METABOLIC AND CARDIOVASCULAR MARKER ALTERATIONS DURING CRITICAL TRAINING IN WILDLAND FIREFIGHTERS

Shae Gurney
University of Montana, Missoula

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METABOLIC AND CARDIOVASCULAR MARKER ALTERATIONS DURING CRITICAL TRAINING IN WILDLAND FIREFIGHTERS

By

SHAE CONNER GURNEY

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Committee Members:

Charles Dumke, Ph.D., FACSM, Chair
Integrative Physiology and Athletic Training

John Quindry, Ph.D., FACSM, FCVS-APS
Integrative Physiology and Athletic Training

Joseph Sol, MS, TSAC-F
USDA Forest Service, National Technology and Development Program

Laurie Minns, Ph.D.
Biological Sciences
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1
Metabolic and Cardiovascular Marker Alterations During Critical Training in Wildland Firefighters

Chairperson: Charles Dumke, Ph.D.

**Introduction:** Wildland firefighters (WLFFs) are confronted with numerous physical and mental stressors. Pre-fire season includes an intense 2-week critical training (CT) period; a preparatory phase of multiple activities that can result in injury, illness, and rhabdomyolysis. The purpose of this study was to identify physiologic changes in metabolic, inflammatory, and oxidative stress biomarkers during 2 weeks of CT in WLFFs.

**Methods:** Eighteen male (29.4 ± 1.1 yr, 182.1 ± 1.6 cm) and three female (26.7 ± 2.6 yr, 169.5 ± 4.2 cm) participants were recruited from a Type I interagency hotshot crew and monitored over their 2-week CT. Fitness was assessed via BLM Fitness Challenge (push-ups, pull-ups, sit-ups, 1.5-mile run). Subjects were asked to fast and abstain from caffeine prior to blood draws collected on days 1, 4, 8, and 11. Plasma was analyzed for changes in the metabolic profile, C-reactive protein (CRP) and oxidative stress markers 8-isoprostane (8ISO), 3-nitrotyrosine (3NT), lipid hydroperoxides (LOOH), and protein carbonyls (PC). A one-way repeated measures ANOVA was used to analyze CRP, 8ISO, 3NT, LOOH, and PC. Paired samples t-tests were used to compare metabolic biomarkers. Data presented as mean ± SEM.

**Results:** As a result of the high physical demands occurring during CT, an observed alteration in total cholesterol (172.6 ± 11.4 to 153.9 ± 8.1 mg·dL⁻¹, p=0.011), hemoglobin A1c (5.2 ± 0.1 to 5.1 ± 0.1 %, p=0.003), and estimated plasma volume (53.8 ± 0.7 to 59.3 ± 1.5 %, p=0.007) from day 1 to 11 occurred. No alteration to CRP (p=0.32) or PC (p=0.73) was observed during this time. A main effect for time was observed in 8ISO (p<0.001), 3NT (p=0.033), and LOOH (p=0.001). Push-up score was significantly correlated with ΔTC (r=0.65, p=0.022) and Δ8ISO (r=0.66, p=0.028).

**Conclusions:** These data suggest the exertion required of WLFFs during CT results in positive alterations to the metabolic profile. The changes in oxidative damage markers is suggestive of an acute stress on the body during CT. The fitness data suggests that WLFF adapt quickly to the physical stresses of CT, with fitness potentially offering protection to metabolic alterations.
Chapter 1: Introduction

Wildland firefighters (WLFFs) are confronted with a number of physical and mental stressors throughout their daily activities in the field and base camp. During a standard shift, a WLFF may work for 12 to 16 hours and expend 4000-5000 kcals, with extreme shifts potentially exceeding 6200 kcals\(^1,2\). Variations in energy expenditure between workdays and individuals can be attributed to sex, environmental temperature and relative humidity, terrain, and the work activity pattern of the given shift, in addition to various other individual characteristics\(^1,3\).

In preparation for these stressors and the long arduous season, WLFFs participate in two weeks of critical training (CT). This training is meant to prepare them for both the mental and physical stresses of the occupation through intense physical training and exposure to fieldwork, including exposure to wood smoke. Of the 58,835 injuries in 2017, 8,380 occurred during training, resulting in 14% of all injuries\(^4\). Furthermore, between 2010 and 2014, 26% of fireground injuries were caused by overexertion or strain\(^5\). Overexertion, particularly during early season activities, can lead to deadly complications through the development of rhabdomyolysis, an excessive breakdown of muscle tissue that can lead to kidney failure and death if left untreated. A recent United States Department of Agriculture Forest Service report showed 26 cases of rhabdomyolysis from 2008-2016, of which 17 occurred during physical training\(^6,7\). During 2016, six of the seven reported cases occurred during physical training, including four instances within four consecutive days. Even though rhabdomyolysis is a concern during CT, it develops in relatively few individuals every year. The most frequent injuries, joint or muscle strains of the extremities, are often the result of
slipping, tripping, or falling\textsuperscript{5,8}. Injuries and illness during CT are documented; however, the physiologic alterations that occur during these two weeks have yet to be elucidated. Studies involving military training frequently focus on alterations occurring over 5+ weeks\textsuperscript{9,10}. To the authors’ knowledge, there are currently no studies documenting physiologic alterations that occur to WLFF during two weeks of CT activities.

The stressors that WLFFs face daily make firefighting a dangerous job. Of the 87 US firefighter deaths in 2017, 12 (14\%) occurred during training-related activities\textsuperscript{7,11,12}. The United States Fire Administration (USFA) attributed 52 of these 87 deaths (60\%) to stress or overexertion; the leading cause, heart attacks, resulted in 50 fatalities\textsuperscript{11}. These data from the USFA is limited in that it only includes deaths from on-duty or recently on-duty (24 hours prior) firefighters. Recently, Smith et al. released a fatality study documenting the relationship between the occurrence of cardiac-related deaths and the presence of coronary heart disease (CHD) in the firefighting community. Results from this autopsy study indicate that those with evidence of CHD died more commonly from sudden cardiac events than their noncardiac trauma controls\textsuperscript{13}.

Working in hot environments challenges WLFFs thermoregulatory abilities through high ambient and radiative temperatures, in addition to the mandatory use of personal protective equipment (PPE). Among the PPE required on shift is a helmet, NOMEX shirt, hood, gloves, pants, boots, and pack. The microenvironment within this PPE has been shown to increase heat accumulation\textsuperscript{14-17}. The combination of these factors contributes to the development of uncompensable heat stress in the field when the body is unable to meet the cooling requirements imposed by the environment\textsuperscript{14}. This compounding of heat can lead to cramping, heat exhaustion, heatstroke, and other
heat-related illnesses, threatening the health of WLFFs. These conditions become even more dangerous and challenging to treat when the work is conducted in remote locations where medical evacuation is difficult.

Additionally, WLFFs frequently inhale ambient smoke filled with particulate matter (PM) and chemicals relative to the environment in which they perform work tasks. In an extensive review, Naeher et al. concluded that the PM makeup best characterizes woodsmoke exposure. During wildfires, significantly more ultrafine (<0.1 μm) and fine (<2.5 μm) PM is produced in proportion to coarse (<10 μm) particulates. Of particular concern are ultrafine and fine PM, due to their ability to penetrate deeper into the lungs. As expected during exercise, an increase in minute ventilation results in a linear increase in PM deposition within alveoli. Exposure to PM has been associated with an increase in hospitalizations and emergency room visits to correct exacerbations of chronic respiratory and cardiovascular diseases and acute cardiorespiratory complications including asthma, leading to increases in cause-specific and all-cause mortality. Recently, the International Agency for Research on Cancer classified air pollution and its associated PM as a Group 1 carcinogen, stating that there is sufficient evidence that it causes lung and is likely involved in the development of other cancers in humans. During controlled burns, a common practice during CT, WLFF can be exposed to PM at a concentration upwards of 300 μg·m⁻³.

Even when back at base camp, WLFFs are faced with stress in the form of sleep deprivation as the result of a noisy environment and extended shifts often lasting late into the night. Even though sleep is of vital importance, controlling for sleep schedules in the field is impractical in practice, making field research largely
observational in nature. In order to learn how sleep deprivation directly affects firefighters in subsequent shifts, simulated firefighting conditions with controlled sleep schedules are primarily conducted in labs \textsuperscript{33-35}. In multi-day fires, Australian firefighters have been reported to sleep 3-6 hours between shifts, whereas US firefighters have reported frequently, \~40\% of nights, getting less than 7 hours of sleep \textsuperscript{36,37}. It is well established that sleep deprivation results in worsened physical and mental performance, especially when less than 7 hours of sleep occurs \textsuperscript{38}. In 2016, AAA concluded that drivers who reported <7 hours of sleep were 1.3 to 11.5 times more likely to have sleepiness as a contributing factor to a vehicular crash as compared to those having slept >7 hours \textsuperscript{39}. Collectively, temperature regulation, smoke inhalation, sleep deprivation, and high energy expenditures are critical stressors to WLFFs during CT that likely result in physiologic alterations.

**Null Hypotheses**

1. Individuals will not undergo alterations to body composition as measured by body weight and body fat percentage.

2. Two weeks of CT involving an increase in intense physical activity will not alter concentrations of total cholesterol (TC), triacylglycerides (TG), low-density lipoprotein-cholesterol (LDL-C), and high-density lipoprotein-cholesterol (HDL-C).

3. No change in blood glucose or hemoglobin A1c (A1C) will occur.

4. Hematocrit, hemoglobin, and estimated plasma volume will not change.
5. Markers of metabolic stress in the form of 8-isoprostan (8ISO), lipid hydroperoxides (LOOH), 3-nitrotyrosine (3NT), protein carbonyls (PC), and C-reactive protein (CRP) will not change from physical activity, exposure to smoke, and altered sleep patterns.

**Significance**

To the authors’ knowledge, this is the first study assessing the impact of activities performed during two weeks of CT on general health markers within WLFFs. Between 2008 and 2016, 17 of the 26 documented cases of exertional rhabdomyolysis occurred during CT, owing to the intensity of this pre-season training endeavor. Due to proper education and subsequent response to the appearance of untoward symptoms, WLFFs are unlikely to die of rhabdomyolysis. However, overexertion and stress frequently result in heart attacks and death in the firefighter community. Importantly, chronic conditions such as heart disease are the result of cumulative stressors including metabolic derangement, ongoing inflammation, and unmitigated oxidative stress. Thus, their cardiovascular risk must be assessed adequately from personal and familial history in addition to a strategic panel of biomarkers in the blood. Extensive cohort studies like the Framingham Heart study have established the relationship between poor metabolic health, as measured by the metabolic profile, and heart disease. Previous studies have confirmed that fighting wildland fires is a difficult job and causes numerous alterations to metabolic processes which may lead to detrimental health effects, but many gaps in knowledge still exist.
Delimitations

1. Subjects were male and female WLFF, 18+ years old from the Lolo IHC, which is employed by the USDA Forest Service.
2. The purpose of this study was to identify alterations to biomarkers of interest from baseline values.
3. Subjects were asked to arrive fasted and uncaffeinated on blood draw days.
4. The serological and descriptive data of interest is focused around the four blood draws.

Limitations

1. Subjects were a convenient sampling of WLFF from the local IHC.
2. Before the study began subjects were not asked to refrain from structured physical activity, thus baseline values may be skewed.
3. Subjects diets and activities were not monitored outside the facility.
4. Activity data of individual WLFFs doing their job-specific tasks is outside the scope of this analysis.

