand whether a subject actually experienced sufficient exhaustion to compare to blood marker analysis.

Summary

Overuse and traumatic injuries are commonplace in individuals who frequently engage in recreational or occupational load carriage. Several epidemiological studies have cited main causes attributed to lower-extremity joint damage involves slips, trips, falls, and in older individuals, overuse. Muscular power and stride length can be predictors of mechanical failure, alongside motor control assessments like the y-balance test and foot clearance test. While metabolomic analysis is relatively new in studying blood markers associated with joint pain, degradation, and inflammation, recent years have shown promise in understanding the physiological and metabolic processes that occur during such incidences.

CHAPTER THREE

THE INFLUENCE OF EXTERNAL LOAD DURING HIKING ON MARKERS OF JOINT STRESS AND MOVEMENT EFFICACY

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

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Contributions: Conceived and implemented the study design. Provided expertise and funding. Aided in data analyses. Provided feedback on the draft of the manuscript.

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Manuscript Information

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Status of Manuscript:
__X_Prepared for Submission to a peer-reviewed journal
____Officially submitted to a peer-reviewed journal
____Accepted by a peer-reviewed journal
____Published in a peer-reviewed journal

THE INFLUENCE OF EXTERNAL LOAD DURING HIKING ON MARKERS OF JOINT STRESS AND MOVEMENT EFFICACY

Introduction

Joint injury, inflammation, and degradation to the lower extremities are some of the most prevalent complications to associated with prolonged external load-bearing exercise (Wang et. al., 2013). The activities that require individuals to carry external loads, primarily on the back, can be generally classified as either occupational or recreational. Occupational pursuits typically encompass individuals whose employment requires traveling for prolonged periods over varied terrain with external loads, which can include special operation warfighters, wildland firefighters, or forest service employees. Recreational external load-carriage involves recreational activities, ranging from mountaineering to backpacking. While the site of injury associated with carrying external load may vary, typical injuries are common in the lower back and lower limb joints (Seay, 2015), these may occur as a result of falling from lost balance while in the field (Gentile et. al., 1992) or may be a product of repeated joint wear and tear. Whether at the occupational or recreational level, the resultant injuries from these activities tend toward either acute skeletomuscular (SKM) trauma or the gradual dysfunction of connective tissues over time (Knapik et. al., 2004).

There are several recognized predictors of joint injury associated with carrying external loads such as age (Mangnuss et. al., 2015), occupation, and fitness level (Drain et. al., 2015) however, there are still several gaps in knowledge of what physiological markers indicate a predisposition to joint degradation or injury. Methods used to investigate biomarkers of joint health are generally related to extraction via blood sampling or synovial fluid, the latter of which is difficult to obtain permissions for. The use of blood cytokine research has elucidated much about inflammatory biomarkers and processes resulting from external load carriage (Jensen et. al., 2019), especially when used in conjunction with radiographic data (Choe et. al., 2011). Advances in metabolomic research has shown promise in recent years as an efficient system of analysis (Miggiels et. al., 2018) of revealing information about metabolic pathways involved in joint health (Southan et. al., 2020), which has potential to be applied toward external load carriage research. An exploratory investigation into the metabolomic differences between hiking with and without loads will provide an observatory platform to direct future research on the topic.

Alongside metabolic methods, the evaluation of movement coordination and efficacy could provide insight to individuals that might be predisposed to acute traumatic injury resultant from slips, trips, or falls through the assessment of dynamic stability (Qu & Yeo, 2011). To evaluate the influence of carrying loads a suitable and specific test of movement efficacy and coordination is required. The y-balance test is a widely accepted dynamic stability test for assessing lower limb neuromuscular control (Schwiertz et. al., 2020), which could indicate an individual’s level of muscular fatigue through diminished control. Similarly, an obstacle clearance assessment has been developed to measure foot clearance variability and compensation that correlates to an individual’s likeliness to slip, trip, or fall when crossing over an obstacle (Brown et. al., 2016). In conjunction with one another, these neuromuscular tests may give some indication of a hiking protocol’s fatiguing effects.

The aim of this study is to examine the biochemical differences between external load-carriage and non-load-bearing conditions through blood metabolomic analysis. Furthermore, the use of motion capture data will measure any differences in neuromuscular control from each condition after hiking that corresponds with physiological changes. It is hypothesized that higher joint stress during external load carriage will elicit a higher upregulation of metabolites relevant to joint stress and joint degeneration over time when compared to non-load-bearing conditions. This research aims to provide quantitative observational differences between conditions that may be translated into future research regarding practical applications of the topic.

Methods

Participant Characteristics and Recruitment

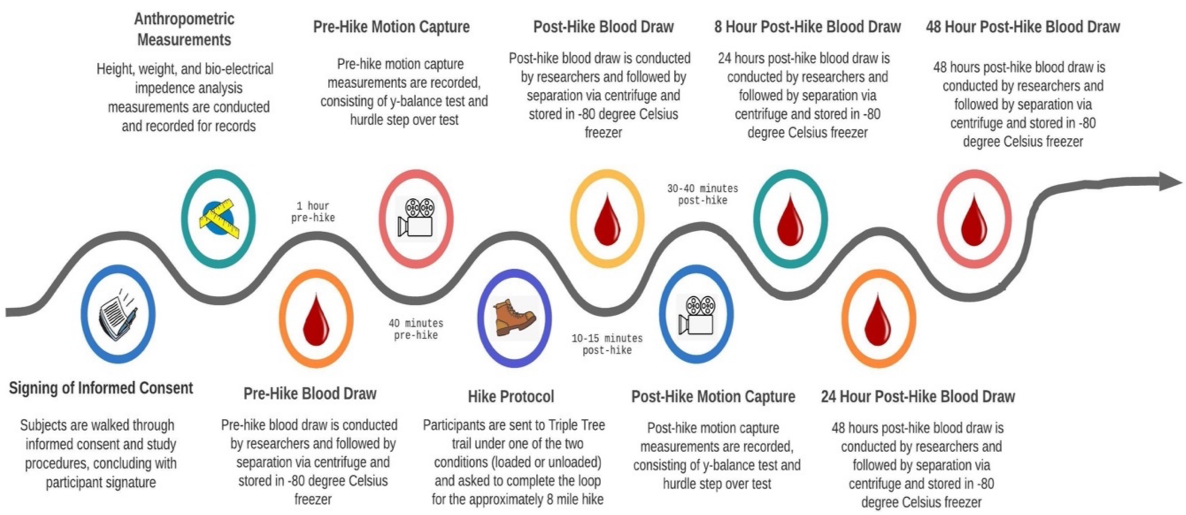
Recreationally active individuals with experience in load carriage from backpacking and other relevant activities were recruited for this study. Inclusion criteria consisted of participants being: 18-50 years of age, active, and healthy with no contraindications to exercise. Exclusion criteria consisted of participants: not meeting guidelines of the health history questionnaire, having other health concerns or conditions that may interfere with participation in the study, and/or any absolute contraindications to exercise as outlined by American College of Sports Medicine guidelines.

This study was approved by the Montana State University Institutional Review Board in October 2020 and was signed by subjects prior to participation, along with a written informed consent (Appendix A). Subjects were recruited by word of mouth throughout the Montana State

University campus as well as throughout Park and Gallatin counties. Interested participants contacted researchers to determine eligibility and a basic briefing of procedures to gauge interest in further participation. Participants were verbally asked about each inclusion and exclusion criteria and informed of the risks and discomforts associated with the study procedure before being scheduled for the first visit.

Experimental Design

Figure 3.1 *Timeline of each activity conducted under each study condition*



The study had a general pre-test/post-test crossover research design with each subject undergoing two separate conditions in randomized order. The research consisted of two conditions which were implemented for each subject with each condition measuring the same variables. For each condition there was a pre-test of the variables, the implementation of a specific exercise protocol, and post-test measurement of the same variables.

The separate conditions consisted of a different weight load carried during the designated hike, one being minimal load and designated as "day packing" weight, the other being 20% bodyweight "backpacking" weight.

Variables measured throughout the study included a blood draw for metabolite analysis pre-hike, post-hike, eight hours post-hike, 24 hours post-hike, and 48 hours post-hike. Biomechanical/motor control measures were taken pre-hike in order to gather baseline under each condition for each subject and repeated post-hike in order to analyze for differences.

The Montana State University Institutional Review Board approved this study which ran from October 2020 to July 2021.

Health History Questionnaires and Body Composition

Participants were asked to complete two separate questionnaires, one which covered each individual's physical activity readiness (Appendix B) and the other covering personal and family joint health history as well as level of physical preparedness prior to participation (Appendix C). Subjects were then taken to have general body anthropometrics recorded such as height, weight, and waist circumference. Upon completion of these measurements a bioelectrical impedance analysis (BIA) (SECA mBCA 515) was completed in order to determine fat tissue content.

Blood Metabolite Analysis

Standard procedures for collection of blood from a forearm vein were used to collect blood samples at five separate time points for each condition of this study: pre-hike, post-hike, 8 hours post-hike, 24 hours post-hike, and 48 hours post-hike. Samples were collected in a single draw fashion which took approximately 5mL of blood with each draw at the designated time points. Blood draws were performed by lab personnel. Samples taken were subsequently centrifuged at 1200xg for 15 minutes and aliquoted into separate tubes for storage in a -80-degree Celsius freezer until analysis.

Metabolites were extracted and analyzed by LC-MS analysis with slight modifications after thawing on ice and centrifuging at 4 degrees Celsius at 500xg for 5 minutes in order to eliminate cells and debris. The supernatant was re-suspended in 50:50 water:acetonitrile at 20 degrees Celsius for 30 min. The samples were vortexed for 3 minutes and centrifuged at 16,100xg for 5 minutes at 4 degrees Celsius. The supernatant was completely evaporated in a vacuum concentrator for ~2 hours, and the dried pellet was re-suspended in 500 mL of acetone to precipitate proteins at 4 degrees Celsius for 30 minutes. The sample was then centrifuged at 16,100xg for 5 minutes. The supernatant was completely evaporated by speedvac, and the pellet was re-suspended in mass spectrometry grade 50:50 water:acetonitrile. Metabolite extracts were analyzed in positive mode using an Agilent 1290 UPLC system connected to an Agilent 6538 Q-TOF mass spectrometer (Agilent Santa Clara, CA). Metabolites were chromatographically separated on a Cogent Diamond Hydride HILIC 150 x 2.1 mm column (MicroSolv, Eatontown, NJ) using an optimized normal phase gradient elution method, and spectra were processed.

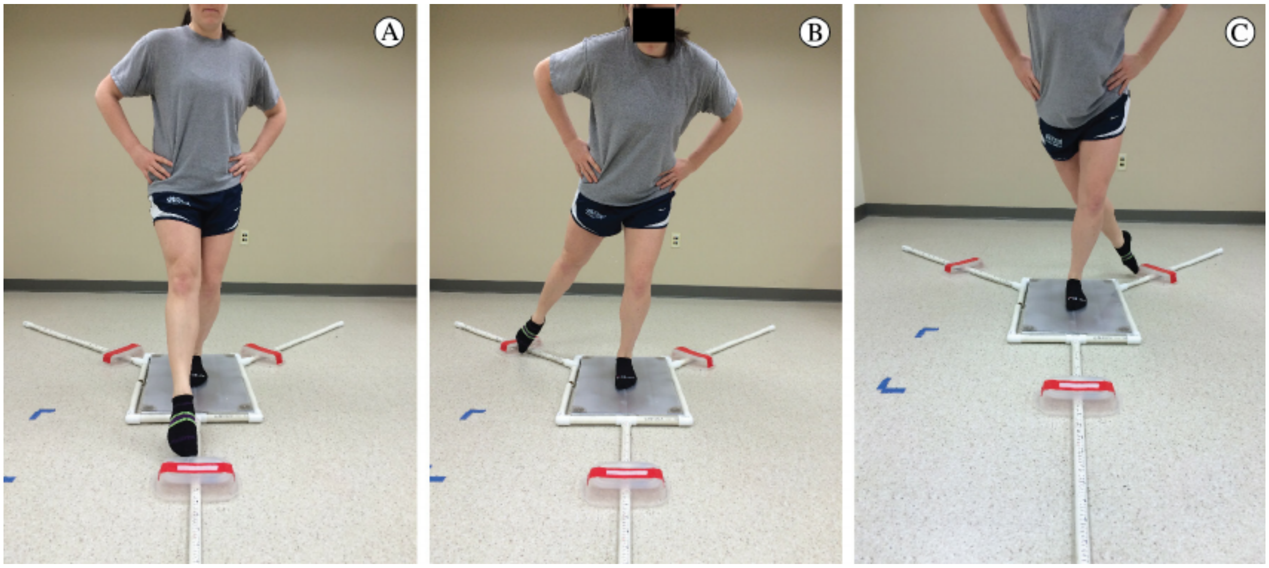
<u>Motion Capture and Evaluation of Movement</u>

Motion during the Y-Balance test and step over test were recorded using a portable motion capture system (XSENS MTw Awinda) within a controlled laboratory environment. Participants were fitted with linked inertial measurement units (IMUs) on Velcro straps at each of the anatomical landmark indicated by the program and all equipment was manually calibrated. Body dimensions were measured and included body height, foot length, shoulder height, shoulder width, elbow span, wrist span, arm span, hip height, hip width, knee height, and ankle height. Straps were placed at the hips, mid thighs, mid calves, and the dorsal region of feet. Once fully prepared, researchers instructed subjects on the task protocols that would be completed, the Y-balance test and a hurdle step-over assessment. The Y-balance test is a functional balance assessment used to evaluate dynamic balance and symmetry, giving an idea of an individual's risk of injury. The hurdle step-over assessment (Figure 2) is an evaluation that is not as widely used in clinical settings but has shown efficacy when determining risk of fall injury in special operation warfighters.