Assumptions

1. The Lolo IHC is a representative sample of WLFF in the United States.
2. The observed baseline values are representative of 'normal' daily values of the participants.
3. The self-reported restraint from food and caffeine is assumed to be true.
4. The work tasks performed by the WLFFs is representative of typical shifts during CT.

Definitions

Bureau of Land Management (BLM) Fire Operation Fitness Challenge:

A voluntary fitness test by which WLFFs can measure their personal fitness, track fitness improvement, and receive national recognition. Participants strive to meet established standards in the four tested exercises – sit-ups, push-ups, pull-ups, and a timed 1.5- or 3-mile run. Each exercise is appropriately scored and compiled into a final composite score that is used for recognition criteria.

Critical Training (CT):

Before being available for assignment, WLFFs must complete 40 hours of operations preparedness training involving field exercises, classroom time, physical fitness, and other fire-related activities. Each day, the crew is allowed to perform 1 hour of fitness training in order to build/maintain their aerobic capacity and strength. All WLFFs must pass a work capacity test, which involves an arduous pack test involving walking 3 miles in under 45 minutes while wearing a 45-pound pack.

Inflammation:

Inflammation is a complex biological response to tissue infection or injury to protect and initiate tissue repair. It is typically characterized by its five overt signs of swelling, heat, alteration in function, redness, and pain. Any combination of these signs may be present at the site of injury and the extent of each depends on the mechanism of injury.

Metabolic Profile:
For the sake of this paper, the measured lipid panel, including measurement of TC, TG, LDL-C, HDL-C, non-HDL-C, and LDL/HDL, in addition to measured blood glucose and hemoglobin A1C is cumulatively identified as a ‘metabolic profile’.

_Oxidative Stress:_

Oxidative stress is an imbalance between free radicals, with their uneven number of electrons that readily react with other molecules, and antioxidants that help stabilize these electrons and prevent cellular damage. Oxidation is a normal and necessary process that helps organisms adapt to their environment, but an imbalance between free radicals and antioxidants can result in oxidative damage to lipids, proteins, and DNA. Accumulation of damage and a concomitant increase in inflammatory processes can result in the development of numerous different disease states.

_Wildland Firefighter (WLFFs):_

WLFFs are any seasonal or full-time crewmembers working on the management and detection of wildland or prescribed burns through the use of a variety of equipment and techniques. This study only considers USDA Forest Service employees, namely the Lolo IHC. An IHC is the most highly trained, skilled, and experienced type of handcrew in the United States and thus has specific qualifications for the initial and extended attack on wildfires. Their assignments are typically the highest priority fires that require extensive work in remote locations with little available support, making their work incredibly challenging.
Chapter 2: Literature Review

Overview

Humans are a highly adaptive species in responses to the stress imposed by their environment. Acutely altering certain biological markers promotes survival from the current insult and the chance to live for another day. In response to these fleeting insults, the body responds with the production of a concert of stress responses, identifiable by the elevation in associated biomarkers. Under normal conditions, acute increases in stress biomarkers quickly return to baseline unstressed levels. Importantly, these acute stressors may alter longer lived mediators to help the individual adapt and become better suited to handle a similar stress in the future. Unfortunately, the continual stress that is abundant in our current ecosystem does not always allow these short-lived mediators to return to true baseline, instead remaining slightly elevated chronically. These chronic elevations may result in disease development and ultimately death if left uncorrected. The literature classically addresses acute (<1 week, often a single bout) and chronic (>1 month) adaptations that occur following exercise training.

Specifically within WLFF populations, research is limited in both the early season and over the duration of a fire season. Single bouts of simulated firefighting and participation in prescribed burns have shown alterations to pulmonary function and acute inflammatory markers\textsuperscript{33,34,49,50}. Additionally, a season of firefighting imposes similar changes to pulmonary function with concomitant increases in upper and lower respiratory symptoms including nose and throat irritation, cough, phlegm, and wheezing\textsuperscript{51}. Recently, Coker et al. identified an increase in total and visceral body fat, total and LDL cholesterol, and total globulin following a season of Alaskan wildland firefighting.
These findings collectively suggest that wildland firefighting can lead to metabolic disruptions both acutely and chronically. Early season firefighting, defined as January – June, from 2003-2007 resulted in 355 (27%) injuries, mainly in the form of musculoskeletal injuries to the extremities as the result of slips, trips, and falls. In comparison, of the 58,835 injuries that occurred during 2017, 14% (8,380) occurred during training activities. Metabolic alterations that arise during this time are mostly unknown, with incident reports frequently describing cases of rhabdomyolysis and heat-related illnesses. To prepare for the rigors of the upcoming season, WLFFs participate in a two week CT assignment that tests their physical fitness and prepares them for their job-specific tasks. This study aims to address how the intermediate duration, high intensity stress imposed upon WLFF during their 2 weeks of CT alters various metabolic biomarkers.

**Oxidative Stress**

Oxidative stress with its acute insults and resultant damage to cellular components, is an essential factor in the development of cardiovascular disease. Homeostasis within complex biological systems can be thought of like a teeter-totter, damaging free radicals on one side and protective antioxidants on the other. Tipping of the balance towards increased free radical generation is termed oxidative stress (OS), nitrative stress if derived from nitrogen species, and ultimately results in a concomitant decrease in antioxidants. Environmental stimuli, such as exercise and radiation, result in free radical generation by various organelles in response to the stresses they encounter. These free radicals can start a cascade of chain reactions unbelievably quickly,
resulting in damage to carbohydrates, proteins, lipids, and nucleic acids as an unpaired electron is passed from one carrier to another in rapid succession. The reactive species that primarily initiate these chain reactions include superoxide, hydroxyl radical (OH-), hydrogen peroxide (H₂O₂), singlet oxygen, nitric oxide, peroxynitrite, and hyperchlorite ⁴⁷. Passing of an unpaired electron is based upon the reductive potential of the carriers and proceeds in a stepwise fashion until either two free radicals are conjugated or a chain-breaking antioxidant terminates the reaction. It is important to note that these chain reactions are often separated into an individual cell via organelle membranes or compartmental space (e.g. plasma) via the cell’s plasma membrane. Transmission of radicals across these membranes is a relatively rare occurrence unless membrane disruption has occurred, allowing the contents of a cell to spill into the intercellular space (or an organelle spilling its contents into the cytoplasm). H₂O₂ is one of the few reactive oxygen species (ROS) that readily diffuse across membranes and is considered an exception to the aforementioned rule. H₂O₂ can inactivate some enzymes directly or in the presence of reduced copper/iron produce the highly reactive and damaging hydroxyl radical ⁴⁷.

Accordingly, identification of OS does not occur through quantification of the incredibly short-lived free radicals but instead focuses on the ‘shadows’ left behind. These ‘shadows’ happen in the form of either antioxidant attenuation or development of damage markers. The accumulation of damage and inability to quench free radicals sufficiently can result in an increased inflammatory state. Due to redundancy in metabolic pathways and the consequent ability of the human body to readily quench free radicals through various mechanisms, antioxidant alterations are difficult to quantify
even if conducted in controlled laboratory settings. Thus, the antioxidant quantification measures Trolox Equivalevent of Antioxidant Capacity (TEAC) and Ferric Reducing Ability of Plasma (FRAP) were excluded from this field-based study. The chain reactions mentioned above result in almost infinite chemical damage marker formation but can be summarized by modifications to proteins, lipids, and DNA, which are especially prone to the toxic effects of radical production. To have confidence in their roles in oxidative damage, markers of choice must be relatively stable end products of radical damage.

In choosing the protein and lipid oxidative damage markers, consideration of their production and clearance rates were necessary. Thus, short and long term markers of lipid, 8-isoprostane (8ISO) and lipid hydroperoxides (LOOH) respectively, and protein damage, 3-nitrotyrosine (3NT) and protein carbonyls (PC), were chosen. Although currently unconfirmed, it is believed that blood is the primary source of measured OS, with oxidative damage markers occurring to specific lipids/proteins in solution. The RBC itself appears to be the primary site of lipid damage. It is currently believed that alterations of 8ISO are related to arachidonic acid metabolism and subsequent signaling through the production of various prostaglandin like molecules. Thus, the rapid spike and subsequent clearance of 8ISO is due to the labile nature of arachidonic acid formation following cleavage from the plasma membrane, with successive metabolism to prostaglandins ultimately resulting in 8ISO formation. Alternatively, LOOH are more reflective of membrane bound molecules and are formed from either a hydrogen abstraction or oxygen radical addition to the membrane-embedded, radically sensitive polyunsaturated fatty acid. This radical addition results in bond rearrangements within the polyunsaturated fatty acid such that it becomes capable of abstracting an additional
hydrogen and initiating a chain reaction. This leads to the propagation of lipid peroxidation until either two free radicals conjugate or a chain-breaking enzyme is encountered. This unique mechanism allows for numerous LOOH to form as the direct result of a single free radical insult. Previous research has shown increases in LOOH formation following long duration and high-intensity exercise, with peaks occurring during recovery from the exercise bout.

Where oxidative lipid damage is more selectively isolated to the RBC lipid membrane, oxidative protein damage markers are associated with specific amino acid residues, instead of particular proteins, in plasma. Measurement of protein oxidation is critical in oxidative damage simply due to the absolute abundance of protein (much greater than lipids, carbohydrates, or free DNA) in plasma and leukocytes, all of which are potential targets of damaging oxidants. The production of 3NT is the result of nitration of the non-essential amino acid tyrosine by reactive nitrogen species or by the creation of tyrosine radicals by metalloenzymes like myeloperoxidase. Over 40 different proteins have been identified as carriers of 3NT modification in blood. This alteration to proteins likely results in a decreased functionality, thus requiring elimination from the bloodstream so a functional protein may take its place. By comparison, PC formation is due to direct oxidation of thiol bonds, largely in the form of cysteine residues. Although the target of protein oxidation is largely unknown, it is reasonable to suspect that albumin, with its high abundance in plasma and a cysteine residue that is readily oxidized during exercise, is the primary source. If this is the case, albumin may serve as a sacrificial protein to aid in protection against oxidative damage.
Damage caused by OS occurs in an intensity and duration-dependent manner, with exercise, altitude, and smoke exposure as potential causal agents. Interestingly, exercise improves control of ROS production as mitochondria fail to increase ROS production in proportion to the energy demand of exercise. Thus, state-III mitochondrial respiration (ADP dependent; exercise) produces proportionally less ROS than state-IV respiration (ATP dependent; rest). Exercise acutely results in OS outside of redox balance that is an adaptive stimulus to upregulate antioxidants and other beneficial gene products as summarized in various reviews. It is important to note that while mitochondrial-derived ROS from acute bouts of exercise are important in activating redox-sensitive gene transcripts and their respective protein products, much of the ROS generated during the bout is enzymatically derived. In response to exercise and other stimulation, the enzymes myeloperoxidase, NADPH oxidase, and xanthine oxidase have been shown to produce significant amounts of hyperchlorite from H$_2$O$_2$ (myeloperoxidase) or superoxide via transfer of an electron to O$_2$ (NADPH oxidase and xanthine oxidase).