Figure 3.2 *Example photo of the hurdle step-over assessment*

The Y-balance requires that a subject stand at the center of a designated “y” that has been marked and reach out to each point of the shape with one leg without losing balance (Figure 3).

***Figure** 3.3 Example photo of Y-balance assessment*



The distance which was reached by each leg is then recorded and used to score the functional balance of an individual. The hurdle step-over assessment requires that a subject have a walking distance of 7 feet before stepping over a 457mm hurdle with one leg. Specific clearance heights indicate the likelihood that an individual will avoid injury when engaging in that task. Participants were first familiarized with the specific tasks for each protocol and allowed to practice until they felt comfortable with the instructions. Each task was completed three times for each leg and the recorded motion capture data was subsequently stored and analyzed at a later time.

Hiking

A trail located near the Montana State University campus was utilized as the track for field testing load carriage vs. non-load carriage in this study. The length of the trail as a single loop is recorded as being ~5.4 miles, but for the purpose of this research participants were asked to complete the upper loop portion twice in order to travel a total distance of ~7.2 miles. Subjects completed field testing at different times of year but consistently when the trail was clear and without hazards that could prove harmful (e.g. ice, mud, fallen trees). Due to the timing of blood draws, all participants were asked to begin data collection in the morning, between 6am and 8am. Scheduling in this manner allowed for uniformity in blood draws across all participants as well as time of hikes under both conditions. All load carriage trials were completed with a subject's own backpack with a payload of 20% of their own bodyweight. Only the consumption of water was allowed before and during the hike under both conditions, with participants getting to eat after the initial post-hike blood draw. All subjects were asked to refrain from heavy exercise and alcohol consumption during the blood draw cycles. While the trail itself was standardized for everyone, weather and trail conditions were unpredictable, and resulted in the use of different foot apparel and clothing between each participant.

Statistical Procedures

Metabolite features were imported into Metaboanalyst (version 5.0) after post-acquisition processing using MSconvert & Mzmine. Feature intensities were then normalized by sum and subsequently autoscaled (mean-centered and divided by the standard deviation of each variable). A volcano plot (fold change (FC) vs. p value) was generated to determine which features showed both a statistical trend ($p < .1$) and substantial abundance change in a given pairwise comparison (FC > 1.3). Therefore, FC values are reported as the quotient of autoscaled feature intensities. P values were generated using a Student's t-test (evaluating the probability that the difference in group means is due to chance, based on the students t distribution), and FC values originated in a fold change analysis (comparing the absolute value change between two experimental group means). Box and whisker plots were generated from the points (features) on the volcano plot that meet the aforementioned p value and FC thresholds and show the distribution of a feature's intensity in each experimental group.

Motion capture data was analyzed using Microsoft Excel for Windows (version 16.0, Microsoft Inc.). Resultant values were first calculated from x and y values and then placed into tables for comparison. A two-tailed T-test was conducted with repeated measures in order to evaluate differences between both pre-hike conditions, both post-hike conditions, and loaded vs. unloaded trials. Statistical significance was set at the alpha = 0.05 level.

Results

Participants

Eight participants, 18- 50 years of age (male = 5, female = 3), enrolled in the study . Participants' age ranged from 23-46 years of age with the mean BMI for the group being 23.34 ± 0.21 kg/m^2. None were smokers, pregnant, or had any family history or personal medical history that contraindicated exercise. Participant anthropometric and demographic characteristics are shown in table 3.1, laying out basic participant demographics and anthropometrics obtained from BIA.

Table 3.1 *Participant Characteristics*

	Males (n=5)	Females (n=3)
Variables	Mean ± SD	Mean ± SD
Age (years)	**30 ± 9.7**	**24.7 ± 0.6**
Height (cm)	**183.7 ± 3.4**	**174.0 ± 4.4**
Body Mass (kg)	**80.9 ± 9.7**	**70.6 ± 4.9**
Body Fat %	**13.1 ± 3.0**	**23.6 ± 1.13**
Waist Circumference (cm)	**82.0 ± 4.9**	**74.9 ± 1.3**
BMI (kg/m²)	**23.5 ± 4.2**	**23.1 ± 0.3**

Y-Balance and Step-Over Test Performance

The local position of the big toe of the stepping foot were recorded in the horizontal plane (x and y coordinate) at their maximum along each arm of the “Y”. The x and y coordinate data were converted to a linear distance using simple Pythagorean theorem, signifying the distance reached by each foot to the three points in the y-balance test which served as the dependent variables from the Y-Balance test. Once mean values for each point were calculated, standard deviations for each were determined and then compared from condition to condition. The maximal vertical height of the big toe of the stepping leg was taken as the dependent variable during the step over test. Tables 3.2 and 3.3 demonstrate pre-hike values and analyze both loaded and unloaded conditions for significant differences. The only findings of significance were between loaded and unloaded lateral measures in the left leg, as represented in table 3.3.

Table 3.2 *Pre-hike Y-balance measures comparing both conditions for the right leg*

	Loaded Anterior	*Unloaded Anterior*	*Loaded Medial*	*Unloaded Medial*	*Loaded Lateral*	*Unloaded Lateral*
Mean (meters)	0.86	0.86	0.80	0.91	0.85	0.81
SD	0.10	0.22	0.15	0.23	0.12	0.16
P-Value	1.00		0.06		0.34	

Table 3.3 *Pre-hike Y-balance measures comparing both conditions for the left leg*

	Loaded Anterior	*Unloaded Anterior*	*Loaded Medial*	*Unloaded Medial*	*Loaded Lateral*	*Unloaded Lateral*
Mean (meters)	0.83	0.81	0.89	0.92	0.81	0.89
SD	0.13	0.08	0.23	0.19	0.14	0.12
P-Value	0.72		0.25		0.01	

Differences between post-hike values of loaded and unloaded conditions were evaluated, as demonstrated in tables 3.4 and 3.5, with no significant difference shown between measures. Analyses were then conducted between pre-hike and post-hike y-balance measures, showing only significant differences between pre- to post-hike right leg lateral values in loaded conditions and between pre- to post-hike left leg lateral values in unloaded conditions.

Table 3.4 *Post-hike Y-balance measures comparing both conditions for the right leg*

	Loaded Anterior	*Unloaded Anterior*	*Loaded Medial*	*Unloaded Medial*	*Loaded Lateral*	*Unloaded Lateral*
Mean (meters)	0.81	0.84	0.84	0.93	0.77*	0.80
SD	0.07	0.05	0.17	0.15	0.12	0.13
P-Value	0.24		0.06		0.26	

**p<0.05 compared to pre-hike value within condition.*

Table 3.5 *Post-hike Y-balance measures comparing both conditions for the left leg*

	Loaded Anterior	*Unloaded Anterior*	*Loaded Medial*	*Unloaded Medial*	*Loaded Lateral*	*Unloaded Lateral*
Mean (meters)	0.78	0.84	0.87	0.87	0.78	0.80*
SD	0.11	0.08	0.20	0.14	0.11	0.15
P-Value	0.28		0.87		0.44	

**p<0.05 compared to pre-hike value within condition.*

The obstacle clearance assessment values, once obtained and converted to meters, were also analyzed for differences between both conditions' pre-hike, post-hike, and then between pre- to post-hike within each condition. Measures for these analyses are found in tables 3.6 and 3.7, with no significant differences found between any of the measures.