Many groups have quantified OS using various biomarkers across many modalities and time durations. Field studies at the 160km Western States Endurance Run can last upwards of 30 hours as researchers wait for runners to stumble across the finish line. Studies examining lipid oxidation with 8ISO, classically called F2-isoprostanes, frequently show augmentation from pre- to post-exercise, with levels remaining elevated for upwards of 90 minutes of recovery before returning to baseline within 24 hours. Additionally, LOOH measurement following exercise bouts lasting 60+ minutes and lasting upwards of 20 hours frequently show an increase in
LOOH, with additional increases during recovery\textsuperscript{59-61}. Supplementation of antioxidants around exercise has been shown to attenuate alterations of 8ISO, but have failed to exert any effect on PC concentrations from baseline\textsuperscript{75,76}. Protein oxidation, as evidenced by increases in 3NT and PC, has been primarily observed following maximal intensity or long-duration exercise, however, wide interindividual variability in PC is frequently observed\textsuperscript{58,77-80}. Training status and gender may influence the extent of 3NT and PC augmentation\textsuperscript{75,78,80}. It would be expected that post-exercise antioxidant measurements, FRAP and TEAC, would be lower than at baseline. However, increased circulating uric acid, a potent aqueous plasma antioxidant from upregulated purine metabolism, causes these measurements to increase\textsuperscript{75,76}. Exogenous supplementation of uric acid followed by 20 minutes of cycling at 80W showed a concomitant increase in TEAC, which completely attenuated 8ISO production\textsuperscript{81}. In exercise lasting anywhere between 20 minutes and 30 hours, either no change or an increase in TEAC and FRAP is frequently observed\textsuperscript{60,61,75,76,82}. Measurement time of selected OS markers must be carefully considered since their different appearance and clearance rates drastically affect the magnitude of change peri-exercise\textsuperscript{82}.

Another critical consideration to keep in mind is the effect of muscle-damaging exercise, often achieved through eccentric exercise, on these oxidative damage and antioxidant status markers. Common blood markers to assess muscular damage include creatine kinase, lactate dehydrogenase, and myoglobin, which are normally found within muscle cells. Thus, their appearance in the blood is suggestive of muscle cell membrane disruption. Though these muscular disruption markers are not directly measured in conjunction with oxidative stress markers in the Western States Endurance
Run and Kona Triathlon World Championship, it is safe to assume based on previous studies that these incredibly strenuous events result in significant muscle damage. These races have shown elevations from baseline in 8ISO, LOOH, and FRAP during and immediately after the event. In a more controlled laboratory setting, heavy and slow eccentric exercise is used to elicit muscle damage from single exercise bouts. A single exercise trial consisting of 3 sets of 50 eccentric contractions on one leg resulted in no alteration to circulating LOOH or TEAC for up to 96-hours following the trial. However, creatine kinase and PC were elevated 24-, 48-, and 72-hours after the acute bout of exercise. This concomitant increase in creatine kinase and PC has been repeatedly observed in elbow flexion/extension and soccer-related muscle damage.

Treadmill max tests to exhaustion have elicited concomitant increases in 3NT, creatine kinase, and lactate dehydrogenase in men and women immediately after exercise. These data suggest that muscle-damaging exercise results in increased oxidative damage markers.

The effects of exercise at high altitude (often >3000m) on OS is examined through either field research in the mountains or well-controlled laboratory studies exposing subjects to normobaric hypoxia in environmental chambers. Altitude studies are complicated by the fact that with increasing altitude there is a proportional decrease in the concentration of inspired O₂. Slow hiking at 8000m can approach a relative intensity of 100% VO₂max, showcasing how the relative intensity of exercise at a given workload is significantly higher as altitude increases. Thus, the measurement of OS as the result of exercise at altitude may be more directly related to the exercise intensity than the altitude itself. In a well-controlled normobaric hypoxic study, Debevec et al.
investigated how twice-daily exercise bouts affects lowland natives during ten days of acclimatization at a 4000m simulated altitude. Exercise participants cycled two 60-minute periods daily at a target heart rate corresponding with 50% altitude specific maximal aerobic power and were compared to sedentary counterparts. After ten days, a marked increase in hemoglobin and hematocrit were observed in both groups that returned to baseline after 24 hours recovery in normoxic conditions. The sedentary group showed higher OS, as evidenced by an increase in 3NT, than exercise subjects following the ten days. Elevations in 8ISO have been observed following hiking and driving ascents to altitude with returns to baseline following days of acclimatization. LOOH is frequently elevated in normobaric hypoxic conditions, particularly during recovery from exercise. Altitude alterations of 3NT are mixed, with either acute increases due to exercise or no change across multiple days. As in normobaric normoxic conditions, alterations to PC are mixed with normobaric hypoxic recovery conditions exhibiting either an increase or no change from baseline. TEAC and FRAP are consistently higher following acute exercise bouts at altitude. Hypoxic exposure appears to attenuate the rise in TEAC when compared to normoxic conditions.

Previously, our group has shown alterations to OS following 90 minutes of simulated firefighting involving controlled woodsmoke inhalation in Missoula, Montana, USA (980m). On three trial days with one-week washout between each, participants walked at a moderate intensity for 90 minutes while breathing either clean air, low exposure (250 μg·m⁻³), or high exposure (500 μg·m⁻³) PM₂.₅ woodsmoke; exposure conditions were pooled for data analysis. Blood was obtained pre-, immediately post,
and 1-hour post-exercise. Immediately post-exercise, an increase in 8ISO, 3NT, and TEAC were observed, while LOOH was decreased at 1-hour post. PC exhibited no change at all time points in all conditions. Only TEAC remained elevated following an hour of recovery, which, when combined with the observed decrease in uric acid, the authors concluded still represented a slight decrease in antioxidant capacity as expected 93. It is concerning that this relatively short duration and low concentration of woodsmoke showed alterations in OS markers, necessitating field trials to better understand the stresses WLFFs experience.

**Inflammation**

Inflammation is characterized by a unique combination of pro- and anti-inflammatory markers that are dependent upon pathologies/disease states, diet, and recent exercise. Low-grade systemic, chronic inflammation is characterized by increased C-reactive protein (CRP) concentrations and blood-borne cytokines 94. Local inflammation is the body’s response to infection or tissue injury that results in the production of cytokines designed to mount the appropriate immune response to heal tissues and clear pathogens. This acute phase response results in a localized several-fold increase in cytokines that are cleared once the tissue is healed and pathogen is cleared. The chronic low-grade inflammation refers to the two- to three-fold higher baseline of these same cytokines that exert their effects systemically instead of locally. This chronic inflammation has repeatedly been associated with cardiovascular disease development 95.
One important cytokine of note, particularly in the field of exercise physiology, is interleukin-6 (IL-6). Muscle derived IL-6 acts like a hormone to mobilize substrates for energy production. When IL-6 activates membrane-bound receptors in adipose tissue and the liver, an increase in lipolysis and fatty acid oxidation and gluconeogenesis is observed in the respective tissues. Importantly, IL-6 is considered a pro-inflammatory mediator but is implicated in both anti- and pro-inflammatory pathways, with its effects mostly dependent on its receptor binding and subsequent signaling cascade in addition to the surrounding milieu of plasma cytokines.

A marked increase in circulating IL-6 from less than 1 pg·mL\(^{-1}\) in trained individuals at rest up to 100 pg·mL\(^{-1}\) during and following an acute bout of exercise is consistently observed across many modalities. This increase is mostly duration dependent and quickly declines following the cessation of exercise as it is rapidly cleared from the plasma. A 10-month exercise program involving 40 minutes of walking/running at ~60%HR\(_{\text{max}}\) with untrained middle-aged women, showed a decrease in overnight fasted IL-6 levels. A similar reduction in resting IL-6 levels has also been observed in elite Taekwondo athletes following four weeks of training. However, 7-days of intensive winter military training resulted in increased IL-6, which can be attributed to the high-intensity work performed. This is supported by an increase in IL-6 during a 5-day combat course that was completed following three weeks of officer school physical training. Since IL-6 is a relatively short lived marker that is generally cleared from circulation within 24 hours of exercise termination, it is considered a non-ideal marker of inflammation for our investigation.
In comparison, a widely accepted marker of systemic inflammation from any number of causative agents is CRP. Thus, chronic elevations of CRP have been associated with various disease states, including atherosclerosis and CVD \cite{110}. CRP concentrations appear unchanged following maximal intensity exercise \cite{111}, but show increases following ultra-endurance events \cite{104}. This is not surprising since increased levels of CRP typically do not manifest until 8-12 hours after the initiation of exercise, likely as the result of increased IL-6 activation of the STAT3 pathway in the liver \cite{99,100}. Exercise programs ranging from 1-18 months have shown efficacy in diminishing resting CRP values in both healthy and obese individuals regardless of whether or not they lost any weight from the intervention, however individual responses are highly variable \cite{112}. Attenuations of CRP following exercise training are maintained for at least one week of detraining \cite{113}. Ten weeks of military training has been shown to significantly increase CRP concentrations in both smokers and non-smokers, with smokers being elevated above non-smokers at all time points \cite{114}. Studies involving WLFFs on a controlled burn and non-burn days have shown nonsignificant increases in pre- to post-shift as well as pre-shift to the morning after CRP values \cite{30,115}. These WLFF were recruited to participate during a prescribed burning season and sampled on burning and non-burning workdays with each individual serving as their control. Blood sampling days were selected randomly throughout these seasons. The observed lack of significance in these studies is likely due to the wide interindividual variability. Taken collectively, these results suggest that this study will not see observable alterations to CRP concentrations during CT.
Metabolic Profile

Measurement of the metabolic profile through blood lipids and glucose is an essential step in stratifying an individual's cardiovascular risk. Lipoproteins are primarily involved in the transportation of fatty molecules throughout the body from both ingested/exogenous and stored/endogenous sources. There are five major categories of lipoproteins, with different functions and sizes, in circulation. These categories from largest to smallest and most dense are chylomicrons and associated chylomicron remnants, very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoprotein (HDL).

In short, chylomicrons are involved in the transport of dietary lipids from the intestines to muscle and adipose tissue via lymphatic drainage from the thoracic duct into systemic circulation. VLDL, IDL, LDL, and HDL particles are involved in the transportation of endogenous lipids from one organ to another to assist in cell maintenance, hormone production, provide fuel for metabolism, and numerous other processes. VLDL particles are produced in the liver and are especially rich in TG, resulting in its relatively low density and large size. As TG is removed from VLDL, the particle decreases in size and increases in density due to the loss of the large hydrophobic fatty acid chains and is gradually transformed into IDL. The continued extraction of TG from these particles along with alterations in apolipoprotein content results in the production of smaller and denser categories of lipoproteins that are further enriched with cholesterol stores. All major categories of the lipoproteins mentioned above, besides HDL, are considered pro-atherogenic with high circulating levels being associated with an increased risk of cardiovascular events. HDL particles that are
formed in the liver play an important role in the removal of cholesterol molecules from peripheral tissues, which may be one mechanism of its anti-atherogenic action, with higher levels associated with a healthy cardiovascular system \(^\text{116}\).

The most heavily studied lipoproteins of interest are the LDL and HDL particles, which are associated with many disease states. However, the number of lipoprotein particles, LDL-P and HDL-P respectively, in circulation has not been the measure classically described in the literature. Instead, the primary focus has been on the total mass of cholesterol carried within the lipoproteins, LDL-C and HDL-C. TC, TG, LDL-C, and HDL-C are considered typical lipid panel markers and are commonly used as screening criteria for health outcomes. The American College of Sports Medicine currently recommends individuals seek to meet the following criteria: TC <200 mg·dL\(^{-1}\), TG <150 mg·dL\(^{-1}\), LDL-C <100 mg·dL\(^{-1}\), and HDL-C ≥ 60 mg·dL\(^{-1}\) (a negative cardiovascular risk factor during assessment) \(^\text{118}\). Besides HDL-C, lipid panel markers are considered ‘better’ or ‘improved’ at lower concentrations. Dyslipidemia, the dysregulation of these lipoproteins away from the optimal levels, in and of itself can be considered a disease and is also associated with cardiovascular disease, atherosclerosis, diabetes, stroke, cancer, and numerous other metabolic diseases \(^\text{119,120}\).