Table 3.6 *Pre-hike step-over measures*

	Loaded Right	*Unloaded Right*	*Loaded Left*	*Unloaded Left*
Mean (meters)	0.67	0.68	0.69	0.69
SD	0.04	0.05	0.05	0.05
P-Value	0.55		0.88	

Table 3.7 *Post-hike step-over measures*

	Loaded Right	*Unloaded Right*	*Loaded Left*	*Unloaded Left*
Mean (meters)	0.69	0.69	0.67	0.71
SD	0.07	0.02	0.06	0.03
P-Value	0.88		0.12	

Metabolomics

After analysis, metabolites were assigned IDs and level under each condition was measured and analyzed for comparison between conditions. Which condition contained a higher upregulation in comparison to the other within a given timepoint was then determined and demonstrated in tables 3.8 through 3.11.

Table 3.8 *Post-hike metabolite IDs, measures, and upregulated condition of significance*

	HMDB ID	*m/z*	FC	p	Upregulated Condition
PE(14:1)	HMDB08855	632.43	5.13	0.03	Backpack
Cortisol	HMDB0063	363.21	2.41	0.03	Backpack
LysoPC(18:1	HMDB02815	522.36	8.21	0.04	Backpack

Table 3.9 *8-hour post-hike metabolite IDs, measures, and upregulated condition of significance*

	HMDB ID	*m/z*	FC	p	Upregulated Condition
PI(16:0/18:2)	HMDB09784	835.51	3.16	1.86E-04	Backpack
PC(15:0/22:5)	HMDB07956	794.57	3.24	0.01	Backpack
PC(15:0/20:4)	HMDB07949	768.56	2.90	0.01	Backpack
PE(22:6)	HMDB09705	836.52	7.10	0.03	Backpack
PC(15:0/22:4)	HMDB07955	775.55	3.03	0.03	Backpack
Glycyrrhetinic acid	HMDB11628	471.34	3.54	0.04	Backpack
trans-Hexadec-2-enoyl carnitine	HMDB06317	398.32	3.87	0.04	Backpack
PC(18:4)	HMDB08240	774.50	2.76	0.05	Backpack
Chenodeoxycholic acid	HMDB00518	393.30	3.23	0.07	Backpack
Uridine 5'-monophosphate	HMDB00288	325.04	3.36	0.08	Backpack
LysoPC(P-16:0)	HMDB10407	480.35	73.11	0.10	Backpack

Table 3.10 *24-hour post-hike metabolite ID, measures, and upregulated condition of significance*

	HMDB ID	***m/z***	**FC**	**p**	**Upregulated Condition**
Cer(d18:1/12:0)	HMDB04947	482.46	0.33	0.06	Daypack
PE(14:1(9Z)/16:1)	HMDB08858	660.45	0.06	0.07	Daypack
17-HDoHE	HMDB10213	345.24	0.20	0.09	Daypack
Palmitic acid	HMDB00220	257.25	0.37	0.11	Daypack
LysoPC(14:0)	HMDB10379	468.32	0.33	0.11	Daypack
PE(14:1)	HMDB08855	632.43	0.02	0.11	Daypack
Lithocholyltaurine	HMDB00722	484.31	0.02	0.12	Daypack
PC(15:0/22:5)	HMDB07956	794.55	0.05	0.12	Daypack
PG(18:1(11Z)/22:6)	HMDB10629	821.52	0.24	0.12	Daypack
12S-HHT	HMDB12535	281.21	0.25	0.13	Daypack
PE(14:0/18:2	HMDB08829	688.49	0.11	0.13	Daypack
PS(16:0/22:6)	HMDB12362	808.52	0.12	0.14	Daypack
DG(14:0)	HMDB07008	513.44	0.06	0.14	Daypack

Table 3.11 *48-hour post-hike IDs, measures, and upregulated condition of significance*

	HMDB ID	*m/z*	FC	p	Upregulated Condition
Thyrotropin-releasing factor	HMDB05763	363.18	14.21	0.08	Backpack
Anserine	HMDB00194	241.13	0.41	0.10	Daypack
PC(18:1)	HMDB00593	786.59	1.96	0.02	Backpack
11beta-Hydroxyprogesterone	HMDB04031	333.21	0.49	0.13	Daypack
LysoPC(16:0	HMDB10382	496.34	0.50	0.02	Daypack

Discussion

The key findings of this investigation include a measurable difference in metabolomic upregulation between external load-bearing and non-load-bearing conditions and potential reliability in movement assessments. While motor control assessments indicated that neither condition induced substantial fatigue, metabolomic analysis identified several differences that provide insights. Clear contrasts between timepoints elucidated differences in the physiological processes occurring in each condition and give some potential insight into joint damage and recovery. While differences in motor control assessments were not significant, they were consistent with previous literature and showed some important numerical divergence in quantitative values. These findings will help refine methodology and considerations for future research regarding external load-bearing exercise. The primary hypothesis of this study stated that higher joint stress during external load carriage would elicit a higher upregulation of metabolites relevant to joint stress and joint degeneration over time when compared to non-load-bearing conditions. Blood metabolomic analyses revealed several differences in upregulation between each condition over 48-hours. No significant differences in the results of the motion capture assessments between both post-hike conditions, thus, this correlation was not assessed.

One of the more prevalent metabolites identified at the post-hike timepoint was upregulated cortisol in the backpacking condition, which has clear consistencies with the physical and mental stress of backpacking (Paul et. al., 2015) compared to daypacking. Aside from the clear stress indicated by its presence, cortisol upregulation has been associated with negative effects on bone mineral density (BMD) (Athimulam et. al., 2020). These findings are consistent with the chronic hypersecretion of cortisol experienced by individuals diagnosed with Cushing's syndrome or under constant mental stress (Furlan et. al., 2005). However, literature on the relationship between cortisol upregulation and BMD in athletes is limited in scope. Cyclists are noted for having poor BMD levels (Abrahin et. al., 2016), yet post-competition observation showed no acute effects on these levels (Mathis et. al., 2013). Despite this, more research on this relationship is needed to study the longitudinal effects of cortisol on athletes, tactical or otherwise, who are constantly and consistently subjected to stressful environments and situations. Thus, the presence of cortisol at this timepoint may indicate more than increased physiological stress brought on by hiking with external load and could indicate acute degradation of bone mineral content.