Alterations to these parameters may occur in either a short- or long-term manner and are dependent upon their roles in circulation. Acute bouts of exercise at 60% \(\text{VO}_{2\text{max}}\) for 2 hours show no changes in HDL-C or LDL-C concentrations \(^\text{121}\). Ironman and half-Ironman competitors have shown improved HDL-C and LDL-C levels during a single race \(^\text{104}\). Still, most literature suggests that more extended exercise programs are
needed to elicit changes in HDL-C and LDL-C. During and immediately following exercise, TG and TC either remain the same or decrease depending on study conditions. Alterations to the metabolic profile from an acute bout of exercise may be significantly altered by plasma volume shifts, which is often not mentioned in studies. For up to 36 hours following a single exercise bout, a decrease in TG and TC can be observed with trained individuals showing a more significant reduction than their untrained counterparts.

Favorable changes in these lipid panel parameters are consistently seen following the implementation of an exercise plan. In as few as four days involving 20-minute training sessions at 75% HR$_{\text{max}}$ in healthy sedentary men, a decrease in TC and TG with a trend toward decreasing LDL-C has been observed without alterations to HDL-C levels. All changes were lost after two days of rest. Increases in HDL-C and decreases in LDL-C are frequently observed from aerobic- and resistance-based programs lasting at least six weeks in duration with alterations appearing to be dose-dependent. Within WLFFs, data about alterations to lipid panel parameters is limited. Across a fire season in IHCs and another crew type, elevations in TC and LDL-C have recently been observed. These alterations, in conjunction with an increase in body weight and body fat percentage, were considered to be maladaptive to metabolic and cardiovascular health. This data is concerning and warrants investigation into potential metabolic alterations resulting from training. Since training in WLFFs is previously unstudied, military training provides a suitable model of training adaptations. Twelve weeks of intense physical training in Israeli military recruits have shown an increase in TC, TG, and HDL-C following six weeks of training reaching energy expenditures of
~11,500 kcals·week\(^{-1}\)\(^9\). At 12 weeks, HDL-C was further augmented while TC and TG remained elevated from the 6-week measurement. LDL-C decreased following a doubling of weekly energy expenditure during the second 6 weeks of the study\(^9\). Following four weeks of physical training, desirable changes in TC (-5.9%), LDL-C (-15.9%), and HDL-C (+31%) have been observed in US Navy SEAL trainees\(^10\). Additionally, five days of severe physical and psychological stress, termed "hell week," resulted in further improvements of TC (-17.2%), LDL-C (-30%), and HDL-C (+12.1%) were observed\(^10\).

In addition to blood lipid composition, blood glucose maintenance is an important determinant of metabolic and cardiovascular risk. During exercise, the body provides the substrate for metabolism mainly in the form of either fatty acids or glucose. At lower intensities, the more effective use of seemingly endless stores of fat allows for energy demands to be met without using up the significantly lesser stores of carbohydrate. As intensity increases, reliance on glucose rises as faster energy production is required through glycolysis. Blood glucose levels increase markedly at high intensities while remaining within a reasonably tight range at moderate and low intensities\(^130\). Aerobic exercise exerts varying effects on plasma glucose levels that are dependent upon intensity and duration\(^131,132\). Following acute bouts of exercise, plasma glucose concentrations can remain elevated for the next 24-72 hours as insulin-dependent and independent transportation of glucose into tissues is upregulated for energy production and glycogen replenishment\(^133\). Consistent exercise bouts result in increased glucose control and more tightly controlled plasma glucose levels during exercise in trained and untrained individuals\(^134\). Exercise programs frequently fail to alter fasting blood glucose
levels, instead altering insulin sensitivity, unless individuals are severely untrained or pre-diabetic \(^{134-136}\). The clinically relevant measure is fasting blood glucose, healthy \(<100 \text{ mg·dL}^{-1}\), which is used to help determine diabetes status \(^{137}\).

Another clinically significant marker for the determination of diabetes and long-term blood glucose levels is glycated hemoglobin or hemoglobin A1c (A1C). The American Diabetes Association classifies a normal A1C level as \(<5.7\%\), pre-diabetes 5.7\%-6.4\%, and diabetes \(>6.4\%\) \(^{137}\). A1C measures the amount of hemoglobin, the oxygen-carrying protein of red blood cells (RBC), that has been irreversibly glycated. Since RBCs have a lifespan of \(\sim 120\) days, A1C is thought to give a weighted average of blood glucose levels over the previous three months \(^{138}\). As a long-term marker of blood glucose, acute exercise does not result in alterations to A1C. Prolonged exercise programs have shown mixed results in changes to A1C, with more significant changes occurring in diabetic subjects \(^{136,139,140}\).

**Rationale**

Free radical production resulting in oxidative damage and acute inflammation can be considered an adaptive stimulus to better prepare an individual for a later stressor. However, if this inflammatory state transitions to a chronic phenotype characterized by increased circulating inflammatory cytokines, the risk of disease development dramatically rises. In particular, the development of cardiovascular disease (CVD) and its potential to result in acute myocardial infarctions is a key consideration in the firefighting community. Of the 87 firefighters that died in 2017, 50 deaths were directly attributed to heart attacks \(^{40}\). Previous investigations have shown associations between
increased short lived oxidative damage markers (namely isoprostanes) and CVD risk \textsuperscript{54,141}. Additionally, cohort studies have identified metabolic panel disturbances associated with CVD \textsuperscript{41}. A combination approach assessing short term radical damage, systemic inflammation, and a metabolic profile will help establish CVD risk in this IHC. This study is the first examining physiologic changes that occur during CT in WLFFs and will continue to grow the body of research by assessing effects on oxidative damage and metabolic biomarkers associated with CVD. Additionally, the investigation takes a novel approach in examining the two-week training period utilized annually in WLFFs, as compared to a single day and seasonal alterations in the literature.
Chapter 3: Methodology

Subjects

Eighteen male and three female participants were recruited from the Lolo IHC in Western Montana and monitored for the duration of their two-week CT period. Participants completed and signed a PAR-Q+ (Appendix E) and informed consent form (Appendix F) approved by the University of Montana Institutional Review Board (#50-19) before participating in the study. Participants were allowed to withdraw from the study in part or in whole at any time.

Experimental Design

Throughout the study, participants were asked to arrive in the morning, both fasted and uncaffeinated for blood draws. Upon daily arrival, they each recorded body weight to the nearest tenth of a kilogram (Salter Brecknell, Fairmont, Minnesota) and completed an upper and lower body soreness scale (Appendix G). Blood collection occurred on four days (1, 4, 8, 11), Monday and Thursday of each week. On the first day of reporting to their crew, participants completed initial paperwork including a questionnaire to evaluate current and recent training practices (Appendix C), PAR-Q+ (Appendix E), informed consent (Appendix F), and an initial muscle soreness scale (Appendix G) for both the upper and lower extremities. This was followed by measurement of height, weight, body composition, and a blood draw. During the study, subjects participated in 2 strenuous training hikes. Day 1 consisted of a 1.3-mile hike and 1795 feet of elevation gain with their 45-pound packs (2.6 miles total). The chainsaw ‘saw appreciation’ hike on day 10 involved a 1.9-mile hike and 1729 feet of
elelevation gain with their packs and chainsaws (3.8 miles total). The schedule for the remainder of the study was as follows in Figure 1.

![Figure 1: Schematic for the two weeks of critical training.](Image)

**Body Composition**

Body composition was obtained on days 1 and 11, as outlined in Figure 1, using a three-site skinfold method measuring tricep, thigh, and suprailliac skinfolds for females, chest, abdomen, and thigh for males. These skinfolds were conducted in rotational order with calibrated Lange skinfold calipers (Beta Technology, Santa Cruz, California) until two measurements within ±2 mm were obtained by the same technician. Body density was calculated using established gender-specific formulas and then converted to body fat using the Siri equation.
**Females**

\[ D_b = 1.0994921 - (0.0009929 \times \sum 3) + (0.0000023 \times \sum 3^2) - (0.0001392 \times \text{age}) \]

(Sum of 3 skinfolds (mm) = triceps + suprailium + thigh)

**Males**

\[ D_b = 1.10938 - (0.0008267 \times \sum 3) + (0.0000016 \times \sum 3^2) - (0.0002574 \times \text{age}) \]

(Sum of 3 skinfolds (mm) = chest + abdomen + thigh)

**Siri**

\[ \% \text{ body fat} = \frac{495}{D_b} - 450 \]

**BLM Fitness Challenge**

To assess the fitness of WLFFs at the beginning of their season, subjects participated in the BLM Fitness Challenge in which they performed as many reps as possible in three minutes for push-ups, pull-ups, and sit-ups in addition to a 1.5-mile run as fast as possible. The exercises were performed in any desired order, while a maximum rest time of 7 minutes between exercises was observed. Full instructions for the BLM Fitness Challenge can be found in Appendix A. Individual results were scored for each event and then totaled using the established score sheet for a maximum score of 100 points per event, 400 points total (Appendix B). In the case of a rep/time having multiple possible point assignments, the highest score was recorded. Individuals can be nationally recognized and fall into one of five categories based on their total score and scores per event: Level 1 – 100 points, ≥20 points/event; Level 2 – 100 points, ≥25 points/event; Level 3 – 200 points, ≥25 points/event; Level 4 – 300 points, ≥25 points/event; Level 5 – 400 points (max score).
**Blood Handling**

Blood was collected from an antecubital venous draw into a 10 mL sodium heparin blood collection vacutainer (Becton, Dickinson and Company, Franklin Lakes, New Jersey). For hematocrit, three heparinized micro-hematocrit capillary tubes (Kimble, Rockwood, Tennessee) were pulled from each vacutainer and spun at 12,000 rpm for 8 minutes in a hematocrit centrifuge (GF Health Products, Inc., Atlanta, Georgia). The plasma volume and RBC volume for each capillary tube were measured using a zeroed electronic digital caliper and then averaged for each participant. Alterations to blood volume, cell volume, and plasma volume were calculated using the previously established lab calculations of Dill & Costill 145. Day 1 changes in blood, cell, and plasma volume were assigned a value of zero based on previous work 146.

From each vacutainer during the four blood draws, 10 μL of whole blood was transferred to a hemoglobin microcuvette (Hemocue Hb 201+, Brea, California) and placed in an analyzer (Hemocue Hb 201+, Brea, California) to determine hemoglobin concentrations. To measure hemoglobin A1c on days 1 and 11, 1 μL of whole blood was transferred into a DCA HbA1c reagent kit (Siemens Healthineers, Malvern, Pennsylvania) and loaded into a DCA Vantage Analyzer (Siemens Healthineers, Malvern, Pennsylvania). On days 1 and 11, 40 μL of whole blood was transferred to a Cholestech LDX lipid profile – GLU cassette (Alere, Waltham, Massachusetts) which was placed into a Cholestech LDX analyzer (Alere, Waltham, Massachusetts) to measure the basic metabolic profile, including TC, TG, LDL-C, HDL-C, non-HDL-C, LDL/HDL, and glucose.
The remaining whole blood was centrifuged at 2,000 rpm for 10 minutes in a vacutainer centrifuge (Ohaus Corporation, Parsippany, New Jersey), plasma was aliquoted into multiple 1.5 mL microcentrifuge tubes (Thermo Scientific, San Diego, California) and stored at -80°C for later analysis.

**Plasma Assays**

8ISO: Commercially available enzyme linked immunosorbent assay (ELISA) was used according to manufacturer specifications for quantification of 8ISO (Cayman Chemical, Ann Arbor, MI) from plasma. Plasma was diluted 1:2 with ELISA buffer (100 µL plasma was added to 100 µL ELISA buffer) and read by spectroscopy at 420 nm.

LOOH: Plasma LOOH was assessed using the ferrous oxidation-xylenol orange assay. This process involves incubation of 60 µL plasma either with/without a reducing agent followed by the addition of a work solution containing xylenol orange, butylated hydroxytoluene, and ferrous ammonium sulfate. The formed ferrous complex was read by spectroscopy at 560 nm and compared to a standard reaction.

3NT: Plasma 3NT was quantified using commercial ELISA kits (Cell Biolabs INC, San Diego, CA) following a Bradford procedure with phosphate buffered saline (PBS) to obtain a dilution of 20 mg·mL⁻¹ protein concentration and read spectrophotometrically at 450 nm, consistent with previous work.

PC: Plasma samples were measured using commercially available ELISA kits (Enzo Life Sciences, Farmingdale, NY). Following a Bradford protein quantification protocol, samples were diluted to 40 mg·mL⁻¹ with PBS, added to the ELISA plate, and read at 450 nm.

CRP: Plasma CRP was analyzed using commercially made ELISA (Cayman Chemical, Ann Arbor, MI) without the
recommended extraction protocol. Plasma was diluted to a total volume of 1 mL using a 1:200 ratio (5 μL plasma was added to 995 μL assay buffer). All solutions were reconstituted/diluted with the appropriately diluted assay buffer. Reconstitution of assay buffer is best achieved by pouring most of the powder into a large media bottle, with the packet subsequently washed thoroughly with previously set aside deionized water and a transfer pipette. The plate was developed according to manufacturer specifications and read at 450 nm. All assays were completed with a coefficient of variation of <10%.

**Statistical Analysis**

The lipid panel, blood glucose, A1C, hematocrit, hemoglobin, plasma volume, and body composition were analyzed using two-tailed paired-sample t-tests. Plasma assays were analyzed using a one-way repeated-measures ANOVA. If a main effect of time was found, a Bonferroni post hoc analysis was done. Two-tailed pearson-correlations were run against fitness scores, pretraining questionnaire responses, changes in blood markers from day 1 to 11, and just day 1 to assess baseline relationships. Results were considered to be statistically significant at p<0.05. Data are reported as mean ± SEM. Statistics were run using SPSS v.26.
Chapter 4: Results

Descriptive Data

Eighteen males (29.4 ± 1.1 yr, 182.1 ± 1.6 cm) and three females (26.7 ± 2.6 yr, 169.5 ± 4.2 cm) participated in CT. Male and female data are pooled for the remainder of the analysis. On the first day of training, subjects completed a pretraining questionnaire (Appendix C) examining their training practices in the months leading up to the initiation of the season. The crew prepared for the season for 3.9 ± 1.0 months while training 4.6 ± 1.3 days per week, with sessions lasting 87.0 ± 28.2 minutes (Appendix D). In the 48-hours leading up to the initiation of CT, individuals were categorized into three groups based upon their self-reported written description of their activities; no physical activity, light physical activity, and moderate-high physical activity. Responses were relatively evenly spread with eight (40%) in no physical activity, seven (35%) in light, and five (25%) in moderate-high (Appendix D).

During CT, WLFFs exhibited an increase in BMI (p=0.044) while decreasing body fat percentage (p=0.002) from day 1 to day 11 (Table 1). A trend, but non-significant increase in body weight was observed during this time (p=0.065).

Table 1: Body composition alterations during 11 days of critical training.

<table>
<thead>
<tr>
<th>Metabolic Profile</th>
<th>Desirable Values</th>
<th>Day 1</th>
<th>Day 11</th>
<th>p</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight, kg</td>
<td>Variable</td>
<td>79.4 ± 2.7</td>
<td>79.9 ± 2.6</td>
<td>0.065</td>
<td>18</td>
</tr>
<tr>
<td>BMI, kg·(m²)⁻¹</td>
<td>18.5 - 25</td>
<td>24.4 ± 0.6</td>
<td>24.6 ± 0.6*</td>
<td>0.044</td>
<td>18</td>
</tr>
<tr>
<td>Body Fat, %</td>
<td>Age/gender dependent</td>
<td>15.2 ± 1.4</td>
<td>14.1 ± 1.3*</td>
<td>0.002</td>
<td>19</td>
</tr>
</tbody>
</table>

Data presented as Mean ± SEM. * significant difference (p<0.05)
Blood Makeup

Subjects exhibited a decrease in hemoglobin ($p=0.003$) and increases in estimated blood volume ($p=0.005$), cell volume ($p=0.005$), and plasma volume ($p=0.007$) (Table 2). A significant increase in the change in blood volume ($Δ 8.1 ± 2.5\%$, $p=0.005$), cell volume ($Δ 6.6 ± 2.0\%$, $p=0.004$), and plasma volume ($Δ 10.5 ± 3.2\%$, $p=0.005$) from day 1 to 11 was also observed.

Metabolic Profile

Many metabolic profile markers were reduced over the course of CT (Table 2). The lipid panel showed a significant decrease in TC ($p=0.011$), LDL-C ($p=0.016$), and Non-HDL-C ($p=0.010$). There were no differences observed in TG ($p=0.11$), HDL-C ($p=0.32$), or the LDL/HDL ratio ($p=0.32$). Fasting blood glucose ($p=0.030$) and A1C were significantly reduced ($p=0.003$) from day 1 to 11.

Table 2: Blood makeup and basic metabolic profile alterations during 11 days of critical training.

<table>
<thead>
<tr>
<th>Blood Makeup</th>
<th>Desirable Values</th>
<th>Day 1</th>
<th>Day 11</th>
<th>p</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g·dL⁻¹</td>
<td>M:13.5-17.5</td>
<td>15.6 ± 0.4</td>
<td>14.4 ± 0.3*</td>
<td>0.003</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>F:12-15.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>M:40-50</td>
<td>46.2 ± 0.7</td>
<td>45.4 ± 0.6</td>
<td>0.082</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>F:35-45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Est. Plasma Volume, %</td>
<td>Not established</td>
<td>53.8 ± 0.7</td>
<td>59.3 ± 1.5*</td>
<td>0.007</td>
<td>16</td>
</tr>
<tr>
<td>Est. Cell Volume, %</td>
<td>Not established</td>
<td>46.2 ± 0.7</td>
<td>49.3 ± 1.4*</td>
<td>0.005</td>
<td>16</td>
</tr>
<tr>
<td>Est. Blood Volume, %</td>
<td>Not established</td>
<td>100.0 ± 0.0</td>
<td>108.1 ± 2.5*</td>
<td>0.005</td>
<td>17</td>
</tr>
<tr>
<td>Metabolic Profile</td>
<td>Desirable Values</td>
<td>Day 1</td>
<td>Day 11</td>
<td>p</td>
<td>n</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>------------------</td>
<td>---------</td>
<td>---------</td>
<td>--------</td>
<td>-----</td>
</tr>
<tr>
<td>Total Cholesterol, mg·dL⁻¹</td>
<td>&lt; 200</td>
<td>172.6 ±</td>
<td>11.4</td>
<td>153.9  ± 8.1*</td>
<td>0.011</td>
</tr>
<tr>
<td>Triacylglycerides, mg·dL⁻¹</td>
<td>&lt; 150</td>
<td>101.8 ±</td>
<td>16.4</td>
<td>84.9 ± 12.4</td>
<td>0.113</td>
</tr>
<tr>
<td>LDL-C, mg·dL⁻¹</td>
<td>&lt; 100</td>
<td>113.8 ±</td>
<td>17.0</td>
<td>93.6 ± 13.0*</td>
<td>0.016</td>
</tr>
<tr>
<td>HDL-C, mg·dL⁻¹</td>
<td>≥ 40 (&gt; 60 better)</td>
<td>56.8 ±</td>
<td>3.6</td>
<td>53.4 ± 4.9</td>
<td>0.319</td>
</tr>
<tr>
<td>Non-HDL-C, mg·dL⁻¹</td>
<td>&lt; 130</td>
<td>114.9 ±</td>
<td>11.4</td>
<td>98.3 ± 9.2*</td>
<td>0.010</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>&lt; 5 (&lt; 3 better)</td>
<td>3.9 ± 1.8</td>
<td>2.1 ± 0.4</td>
<td>0.315</td>
<td>8</td>
</tr>
<tr>
<td>Glucose, mg·dL⁻¹</td>
<td>&lt; 100</td>
<td>93.1 ± 2.2</td>
<td>88.4 ± 2.2*</td>
<td>0.030</td>
<td>17</td>
</tr>
<tr>
<td>Hemoglobin A1c, %</td>
<td>&lt; 5.7</td>
<td>5.2 ± 0.1</td>
<td>5.1 ± 0.1*</td>
<td>0.003</td>
<td>17</td>
</tr>
</tbody>
</table>

Data presented as Mean ± SEM.
* significant difference (p<0.05)

Inflammation

There was not a significant change in CRP observed during CT (Figure 2)

(p=0.32).
Figure 2: Plasma CRP concentrations across critical training. There was no main effect for time (p=0.32). Mean ± SEM (n=16).

Plasma Oxidative Damage

Biomarkers of oxidative damage to protein, 3NT and PC, and lipid markers, 8ISO and LOOH, were assessed. Short-term, 8ISO, and long-term, LOOH, lipid damage markers are presented in Figure 3. Alternatively, the short- and long-term markers of protein damage, 3NT and PC respectively, are shown in Figure 4. 8ISO (p<0.001), LOOH (p=0.001), and 3NT (p=0.033) exhibited a main effect for time. PC did not show a main effect for time (p=0.73).
Figure 3: Short (A) and long (B) term markers of lipid oxidation; all measures exhibited a main effect for time. * different than Day 1 (p<0.05), # different from Day 4 (p<0.05), ‡ different from Day 8 (p<0.05). Mean ± SEM (n=16).

Figure 4: Short (A) and long (B) term markers of protein oxidation; only 3NT exhibited a main effect for time (p=0.033). # different from Day 4 (p<0.05). Mean ± SEM (n=16).

BLM Fitness Challenge

Raw and scored results from the BLM Fitness Challenge are found in Table 3. Pearson-correlations between fitness parameter scores and changes in various blood markers are reported in Table 4. Interestingly, push-ups score was significantly related to all other fitness parameter scores (Table 4), while all other fitness parameter scores only correlated with total score (data not reported). Deltas were calculated as day 11 minus day 1. Thus, a positive number indicates an increase from day 1 to 11. The
presented correlations suggest that fitness is protective against metabolic perturbations, with fitter individuals having a lesser change to their metabolic profiles.

Table 3: BLM Fitness Challenge results and scores (n=13).

<table>
<thead>
<tr>
<th>Repetitions (#) / Time (s)</th>
<th>Push-ups</th>
<th>Pull-ups</th>
<th>Sit-ups</th>
<th>1.5-mile Run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>53.3 ± 4.8</td>
<td>15.3 ± 2.3</td>
<td>76.4 ± 6.7</td>
<td>580.8 ± 10.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(30 - 85)</td>
<td>(6 - 37)</td>
<td>(36 - 126)</td>
<td>(541 - 647)</td>
<td></td>
</tr>
<tr>
<td>Score</td>
<td>59.8 ± 3.2</td>
<td>61.4 ± 7.2</td>
<td>57.2 ± 4.6</td>
<td>51.8 ± 4.7</td>
<td>230.2 ± 15.1</td>
</tr>
<tr>
<td></td>
<td>(37 -79)</td>
<td>(21 - 100)</td>
<td>(20 - 86)</td>
<td>(28 - 82)</td>
<td>(146 - 313)</td>
</tr>
</tbody>
</table>

Mean ± SEM. (minimum, maximum).