Metabolites identified for this study at the 8-hour post-hike timepoint were all significantly more upregulated in the backpacking condition compared to daypacking. While there was some variety between features, a majority were identified as being different forms of glycerophospholipids. These molecules are glycerol-based phospholipids responsible for the integrity and functionality of in biological cell membranes (Castro-Gomez et. al., 2015). The upregulation of glycerophospholipids and sphingolipids has a noted relationship with early and late-stage osteoarthritis (Kosinska et. al., 2014), suggesting a potential mechanistic relationship with joint degradation. Abnormal pathways of glycerophospholipid metabolism are linked pro-inflammatory processes when measured in synovial fluid (Zhan et. al., 2020), thus an increase in systemic circulation could be an indicator of damage or inflammation taking place at a higher rate when participating in external load-bearing exercise. Further investigation into the relationship between the presence of upregulated systemic glycerophospholipids synovial glycerophospholipids is required.

A dramatic switch occurred at the 24-hour timepoint, in which the condition with significant metabolite upregulation became daypacking. While a variety of molecules were identified, including lipids and bile salts, the four that may illicit insight were ceramide(d18:1/12:0), 17-HDoHE, palmitic acid, and DG(14:0). Ceramide is a lipid based signaling molecule that manages and regulates cellular proliferation and apoptosis (Kolesnik & Kronke, 1998). 17-HDoHE is a DHA omega-3 essential fatty acid responsible for antioxidative effects that combat cellular inflammatory damage (Swanson et. al., 2012). Palmitic acid is a saturated fatty acid responsible for maintaining cellular balance with unsaturated fatty acids (Carta et. al., 2017), while DG(14:0) is an identified precursor to triglycerides. The combination of palmitic acid and triglycerides resulting from DG(14:0) may signify the beginning of triglyceride/fatty (TG/FA) acid cycling (Miyoshi et. al., 1988), which increases with exercise

(Bahr et. al., 1990) as a of controlling lipid metabolism (Lovsletten et. al., 2018). While seemingly unrelated in their physiological roles, these molecules may provide a broader picture of what is occurring at this particular timepoint. Because physiologic recovery from exercise tends to peak between 24-48 hours (Hausswirth & Meur, 2011), these metabolites working in conjunction with one another may suggest a more efficient recovery for the daypacking group compared to backpacking.

The final timepoint of 48-hours post-hike contained a handful of metabolites that were a mix between which conditions were more upregulated. While it was difficult to discern which ones may be important, anserine seemed to shed the most light on what was occurring for the daypacking condition. Anserine is a methylated derivative of carnosine, associated with strong antioxidant effects that mitigate cellular damage via non-enzymatic glycation (Szwergold, 2005) and lipid peroxidation (Wu et. al., 2003), as well as contribute to restoration of muscle contraction via calcium metabolism (Petukhov et. al., 1976). The presence of this metabolite's upregulation at this particular timepoint may contribute to the indication that the recovery from exercise is occurring either at a higher rate or at higher efficiency in the daypacking condition.

Only three comparisons in the analysis of the Y-balance and step-over tests reached the threshold for significance, all of which involved lateral leg movement in the y-balance test. It has been determined that performing this neuromuscular assessment after fatiguing exercise tends to revert to normalized values in anterior scores within 10 minutes, medial scores within 20 minutes, and lateral scores taking greater than 20 minutes (Johnston et. al., 2018). Because it took 15-20 minutes for participants to return to the lab after the hiking protocol, 50-10 minutes to get blood drawn, and another 5-10 minutes to get set up for motion capture recording, the scores of significance are in line with the aforementioned literature. Despite the lack of significance between scores in the post-hike y-balance for backpacking conditions, the distances reached were quantitatively lesser, which could be explained by smaller movements as compensation for slight muscular fatigue (Gonell et. al., 2015). Future analysis of leg movement while reaching for each point may produce data on the stability of an individual after hiking under external load-bearing conditions.

While reliability of the y-balance test has been established as a method of assessing lower body neuromuscular control (Shaffer et. al., 2013), lack of significant differences between pre-hike values under both conditions indicates some potential reliability in the obstacle clearance test as well. Brown et. al. (2015) conducted the obstacle clearance methodology that guided this research but did not specify whether dominant or nondominant legs were assessed. The lack of specification in this and other research utilizing these methods seems to show results applicable to dominant leg clearance, showing that fatigue in healthy individuals results in compensatory higher leg clearance. Nondominant legs tend to have less motor control post fatigue (Haaland & Hoff, 2002), which may explain the lower clearance performed in the step over for the left leg. However, all of this is speculative and requires further investigation in future research.

There were several considerations to take into account for this study design. First, the sample size was limited to eight participants providing blood samples for metabolomic analyses while only six were able to provide motion capture data. Therefore, such a small sample of could not elicit applicable results to the larger population. Technical difficulties and scheduling constraints did not allow adequate time for top metabolomic features to be identified, instead relying on what could currently be identified among both conditions. Trail conditions have shown variability throughout the duration of data collection, which could affect physiological effects due to energy expenditure differences. The amount of weight used for external load may not have been enough to provoke muscular exhaustion, which may also be a function of the rate of work performed by separate individuals. Future research will need to take these into account for protocol development. The results of this study cannot be easily applied to occupational load carriage over prolonged periods, which could last several days in the field. It is also worth noting that untargeted metabolomic analyses were not direct measures of joint degradation, which can only be accomplished through targeted analyses and/or directly through synovial fluid sampling. Despite these considerations, the findings in this study show promise for further investigation in future research regarding metabolomics and movement control and efficacy.

In conclusion reliability in motor control testing with the y-balance test and step-over assessment seems apparent and may prove of use in future studies. The incorporation of 20% of an individual's bodyweight as a load-carriage exercise to induce fatigue may need to be increased for physically active participants. Identifiable differences were noted when comparing the metabolomic analyses of both conditions. Backpacking elevated cortisol when compared to daypacking, relating the increased physiological stress it places on an individual. Based on previous research, there may be a relation to bone degradation with increased levels of cortisol when chronically exposed. Glycerophospholipid metabolism pathways are linked with bone damage, with levels being particularly increased under backpacking conditions for individuals. The upregulation of metabolites associated with apoptosis, omega-3s, and TG/FA cycling during the daypacking condition when compared to backpacking can be linked to recovery after exercise. Furthermore, the development of anserine, a carnosine derivative, suggests recovery from muscular damage and metabolism of calcium. This study has expanded the potential for identifying important metabolic processes during load carriage exercise that is performed at occupational and recreational levels. Once these methodologies are refined, the research can turn to practical application studies of injury determinants and prevention in individuals like wildland firefighters, special operation warfighters, or weekend backpackers.

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CHAPTER FOUR

GENERAL CONCLUSION

The purpose of this study was to enhance our understanding of bone and joint stress induced by external load bearing exercise performed in the field using untargeted metabolomic analysis, as well as to discover the efficacy of comparing resulting biochemical markers to motor control measures. It was hypothesized that higher joint stress caused by external load would elicit increased upregulation of metabolites relevant to joint inflammation and degradation when compared to non-load-bearing conditions. Significant differences between metabolite upregulation at each timepoint were identified for each study condition, showing some pattern between physiological differences. Metabolites of note include cortisol at post-hike, a series of glycerophospholipids at 8-hours post-hike, markers of cell turnover and proliferation at 24-hours post-hike, and anserine at 48-hours post-hike. Thus, the primary hypothesis was confirmed, but further investigation must take place once more features have been identified, particularly the top features. There were no significant differences in motor control measures to infer a relationship between muscular fatigue and blood metabolites identified, thus the secondary hypothesis was not confirmed.