Table 4. Pearson-correlations (two-tailed) between fitness and other measures.

<table>
<thead>
<tr>
<th>Fitness Parameter (score)</th>
<th>Measure</th>
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<th>Significance</th>
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<td>Push-up</td>
<td>Δ TC</td>
<td>0.650</td>
<td>0.022</td>
<td>12</td>
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<tr>
<td>Push-up</td>
<td>Δ HDL-C</td>
<td>0.687</td>
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<tr>
<td>Push-up</td>
<td>Δ Glucose</td>
<td>0.677</td>
<td>0.016</td>
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</tr>
<tr>
<td>Push-up</td>
<td>Δ 8ISO</td>
<td>0.658</td>
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<tr>
<td>Sit-up</td>
<td>Δ BMI</td>
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</tr>
<tr>
<td>Sit-up</td>
<td>Δ 8ISO</td>
<td>0.637</td>
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<tr>
<td>Run</td>
<td>Δ Hemoglobin</td>
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<tr>
<td>Run</td>
<td>Δ TC</td>
<td>0.670</td>
<td>0.017</td>
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</tr>
<tr>
<td>Run</td>
<td>Δ Non-HDL-C</td>
<td>0.624</td>
<td>0.040</td>
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</tr>
<tr>
<td>Total</td>
<td>Δ TC</td>
<td>0.583</td>
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</tr>
<tr>
<td>Total</td>
<td>Δ 8ISO</td>
<td>0.602</td>
<td>0.050</td>
<td>11</td>
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<table>
<thead>
<tr>
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<tr>
<td>Δ Hemoglobin</td>
<td>Δ LOOH</td>
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<td>0.023</td>
</tr>
<tr>
<td>Δ Hemoglobin</td>
<td>Δ BMI</td>
<td>-0.492</td>
<td>0.045</td>
</tr>
<tr>
<td>Δ Hematocrit</td>
<td>Δ BMI</td>
<td>-0.591</td>
<td>0.016</td>
</tr>
<tr>
<td>Δ 3NT</td>
<td>Δ PC</td>
<td>0.611</td>
<td>0.012</td>
</tr>
<tr>
<td>Δ 3NT</td>
<td>Δ BMI</td>
<td>-0.531</td>
<td>0.034</td>
</tr>
<tr>
<td>Δ PC</td>
<td>Δ HDL</td>
<td>-0.704</td>
<td>0.002</td>
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### Day 1 Correlations

<table>
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<th>n</th>
</tr>
</thead>
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<tr>
<td>Push-up score TC</td>
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<tr>
<td>Push-up score TG</td>
<td>-0.817</td>
<td>0.025</td>
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<td>Push-up score LDL-C</td>
<td>-0.874</td>
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<td>Sit-up score Body Weight</td>
<td>-0.698</td>
<td>0.008</td>
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</tr>
<tr>
<td>Total score Body Weight</td>
<td>-0.591</td>
<td>0.033</td>
<td>13</td>
</tr>
<tr>
<td>Total score Non-HDL-C</td>
<td>-0.611</td>
<td>0.046</td>
<td>11</td>
</tr>
<tr>
<td>BMI TC</td>
<td>0.541</td>
<td>0.017</td>
<td>19</td>
</tr>
<tr>
<td>BMI Non-HDL-C</td>
<td>0.613</td>
<td>0.007</td>
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<td>LDL-C Glucose</td>
<td>0.647</td>
<td>0.017</td>
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<tr>
<td>LOOH Hematocrit</td>
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<tr>
<td>PC Hematocrit</td>
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### Preparedness Questionnaire (Question)

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<th>(1) Months preparing</th>
<th>(2) Days/week</th>
<th>Pearson Correlation</th>
<th>Significance</th>
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</thead>
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<tr>
<td>(1) Months preparing</td>
<td>(2) Days/week</td>
<td>0.564</td>
<td>0.010</td>
<td>20</td>
</tr>
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<td>(1) Months preparing</td>
<td>(3) Session duration</td>
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<td>0.012</td>
<td>20</td>
</tr>
<tr>
<td>(2) Days/week</td>
<td>(3) Session duration</td>
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<td>0.039</td>
<td>20</td>
</tr>
<tr>
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<td>9</td>
</tr>
<tr>
<td>(2) Days/week</td>
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<td>0.557</td>
<td>0.025</td>
<td>16</td>
</tr>
<tr>
<td>(3) Session duration</td>
<td>Δ Hemoglobin</td>
<td>-0.521</td>
<td>0.032</td>
<td>17</td>
</tr>
<tr>
<td>(1) Months preparing</td>
<td>Sit-up score</td>
<td>0.567</td>
<td>0.044</td>
<td>13</td>
</tr>
<tr>
<td>(3) Session duration</td>
<td>Push-up score</td>
<td>0.566</td>
<td>0.044</td>
<td>13</td>
</tr>
<tr>
<td>(3) Session duration</td>
<td>Sit-up score</td>
<td>0.636</td>
<td>0.019</td>
<td>13</td>
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<tr>
<td>(3) Session duration</td>
<td>Total score</td>
<td>0.672</td>
<td>0.012</td>
<td>13</td>
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</table>

### Fitness Score Correlations

<table>
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<th>n</th>
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<tbody>
<tr>
<td>Push-ups Sit-ups</td>
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<td>0.037</td>
<td>13</td>
</tr>
<tr>
<td>Push-ups Pull-ups</td>
<td>0.669</td>
<td>0.012</td>
<td>13</td>
</tr>
<tr>
<td>Push-ups Run</td>
<td>0.563</td>
<td>0.045</td>
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</tr>
<tr>
<td>Push-ups Total</td>
<td>0.881</td>
<td>&lt;0.001</td>
<td>13</td>
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</tbody>
</table>

Δ is change from day 1 to 11 calculated as: Day 11 – Day 1
Chapter 5: Discussion

The observed alterations to various markers confirms that CT is a stressful time. We accept the null hypothesis that body weight would not change. Additionally, we reject the null hypothesis that body fat percentage would not change during CT. From our whole blood measurements, we accept the null hypotheses that TG, HDL-C, and hematocrit would not change. We reject the null hypotheses that TC, LDL-C, glucose, A1C, hemoglobin, and estimated plasma volume would not change. Following plasma separation and storage, we accept the null hypotheses that PC and CRP would not change. Finally, we reject the null hypotheses that 8ISO, 3NT, and LOOH would not change during CT.

This study is the first of its kind to examine the cardiovascular related physiologic alterations that occurred in an IHC during their 11 days of CT. The observed changes to body composition, blood lipids, glucose, and oxidative damage suggest that the CT period is stressful to WLFFs as they adjust from more classic physical fitness movements to the job-specific tasks required in the field. The novelty of this study is highlighted by the job specificity of the participants in addition to the relatively short, 11-day, training duration.

Previous research has established that variable and unpredictable alterations to body composition measures regularly occur during intensive military training programs \(^9,10^9,11^4\). In this study, there was an observed increase in BMI, with a decrease in body fat percentage. These acute alterations are unlikely to be the result of changes in lean body mass and are instead indicative of adaptive body water alterations.
In conducting biomarker analysis, one important consideration is assessment of potential plasma volume shifts so hydration status can be determined and biomarker concentrations may be corrected. In field and multi-day laboratory studies, the Dill & Costill technique is generally considered indicative of adaptive shifts in plasma volume. Throughout CT, there was a significant decrease in hemoglobin with observed increases in estimated blood, cell, and plasma volume. These findings are in line with short term heat acclimation and dehydration studies conducted over the course of two weeks, and are indicative of plasma volume expansion which has protective effects against the development of heat related illness. Bookended by heat stress trials, three dehydrating acclimation trials (~2.4% dehydration) in WLFF PPE, with four heat exposures within 7 days, resulted in an increase in body weight and plasma volume. This 0.5 kg increase in body weight and 7% increase in plasma volume across 11 days was similar to the results found in this study. Taken in conjunction, the observed alterations to body composition, estimated blood volume, cell volume, and plasma volume may be the result of plasma volume expansion in addition to compartmental water shifts to maintain homeostasis.

A basic metabolic profile, including blood lipids, blood glucose, and A1C measurement, is an integral part of helping determine the risk of cardiovascular disease development/progression. As expected in relatively young, fit individuals with highly active jobs, the participants were overall ‘normal’ in all parameters. Similar to previous research, a decrease in TC, LDL-C and Non-HDL-C (which includes LDL-C) was observed. Following CT, HDL-C remained unchanged, which is anticipated if the training load is not increased above current workload capacities.
Military training involving weeks of training followed by at least one week of elevated energy expenditure have shown increases in HDL-C following the increase in physical activity. Additionally, fasting glucose and A1c was significantly decreased, which is in accordance with physically active non-diabetic individuals. Though statistically significant, the observed alterations to glucose and A1C are clinically insignificant as our subjects are not classified as prediabetic/diabetic.

A recent investigation of WLFFs showed detrimental alterations to cardiovascular and metabolic health over the course of an entire fire season. In comparison to our increased BMI and concomitant decrease in body fat percentage, Coker et al. showed an increase in body weight, BMI, and total fat mass via dual xray absorptiometry. In the current study, the observed alterations to body composition, when taken with the increase in plasma volume, are indicative of positive adaptations to combat thermal strain in contrast to the negative metabolic alterations demonstrated across the season. In opposition to our data, Coker et al. also showed deleterious increases in TC and LDL-C. Together, this suggests that positive alterations over the course of CT may not extend over the entire season. It remains to be shown whether this reflects a lower activity level during the actual fire season, dietary influences, or a combination of both.

A long-lived marker of systemic inflammatory processes in both a nonpathological and disease state is CRP. Following exercise, CRP may be upregulated by IL-6 activation of the STAT3 pathway in the liver. However, the interindividual variability of circulating CRP is problematic within scientific literature in achieving statistical significance. As in our investigation, many studies peri-exercise/work conducted within athletic and WLFF populations show no alteration to
resting CRP values. It is during novel exercise bouts or in cases of high energy expenditure in trained individuals, that an acute increase in CRP may be anticipated. Thus, the lack of alteration to CRP in the young, athletic population of this study is unsurprising.

Disruption away from homeostasis is a common characteristic of exercise, woodsmoke, and altitude, both independently and congruently. The increased generation of free radicals from these potential insults leads to accumulative oxidative damage which can result in an increased inflammatory state. Currently, it is believed that blood is the primary source of measured oxidative stress, with measured damage occurring to lipids and proteins in solution. The positive relationship between Δhemoglobin and ΔLOOH from day 1 to 11 suggest that alterations to red blood cells (as measured by hemoglobin) results in concomitant changes to LOOH. Similarly, the negative relationship between hematocrit and LOOH/PC on day 1 indicates that the greater the plasma volume, the lower the concentration of oxidative damage markers. Taken in conjunction, correlations between hemoglobin/hematocrit and oxidative damage markers both at baseline and as changes from day 1 to day 11 support that blood is the primary source of measured OS.

The red blood cell itself appears to be the primary site of lipid damage. Previous research investigating 8ISO, classically F2 Isoprostanes, has primarily investigated alterations within a single day, with elevations consistently occurring during and immediately after exercise with returns to baseline within 2 hours. Investigations across multiple days have been done around ultra-endurance races and have shown acute increases with return to baseline within 24 hours. Our results
indicating minimal significant alterations across the training period are unsurprising since blood collections was performed in the mornings. This is in line with the current idea that 8ISO is a short lived signaling molecule created from arachidonic acid metabolism following cleavage from the plasma membrane.\(^{56}\)

Alternatively, LOOH are more reflective of membrane bound polyunsaturated fatty acid modifications, owing to their longer half-life in plasma.\(^{57}\) Previous research has shown increases in LOOH formation following long duration and high-intensity exercise, with peaks occurring during recovery from the exercise bout\(^ {58-61}\). Individuals participating in the Western States 160km endurance run increased LOOH concentrations from baseline at both the 90km and 160 km marks.\(^ {61}\) Additionally, 3-hours of treadmill running showed a similar increase from pre- to post-exercise.\(^ {60}\) Exercise lasting between 60 and 90 minutes followed by normoxic or hypoxic recovery has shown a stepwise increase in LOOH for up to 6 hours (when the last sample was collected) following the exercise bout in both recovery conditions.\(^ {58,59}\) This delayed increase in LOOH is consistent with the observed stepwise increase in the current study, suggesting an accumulation of lipid damage throughout CT.

Measurement of protein damage is critical in oxidative damage determination due to the relative abundance of protein in plasma.\(^ {62}\) Studies have identified acute spikes in 3NT following high-intensity exercise bouts, which return to baseline in 2-24 hours.\(^ {77,78,80,93}\) Singular exercise bouts conducted until volitional fatigue (lasting ~15 minutes in duration) have shown an increase in 3NT from pre- to post-exercise.\(^ {77,78,80}\) This response was similar between men/women\(^ {78}\), even when conducted in normoxic/hypoxic conditions.\(^ {77}\) Peak 3NT concentrations following a max protocol were
found to occur 1-hour post-exercise, with loss of statistical significance at 2 hours and complete return to baseline at 24-hours post-exercise. Simulated WLFF working conditions involving 90 minutes of walking at <57% VO$_{2\text{max}}$ and woodsmoke exposure resulted in increased 3NT immediately post-exercise in only the woodsmoke exposure conditions. A return to baseline was observed at 1-hour post-exercise. This study’s relative lack of alteration to 3NT is supported by the literature in that any acute spikes from training would be expected to return to normal by the next morning.

By comparison, the current study found no alteration to PC levels across the CT period. Previous work has established that either no change or an increase in PC concentrations occurs following high intensity and long duration exercise bouts. The lack of observed alterations may be due to elevated baseline measures from previous training or insufficient sampling time post-exercise to see a spike in values. A single bout of exercise at ~70% VO$_{2\text{max}}$ followed by an exercise to exhaustion has shown PC peaking at 4-hours with recovery to baseline by 24-hours of recovery. Unsurprisingly, changes to 3NT and PC were positively correlated during CT.

Coming into the season, crew bosses frequently strive to have their crew average above a 200 score on the BLM fitness challenge, which the observed crew achieved. Of the 13 individuals to complete the challenge, eight achieved a level 3/4 national recognition and all met at least level 1 recognition. Overall, heavier individuals tended to score worse on the challenge. The observed positive correlations between push-ups/total score and changes from day 1 to 11 in TC, HDL-C, glucose, and 8ISO suggests that the lowest fitness scoring individuals had the greatest decrease (TC and glucose) or smallest increase (HDL-C and 8ISO) in these markers. Additionally, fitness
scores were negatively associated with day 1 TC, TG, LDL-C, Non-HDL-C, and body weight. Previously, an increased ability to perform push-ups was associated with a decreased risk of future cardiovascular events, suggesting that push-ups can be a relatively good proxy of overall physical fitness in relation to CVD development.

In addition to the hard numbers from the fitness challenge, personal assessment of the training practices of the crew members was obtained from the pretraining questionnaire (Appendix C). Individuals prepared for the upcoming season for ~4 months prior at a frequency of ~5 days per week and duration of ~90 minutes per session (Appendix D). In the 48 hours before the initiation of CT, individuals were widely variable in their daily activities from being sick and sleeping all day to weighted hiking uphill. For the most part, individuals relaxed and only did activities <6 METS (75%) in their final days before the season. Unsurprisingly, an individual's answers to questions 1-3 of the pretraining questionnaire (1. Months preparing for season, 2. Days of training per week, 3. Individual session duration) were positively associated with fitness scores and the other training questions. Thus, the crew was mostly in good physical condition and rested at the initiation of CT as evidenced by their BLM fitness challenge scores and self-reported pretraining questionnaire responses.

In conclusion, this study is the first to examine biomarkers of oxidative stress, inflammation, dyslipidemia, and glucose regulation in a comprehensive field study of WLFF during CT. The challenges presented to WLFFs during CT are expected to be difficult, but should fall short of causing undue harm to the crew. Proper preparation both mentally and physically in the weeks and months before critical training is imperative to the success of a WLFF in performing their duties on a daily basis in the
field. The acute challenges observed during CT are likely reflective of the ongoing stressors a WLFF faces during the remainder of the fire season. This high-level IHC appeared adequately prepared for their upcoming season, but the evidence is still lacking in lower-level, potentially less prepared crews. An investigation into other crews in different geographic regions with different physical fitness and years of experience are interesting possible future areas of research.

**Acknowledgements**

The authors are appreciative of the participants for their time and participation. I would like to thank my committee for their valuable suggestions and concerns throughout the entirety of the project. Special thanks are necessary to Tiffany Quindry for her assistance and sharing of lab space and equipment. I am especially thankful to Katie Christison, Cassie Williamson-Reisdorph, and Kathryn Tiemessen for their help in data collection and analysis. My sincere gratitude is given to my advisor Charles Dumke for his incredible guidance in this work. He has been an excellent mentor, providing valuable insight at every stage of my education.

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Appendices

Appendix A – BLM Fitness Challenge Instructions

Test Administration
A medical plan must be in place prior to the test in case first-aid assistance is needed. The number of assistants should be based upon the number of participants. Test administrators shall read a description of each exercise (see below) and demonstrate proper form prior to the test.

The individual test exercises are performed as follows:

**Pull-ups:** Starting position is hanging from a bar, hands approximately shoulder width apart, arms fully extended with elbows locked. Hands can be palms away or palms facing the individual. Individual lifts the body until the chin is above the bar and returns to the starting position. This is one repetition. On each repetition the arms must be fully extended, and the chin must clear the bar. No kipping or kicking is allowed. Count the number of pull-ups completed in three minutes or when the individual cannot maintain the starting position (lets go of the bar).

**Push-ups:** Starting position is back straight and parallel with the ground, arms straight with hands approximately shoulder width apart and elbows locked. Individual lowers the body until the arms form a ninety-degree angle and returns to the starting position with the arms fully locked. This is one repetition. The back must remain straight throughout the exercise. All resting must occur in the starting position. The buttocks are not allowed in the air in the starting position. The arms must be fully extended (elbows locked). Count the number of repetitions successfully completed in three minutes or when the starting position can no longer be maintained (arms collapsing, buttocks in the air).

**Sit-ups:** Starting position is hands behind the ears, back on the ground, legs bent at a forty-five-degree angle. Feet can be held by a person or a fixed object. The individual raises the back until the elbows touch the legs, then returns to the starting position (shoulder blades touch the ground). This is one repetition. Exercise mats may be used for padding. Count the number of repetitions completed in three minutes.

**1 ½ mile or 3-mile run:** Conducted on flat, smooth surface.

Exercises may be completed in any order. Each callisthenic event must be completed within three minutes. Maximum break between callisthenic events is seven minutes. A ten-minute warm-up is allowed for the run. An individual may test multiple times, but scoring will always be based on the results of a single testing event, not on amalgamated individual exercise scores from separate testing events.

From: [https://www.nifc.gov/training/trainingFitness.html](https://www.nifc.gov/training/trainingFitness.html)
## Appendix B – BLM Fitness Challenge Score Sheet

### BLM Fire Operation Fitness Challenge Score Sheet

<table>
<thead>
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<th>3 Mile</th>
<th>1.5 mi</th>
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Appendix C – Pretraining Questionnaire

The National Technology and Development Program, in cooperation with the University of Montana, is collecting information on offseason training performed by wildland firefighters (WLFFs). This information will be used to identify common components of WLFF offseason training. Your participation is voluntary, you do not have to respond to all of the questions, and all answers will be anonymous. Please answer each question to the best of your abilities.

1. How long ago did you begin training specifically for this upcoming season?  
(Please select one answer only)  
- Less than one month  
- One to two months  
- Two to three months  
- Three to four months  
- Four or more months

2. How many days per week, on average, did your preseason training consist of usually?  
(Please select one answer only)  
- One day per week  
- Two days per week  
- Three days per week  
- Four days per week  
- Five or more days per week

3. On average, which was the duration of a daily session?  
(Please select one answer only)  
- Less than 30 minutes  
- 30 minutes to 60 minutes  
- 60 minutes to 90 minutes  
- 90 minutes to 120 minutes  
- 120 or more minutes

4. How many individuals were typically involved, or participated, in your workouts? Choose the answer that best describes your training.  
(Please select one answer only)  
- Individually or by oneself  
- Individually and sometimes with a partner or group.  
- Individually or with a group.  
- With a partner most of the time.  
- With a partner or with a group, rarely individually.

5. Where did the majority of your physical training occur?  
(Please select one answer only)  
- Home  
- Outdoors  
- Work facility  
- Fitness Center (gym membership)  
- Fitness Classes

6. Did these workouts typically consist of a warmup and cooldown session each ranging from 5 to 15 minutes in length?  
(Please select one answer only)  
- Yes  
- No

PLEASE TURN TO NEXT PAGE
7. Of those training days per week, how many days were primarily anaerobic (resistance/strength/power/speed) focused?

(Please select one answer only)

Note: If you changed emphasis throughout your training, answer in general terms.

- None
- One day per week
- Two days per week
- Three days per week
- Four or more days per week

8. Of those training days per week, how many days were primarily aerobic (cardio/endurance) focused?

(Please select one answer only)

Note: If you changed emphasis throughout your training, answer in general terms.

- None
- One day per week
- Two days per week
- Three days per week
- Four or more days per week

9. Please select the forms of activity included in your training:

(Please select all that apply)

- Running
- Weightlifting
- Hiking (w/o weight)
- Hiking (w/weight)
- High-Intensity Circuit Training
- CrossFit
- Swimming
- Biking (Mountain/Road/Touring)
- Recreational Sports/Activities
- Yoga
- Plyometric
- Recovery Workouts
- Flexibility/Stretching
- Other(s): Please Specify

10. In your own words, describe the goal(s) of your physical training. Please include specific goals that you may have had to prepare for this season.

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

11. Please explain what you have been doing physically for the last 48 hours. Include time spent flying, driving, workouts, other exercises, injuries, amount of sleep, etc.