Despite the current findings, several considerations about the sample size, experimental design, and potentially relevant data for future analysis. One of the more important aspects to consider in this research involves the number of participants that volunteered for data collection in this study. While the blood metabolomics involved an n=8 sample size, the efficacy of motor control values is limited to a sample of n=6, requiring future research to recruit a larger number of participants. When experimental design is considered, it may be of consequence to have all participants complete both conditions of the protocol under the same trail conditions and general time of year. Hiking through a muddy trail versus a dry trail has energetically different expenditures versus a dry trail, which could in turn affect metabolic processes measured in the blood. While the amount of work achieved by all subjects was numerically equal, the rate was not controlled or measured (speed at which the hiking protocol was completed), which could also create measurable differences in physiological processes. Lastly, relevant data to this study must be considered as it has not given a full and complete picture of what has occurred as a result of the procedures. While some metabolites have been identified and shown clear differences between conditions, the top metabolite features and their role played under each condition have yet to be identified. Motor control analyses demonstrated that there were no significant differences between each condition after the protocol as far as distances reached by each foot, but stability during each attempt may lead to further insight about fatigue and neuromuscular control. Future research may indicate correlations between the biochemical and biomechanical measures recorded.

This initial investigation provides many lessons about participant recruitment, experimental design, and potential analyses that can be built upon in future research. More time and screening can be put into recruitment of healthy, active participants who are willing and able to dedicate time to this protocol. Separating individuals by age group and/or sex may provide further insight into demographic differences and effects of external load carriage. Furthermore, investigating individuals based in different occupations may prove more useful for practical and applicable results. Development of a protocol that exposes participants to continuous load carriage may provide insight into the effects of occupational load carriage when considered with metabolomic data. A closer look at motor control data to measure stability during assessments and dominance of leg could elicit a better understanding of minimal weight required to induce muscular fatigue.

In conclusion, this study has shown promise in demonstrating clear differences at a physiological level and potential for assessment reliability in future protocols. While adjustments must be made to the sample sizing, methodology, and deciding what resulting data may be relevant, there is promise in utilizing this line of thought for further research in the influence of external load during hiking on markers of joint stress and movement efficacy.

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APPENDICES

APPENDIX A

IRB APPROVAL LETTER & INFORMED CONSENT

MONTANA STATE UNIVERSITY

INSTITUTIONAL REVIEW BOARD

For the Protection of Human Subjects
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MEMORANDUM

TO: Isaac Rowland, Mary Miles, David Graham, Ron June

FROM: Mark Quinn Mark Quinn CH
Chair, Institutional Review Board for the Protection of Human Subjects

DATE: April 14, 2020

SUBJECT: *"Physiological and Motor Control Characteristics of Wildland Firefighters as Determinants of Lower Extremity Injuries and Discomfort"* [IR041420]

The above proposal was reviewed by expedited review by the Institutional Review Board. This proposal is now approved. See below for follow up instructions.

Please keep track of the number of subjects who participate in the study and of any unexpected or adverse consequences of the research. *If there are any adverse consequences, please report them to the committee within 3 calendar days*. If there are serious adverse consequences, please suspend the research until the situation has been reviewed by the Institutional Review Board.

Any changes in the human subjects' aspects of the research should be approved by the committee before they are implemented.

It is the investigator's responsibility to inform subjects about the risks and benefits of the research. Although the subject's signing of the consent form, documents this process, you, as the investigator should be sure that the subject understands it. Please remember that subjects should receive a copy of the consent form and that you should keep a signed copy for your records.

Due to the change in the Common Rule effective January 21, 2019, you will not receive follow-up renewal forms unless your research was reviewed via full committee or is sponsored by the FDA. Enclosed is a "Project Closure Form" which we would like you to fill out and submit to our office upon completion of the research project so that we can close out the protocol. Please keep this form with your application and approval letter so that you remember to submit it at the termination of your project.

Enc.

SUBJECT CONSENT FORM FOR PARTICIPATION IN HUMAN RESEARCH AT MONTANA STATE UNIVERSITY

Study Title: Physiological and Motor Control Characteristics of Backpacking as Determinants of Lower Extremity Injuries and Discomfort

Investigators: Isaac Rowland, BS; Mary Miles, Ph.D.; David Graham, Ph.D.; Ron June, Ph.D.
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Summary: Backpacking is a physically arduous activity that leaves individuals who participate in it prone to discomfort and/or injury. A large amount of these insults to musculoskeletal integrity typically occur in the lower extremities, particularly the knee joints. Several studies have been conducted on special operation forces in the military on predictive measures of injury to warfighters who deal with a great amount load carriage. However, there are no general measures or indicators that can be utilized by the general population in order to predict and prevent lower extremity injuries. We are interested in cross-referencing past history of injury, physical training, blood metabolites, and motor-control measures in recreational backpackers and hikers.

Purpose: The purpose of this study is to determine if motor control performance and metabolites link to joint damage change while backpacking with a heavy load over long distances on varied terrain. We will also observe if physiological and motor-control measures change or can be utilized to predict likelihood of lower-extremity injury or any general changes with fatigue in the average hiker/backpacker. More specifically, we are asking the following questions: Do motor control performance and metabolites linked to joint damage change when hiking with heavy loads? Are there predictive measures under different conditions (load) that can be taken to find joint discomfort or damage for the general population? How do joint inflammatory markers, motor control measures, and past history of injury and/or discomfort relate to incidence of discomfort and/or injury while hiking? Information from this study will allow us to better understand and develop methods of preparing and preventing injury that may occur as a result of hiking/backpacking with heavy loads.

Participants in the Study: You have been asked to participate in this study because you meet the following criteria: 18-50 years of age and physically healthy with no contraindications to participation in exercise. You may not be a participant if you do not meet eligibility requirements of our screening questionnaire because of health history, symptoms, issues or risks, have a pacemaker, or if you have other health concerns or conditions that may interfere with your participation in the study.