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

This is the end of the questionnaire. Thank you for your participation.
## Appendix D – Pretraining Questionnaire Responses

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<td>3) Duration of training sessions (minutes)?</td>
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<td>5 (25%)</td>
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</tbody>
</table>
# Appendix E – 2019 PAR-Q+

## 2019 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.

<table>
<thead>
<tr>
<th>Question</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Has your doctor ever said that you have a heart condition? OR high blood pressure?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if you think was associated with over-breathing (including during vigorous exercise).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? PLEASE LIST CONDITIONS HERE:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5) Are you currently taking prescribed medications for a chronic medical condition? PLEASE LIST CONDITIONS AND MEDICATIONS HERE:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>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 CONDITIONS HERE:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7) Has your doctor ever said that you should only do medically supervised physical activity?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 International Physical Activity Guidelines for your age (www.who.int/dietphysicalactivity/en/).
- 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 under 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 _______________________________________________________

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _______________________

WITNESS _________________________________________________________

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 ePARmedX 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.
2019 PAR-Q+

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
   (Answer NO if you are not currently taking medications or other treatments)
   1a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?
       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/pairs 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
   (Answer NO if you are not currently taking medications or other treatments)
   2a. Does your cancer diagnosis include any of the following types: lung/breast, multiple myeloma (cancer of plasma cells), head, and/or neck?
       YES ☐ NO ☐ ☐
   2b. Are you currently receiving cancer therapy (such as chemotherapy 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
   (Answer NO if you are not currently taking medications or other treatments)
   3a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?
       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 a diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?
       YES ☐ NO ☐ ☐

4. Do you have High Blood Pressure?
   If the above condition(s) is/are present, answer questions 4a-4b
   (Answer NO if you are not currently taking medications or other treatments)
   4a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?
       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
   (Answer NO if you are not currently taking medications or other treatments)
   5a. Do you have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?
       YES ☐ NO ☐ ☐
   5b. Do you have any signs 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 sleeplessness.
       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 ☐ ☐
2019 PAR-Q+

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
(Skip this section if you are not currently taking medications or other treatments)

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)

6b. Do you have Down Syndrome AND back problems affecting nerves or muscles?

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
(Skip this section if you are not currently taking medications or other treatments)

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)

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?

7c. Are you currently suffering from 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?

7d. Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?

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
(Skip this section if you are not currently taking medications or other treatments)

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)

8b. Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?

8c. Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?

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
(Skip this section if you are not currently taking medications or other treatments)

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)

9b. Do you have any impairment in walking or mobility?

9c. Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?

10. Do you have any other medical condition not listed above or do you have two or more medical conditions?
If the above condition(s) is/are present, answer questions 10a-10c
(Skip this section if you are not currently taking medications or other treatments)

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?

10b. Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?

10c. Do you currently live with two or more medical conditions?

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.
2019 PAR-Q+

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 and 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

SIGNATURE

DATE

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER

WITNESS

For more information, please contact

www.eparmedx.com

Email: eparmedx@gmail.com

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. John E. R. Winburn with Dr. Norma Glidewell, Dr. Veronica Jarmak, and Dr. Daniela 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.

Key References
Appendix F – Consent Form

SUBJECT INFORMATION AND CONSENT FORM

PROJECT IN BRIEF: The Physiologic Stress of Two Weeks Critical Training in WLFF

SPONSOR: United States Forest Service (NTDP)

RESEARCHERS: Dr. Charles Dumke (406) 243-6176
The University of Montana
32 Campus Drive
203 McGill Hall – HHP
Missoula, MT 59812

Please read the following information carefully and feel free to ask questions. Only sign the final page when you are satisfied procedures and risks have been sufficiently explained to you.

REQUIREMENTS
This research study requires that you meet the following criteria:

➢ Participants must be 18 years or older.
➢ Participants must be part of a WLFF crew involved in critical training.

PURPOSE OF THE STUDY
Wildland firefighters (WLFF) are at risk for early season overtraining and muscle soreness in the first two weeks upon reporting to work. The purpose of this study is to survey muscle soreness, physiologic, and cardiovascular markers of physiologic stress during critical training.

TEST PROCEDURES:
Data will begin to be collected on the first day crews report for critical training in the spring. For two weeks select measures will be repeated on particular days. Measurements will be done in the morning prior to deployment, and not take longer than 30 minutes.

A screening assessment which involves a health/exercise questionnaire (Par-Q) and family history questionnaire.

Prior to any testing, you will complete a physical activity readiness questionnaire (PAR-Q) to screen for known risk factors of coronary heart disease and a family history questionnaire to screen for history of heat related illness or death.

If you successfully complete the PAR-Q, you will then provide written informed consent following the reading of this document.

MEASUREMENTS:

a. A questionnaire to evaluate current and recent training practices.
This questionnaire will ask about your recent training practices. The questionnaire will be done once and take approximately 5-7 minutes.
b. Measure of body weight and percent body fat obtained using skinfolds
   This test will take approximately 5 minutes. Body weight will be collected on a calibrated scale without shoes. Skinfolds will require a measurement of a pinch of your skin. In men this is done on the chest, abdomen and thigh. In women, it’s done on the triceps, hip and thigh. Body weight will be collected multiple times during the two weeks; skinfolds will be done once.

c. Heart rate and activity monitoring
   You will be outfitted with two monitors, a heart rate chest strap, and a triaxial activity monitor. Heart rate will be measured using an elastic chest strap that is worn on the skin under your shirt around your chest. The activity monitor will be worn like a watch. These will help us assess the amount of work and cardiovascular stress you undertake in your training over the next two weeks. It will take approximately 2 minutes to outfit you with monitors, but you are asked to wear these monitors for the full two weeks of critical training.

d. Blood Sample
   We will take a total of 4 blood samples (Monday and Thursday in each of the two weeks) collected using a venipuncture technique from your arm. The site will be cleaned with alcohol prior to the blood draw, and wiped clean afterwards. Each blood sample (~10 ml or 1/2 tablespoon) will be taken from your arm under the direction of Dr. Charles Dumke, Ph.D. These samples will be collected to measure changes in muscle damage, and markers of physiologic and cardiovascular stress.

e. Questionnaire to evaluate muscle soreness
   Each morning over the two weeks, you will rate your level of soreness on a scale. This will take approximately 5 minutes.

f. Pack test results
   We will ask you to report the results of your arduous pack test. This includes total time, heart rate, and rating of perceived exertion.

g. Muscle activation
   A subset of the volunteers will wear surface electrodes that attach to the skin over your muscles. The skin will be shaved, and the electromyographic (EMG) sensor and electrodes will be wrapped in athletic tape to prevent the equipment from falling off. You will be asked to carry a microcomputer about the size of a cell phone that records the EMG data from the electrodes. This will take approximately 5 minutes. If you volunteer for this subset of data, you will be asked to do this for one day of the two weeks of training.

h. Pulse wave velocity and heart rate variability
   A subset of the volunteers will be asked to sit or lay quietly while connected to a device that monitors your heart rate, rhythm, and heart rate variability. All the while an automated device will record blood pressure in your arm and leg. As this test is conducted, an assistant will gently place a “transducer” (a soft probe that will measure blood pulses with each heart beat) over your carotid artery (in your neck). All of these tests are painless and will be performed while you lie on a padded table. This will take approximately 30 minutes. If you volunteer for this subset of data, this may mean pre and post daily measurements on up to three days during the two weeks of training.
RISKS AND DISCOMFORTS
1. Mild discomfort may result during and after exercise. These discomforts include shortness of breath, tired or sore legs, nausea and possibility of vomiting.
2. Exercising may result in profuse sweating and the perception of feeling very hot.
3. Muscle soreness may occur as a result, but should not persist.
4. Certain changes in body function take place when any person exercises. Some of these changes are normal and others are abnormal. Abnormal changes may occur in blood pressures, heart rate, heart rhythm or extreme shortness of breath. Very rare instances of heart attack have occurred. Every effort will be made to minimize possible problems by the preliminary evaluation and constant surveillance during testing.
5. Mild symptoms of dehydration such as headache and general fatigue may result during and after the exercise.
6. You will be informed of any new findings that may affect your decision to remain in the study.
7. Should symptoms, such as chest discomfort, unusual shortness of breath or other abnormal findings develop, alert the immediate supervisor available. Guidelines by the American College of Sports Medicine will be followed to determine when exercise should be stopped. These symptoms include moderate to severe angina (chest pain), increased dizziness, shortness of breath, fatigue and your desire to stop.
8. When blood samples are collected for this study, participants may feel a slight sting or "pinch" in their arm, they may suffer a small bruise, and there is a very slight possibility of infection. Should participants notice unusual redness, bruising, or swelling at the blood sampling site they should seek medical attention and contact the study director, Dr. Charles Dumke. During the blood draw, precautions (cleaning the site with alcohol, sterile supplies, and wearing a band-aid) will be taken to minimize deleterious effects.
9. Certain medications could increase the risk for adverse effects during this heat related study. If you are taking any medications, you must check with your physician before participating in the study.

BENEFITS OF PARTICIPATION
The information from these tests will provide you with an assessment of your fitness and general health. There are no other direct benefits to the participants in the study. Once completed the researchers will share their generalized findings with the subjects.

The scientific benefit includes expanding current understanding of how early season training affects WLFF stress. This information may contribute to the reduction of overuse injuries and extreme soreness.

CONFIDENTIALITY
1. Your records will be kept confidential and not be released without consent except as required by law.
2. Only the researcher and his research assistants will have access to the files.
3. Your identity will be kept private.
4. If the results of this study are written in a scientific journal or presented at a scientific meeting, names will not be used.
5. All data, identified only by an ID #, will be stored in our laboratory.
6. The signed consent form and information sheet will be stored in a locked cabinet separate from the data.
COMPENSATION FOR INJURY
In the event that you are injured as a result of this research you should individually seek appropriate medical treatment. If the injury is caused by the negligence of the University of Montana or any of its employees, you may be entitled to reimbursement or compensation pursuant to the Comprehensive State Insurance Plan established by the Department of Administration under the authority of M.C.A., Title 2, Chapter 9. In the event of a claim for such injury, further information may be obtained from the University’s Risk Manager (406-243-2700; jason.sloat@msou.montana.edu) or the Office of Legal Counsel (406-243-4742; legalcounsel@umontana.edu). (Reviewed by University Legal Counsel, December 31, 2018)

VOLUNTARY PARTICIPATION AND WITHDRAWAL
It is important that you realize that you are free to withdraw from the study at any time. If you decide to drop out of the study you will not receive compensation.

FUTURE RESEARCH
Identifiers might be removed from the identifiable private information or identifiable biospecimens and could then be used for future research studies or distributed to another investigator for future research studies without additional informed consent from you or your legally authorized representative.

QUESTIONS
You may wish to discuss this with others before you agree to take part in this study. If you have any questions about the research now or during the study contact Dr. Charles Dumke, PhD at (406) 243-6176 (office). If you have any questions regarding your rights as a subject, you may contact the chair of the IRB through the University of Montana Research Office at (406) 243-6672.

STATEMENT OF CONSENT
I have read the above statements and understand the risks involved with this study. I authorize Dr. Charles Dumke, PhD, and such assistants that he may designate, to administer and conduct the testing as safely as possible with a minimal amount of discomfort. If I have additional questions, I may contact Dr. Charles Dumke, PhD, at (406) 243-6176.

Participant (print) ____________________________________________
Signature ________________________________________________
Date _______________________________________________________

STATEMENT OF CONSENT TO BE PHOTOGRAPHED DURING DATA COLLECTION
During the study, I understand that pictures may be taken. I provide my consent to having my picture taken during the course of the research study. I provide my consent that my picture may be used in some presentations related to this study. If pictures are used at any time for presentation, names and/or physiological data will not be associated with them.

Signature ______________________________________ Date __________
Appendix G – Muscle Soreness Scale

PAIN SCORE 0-10 Numerical Rating Scale (NRS)

0 1 2 3 4 5 6 7 8 9 10

No pain Mild Moderate Severe

Worst pain imaginable