Procedures: Participation is voluntary. If you agree to participate in this study, you will be asked to complete two visits to the Nutrition Research Laboratory to complete the procedures of the investigation. All visits (Visit 1 and Visit 2) will take 1-2 hours and include one standard blood draw to collect 1 teaspoon of blood in addition to a health history questionnaire and battery of motor control tests. The motor control tests will be performed before and after completion of the hikes under both conditions. There will be roughly 1-3 weeks in between each visit, during which participants will go about their occupational routine as normal. Completion of the study should take approximately 1-3 weeks, depending on the scheduling of the visits. During these visits you will be asked to do the following things:

Visit 1: This visit will take 1-2 hours and include the following activities:

1) Informed consent. Read and provide written informed consent. We will also provide you with a copy of this form and discuss it with you
2) Health screening questionnaire. Complete a health history questionnaire that asks questions about your health, particularly joint health and past injuries and/or discomforts. Patient's resting blood pressure and heart rates will also be measured.
3) Physical activity questionnaire. Complete a survey that asks questions about any physical preparations or training taken prior to the start of the season.
4) Body size measurements. The researcher will take baseline measurements of height, and circumferences of your waist. You will remain clothed during these measurements; however, you will be asked to remove extra clothing items such as coats or sweatshirts.
5) Measurement of muscle and fat tissue using bioelectrical impedance analysis. This test simply involves standing on a scale with your feet and hands on sensors for a few seconds. A very low electrical current that you cannot feel and that is not dangerous is transmitted and received across the sensors. The technology is commonly used in a variety of settings such as gyms and health screenings, but the instrument that we will use is more sophisticated in being able to estimate muscle and fat tissue in different regions of your body, including the abdominal cavity.
6) Completion of designated hike. Participants will travel the designated hiking trail as pre-determined by researchers on the scheduled day under the appropriate condition.
7) Pre-hike blood collection. Standard procedures for collection of blood from a forearm vein will be used to collect blood. This will allow us to measure untargeted metabolites in the blood.
8) Pre-hike motion capture Participants will be fitted with motion capture markers in the lower extremities and asked to perform a set of balance and motor control tasks. Participants will be prepared for motion analysis in a similar manner for all visits. Skin will be prepared in the same manner and a set of surface electrodes and number of reflective makers will be placed on anatomical landmarks. Once markers and electrodes have been placed and the participant has been prepared, they will begin a battery of used exercises and activities commonly used for evaluation in biomechanics research. To evaluate the motion of the participant researchers will record movements using a set of infra-red cameras that track the motion of the retroreflective markers on the participant. The cameras and software used do not collect typical "video" images of a participant, rather the image is only of the markers which display as a cluster of reflective dots. No identifying images are collected during this process. A Y-balance test, as shown in figure 1 below, will be administered to evaluate dynamic balance and functional symmetry in lower-extremity and core musculature. An obstacle-crossing task will then be performed in which participants walk across a 10-meter walkway and step over a 457 mm obstacle placed 5 meters from the start to measure foot clearance. Once all exercises have been completed surface electrodes and reflective markers will be removed and any residue left on the participants skin from the markers or athletic tape will be cleaned and all markers,

electrodes or equipment contacted by researchers or participants will be cleaned with disinfecting spray and/or wipes.

9) participants walk across a 10-meter walkway and step over a 457 mm obstacle placed 5 meters from the start to measure foot clearance. Motion capture involves filming you so that the markers attached to your body can be digitally analyzed. Faces are blocked or blurred so that your identity is protected.

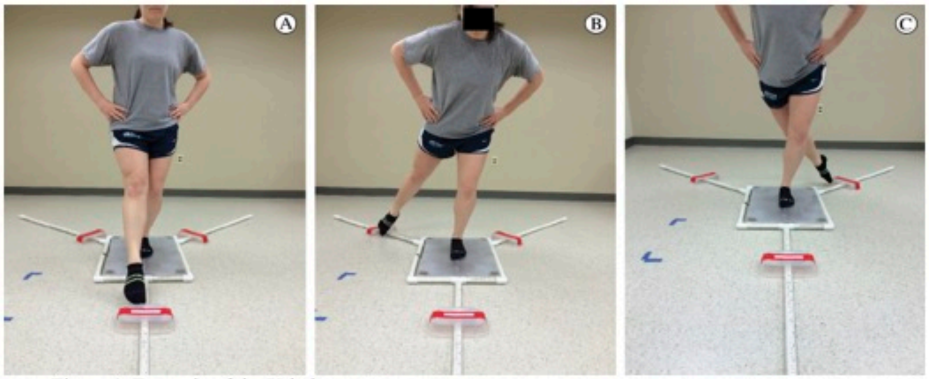

Figure 1. Example of the Y-balance test


Figure 2. Example of the 457 mm obstacle cross

10) Post-hike blood collection. Same procedures as pre-hike blood draw.
11) Post-hike motion capture. Same procedures as pre-hike motion capture.
12) 8-hour, 24-hour, 48-hour post-event blood collection. Same procedures as all other blood draws.

Visit 2 (separated by 1-2 weeks): Will follow the same procedures as visit 1 under different condition

Risks: There are side effects and risks involved from having blood drawn or doing certain exercise activities. These side effects are often called risks, and for this project, the risks are:

1) Approximately 5 ml of blood (1 teaspoon) will be taken from you by placing a needle into one of your veins on two occasions, once at each visit. This is the standard medical method used to obtain blood for tests. There is momentary pain at the time the needle is inserted into the vein, but other discomfort should be minimal. In about 10% of the cases there is a small amount of bleeding under the skin which will produce a bruise. The risk of infection is less than 1 in 1,000.
2) Although the repeated high-intensity exercise test is not any more dangerous or difficult than your typical occupational tasks, there are inherent risks with exercise performance. This presents a risk that a) the participant will sustain a fall and b) the participant will sustain large internal forces on the joints. We mitigate these risks in the following way: Firstly, participants are screened for general health, musculoskeletal health, general mobility and cognitive function. Thirdly, because large internal joint forces could be experienced during the Y-balance and foot clearance tasks, care is taken to minimize the volume of perturbations (the overall number of times that a participant experiences significant disruption to their posture or balance) any one participant experiences. Participants are progressively introduced to the demands of the test by habituating them to the tasks gradually and with warmup. We also refer you to our experience with the method and population of interest: one investigator, Dr David Graham, has 15 years of human biomechanics research in applications which includes the balance recovery methods.
3) There is a risk of breach of confidentiality, and it will be minimized by keeping all documents with identifiable participant information in a filing cabinet in the MSU nutrition laboratory. All other data (motion capture data) will be recorded in an un-identifiable manner. Motion-capture data records movement of the individual markers, and not the participants themselves.
4) The exercise stress test is a standard clinical procedure which is routinely performed on many people every year for diagnosis and evaluation of their condition. Adverse effects are very rare but have occurred. The mortality of this test is approximately 1 in 10,000 tests, and the incidence of serious complications including prolonged abnormality of heart rhythm or prolonged chest pain is approximately 4 in 10,000 tests. The exercise performed in this study is a high intensity functional test of occupational tasks rather than an exercise stress test, but the risk of adverse effects is expected to be comparable to the stress test. High-intensity exercise training has been associated with shortness of breath,

fatigue, light-headedness, and in some cases nausea. In order to minimize these effects, you will do a 5-minute warm-up and be instructed through an active recovery during each exercise session. Active recovery has been associated with a reduction in the afore-mentioned risks. Furthermore, each exercise session will be supervised by the principle investigator and both heart rate and performance time will be recorded. At any point during the study, you are allowed to stop testing.

5) You may also experience local muscle and/or joint soreness following exercise. To reduce (but not completely avoid) the effect of muscle/joint soreness, you will be allowed a time for a warm-up and cool-down, and will be advised about stretching exercises.

Benefits: You may gain some benefits by receiving information from your own personal data collected by investigators. No other benefits are promised to you.

Compensation: You will receive up to $100 upon completion of your testing, $50 for completion of each of the two visits. You may withdraw from the study at any time. If you choose not to complete a condition, then the amount of money paid to you will be prorated depending on how much of the condition is completed.

Freedom of Consent: You have the right to withdraw from participating in the study at any time with a no questions asked policy. You may withdraw in writing, over the phone (to Isaac Rowland at (972) 795-1713), or in person. If you withdraw, you will not lose any benefits you incurred up to the time of withdrawal. Your participation in this study is completely voluntary.

Funding: This study is not externally funded.

Please ask any questions: You are encouraged by the researcher to ask any and all questions you may have, as well as addressing any concerns about the study. The researcher will answer your questions as fully and as accurately as possible. Your peace of mind and comfort in the study is of utmost importance to the researcher.

Confidentiality: All data and information received from you for this study will be kept completely confidential. You will be given a subject identification number that will be used to describe all data. This data will be kept locked in a file cabinet in the Nutrition Research Laboratory. This information could be published in scientific and/or medical journals, but your identity will remain confidential. If you withdraw from the study at any time, all of your information will be deleted from the study records, and you will not be contacted again regarding the study. There are absolutely no penalties for withdrawing.

In the unlikely event of injury to you due to participation in this study, medical treatments such as first aid and help getting to adequate health care providers (such as transport to Bozeman Deaconess Hospital) will be provided, however, there is no compensation for any of this provided by Montana State University. You can access further information involving this policy and treatment by contacting Mary Miles at 994-6678, or emailing her at mmiles@montana.edu).

Any other questions you may have regarding your rights as a participant may be answered by the chairman of the Human Subjects Committee, Mark Quinn. He can be reached at 406-994-4707 or mquinn@montana.edu.

STATEMENT OF AUTHORIZATION

Study Title: Physiological and Motor Control Characteristics of Wildland Firefighters and their Associations with Lower Extremity Injuries and Discomfort

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AUTHORIZATION: I have read the above and understand the discomforts, inconveniences, and risk of this study. I, ______________________(name of subject), agree to participate in this research. I also agree that my health information can be collected and used by the researchers and staff for the research study described in this consent form. I understand that I may later refuse participation and that I may withdraw from the study at that time. I have received a copy of this consent form for my own records.

Signed: ______________________________ Date: ______________________________

Investigator: ___________________________ Date: ______________________________

APPENDIX B

PARQ+ QUESTIONNAIRE

2020 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1) Has your doctor ever said that you have a heart condition ☐ **OR** high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:** ______	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:** ______	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it **does not limit your current ability** to be physically active. **PLEASE LIST CONDITION(S) HERE:** ______	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.
Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow Global Physical Activity Guidelines for your age (https://apps.who.int/iris/handle/10665/44399).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

PARTICIPANT DECLARATION
If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for its records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME ______________________ DATE ______________

SIGNATURE ______________________ WITNESS ______________

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER ______________________

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

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FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1.	**Do you have Arthritis, Osteoporosis, or Back Problems?** If the above condition(s) is/are present, answer questions 1a-1c — If **NO** ☐ go to question 2	
1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	YES ☐ NO ☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES ☐ NO ☐
2.	**Do you currently have Cancer of any kind?** If the above condition(s) is/are present, answer questions 2a-2b — If **NO** ☐ go to question 3	
2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	YES ☐ NO ☐
2b.	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	YES ☐ NO ☐
3.	**Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm** If the above condition(s) is/are present, answer questions 3a-3d — If **NO** ☐ go to question 4	
3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES ☐ NO ☐
3c.	Do you have chronic heart failure?	YES ☐ NO ☐
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	YES ☐ NO ☐
4.	**Do you currently have High Blood Pressure?** If the above condition(s) is/are present, answer questions 4a-4b — If **NO** ☐ go to question 5	
4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)	YES ☐ NO ☐
5.	**Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes** If the above condition(s) is/are present, answer questions 5a-5e — If **NO** ☐ go to question 6	
5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	YES ☐ NO ☐
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	YES ☐ NO ☐
5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	YES ☐ NO ☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES ☐ NO ☐
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES ☐ NO ☐

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6.	**Do you have any Mental Health Problems or Learning Difficulties?** This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome	
	If the above condition(s) is/are present, answer questions 6a-6b	If **NO** ☐ go to question 7
6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
6b.	Do you have Down Syndrome **AND** back problems affecting nerves or muscles?	YES ☐ NO ☐
7.	**Do you have a Respiratory Disease?** This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure	
	If the above condition(s) is/are present, answer questions 7a-7d	If **NO** ☐ go to question 8
7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	YES ☐ NO ☐
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES ☐ NO ☐
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	YES ☐ NO ☐
8.	**Do you have a Spinal Cord Injury?** This includes Tetraplegia and Paraplegia	
	If the above condition(s) is/are present, answer questions 8a-8c	If **NO** ☐ go to question 9
8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	YES ☐ NO ☐
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES ☐ NO ☐
9.	**Have you had a Stroke?** This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event	
	If the above condition(s) is/are present, answer questions 9a-9c	If **NO** ☐ go to question 10
9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
9b.	Do you have any impairment in walking or mobility?	YES ☐ NO ☐
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	YES ☐ NO ☐
10.	**Do you have any other medical condition not listed above or do you have two or more medical conditions?**	
	If you have other medical conditions, answer questions 10a-10c	If **NO** ☐ read the Page 4 recommendations
10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months?	YES ☐ NO ☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES ☐ NO ☐
10c.	Do you currently live with two or more medical conditions?	YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: ______________________

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

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If you answered NO to all of the FOLLOW-UP questions (pgs. 2-3) about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered **YES** to **one or more of the follow-up questions** about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+ at www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ **at www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME ______________________ DATE ______________________

SIGNATURE ______________________ WITNESS ______________________

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER ______________________

For more information, please contact

www.eparmedx.com
Email: eparmedx@gmail.com

Citation for PAR-Q+
Warburton DER, Jamnik VK, Bredin SSD, and Gledhill N on behalf of the PAR-Q+ Collaboration. The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+). Health & Fitness Journal of Canada 4(2):3-23, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

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11-01-2019

APPENDIX C

PHYSICAL ACTIVITY & INJURY HISTORY QUESTIONNAIRE

Physical Activity Level and Injury History Questionnaire

Have you ever suffered from a joint injury to your lower extremities?
If so, please describe:

Have you ever suffered from joint discomfort in your lower extremities?
If so, please describe:

Do you regularly participate in exercise? Approximately how many hours per day and how many days per week?

If you participate in training, what has it mainly consisted of (e.g.: weightlifting, hiking, etc.)?