The combined effects of exercise-induced muscle damage and heat stress on acute kidney stress and heat strain during subsequent endurance exercise

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THE COMBINED EFFECTS OF EXERCISE-INDUCED MUSCLE DAMAGE AND HEAT STRESS ON ACUTE KIDNEY STRESS AND HEAT STRAIN DURING SUBSEQUENT ENDURANCE EXERCISE

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ABSTRACT

Purpose: The purpose of this study was to investigate the combined effect of downhill running and heat stress on muscle damage, as well as on heat strain and kidney stress during subsequent endurance exercise in the heat. Methods: Using a crossover design and randomized order, ten non-heat-acclimated, physically active males completed downhill running in a cool (EIMD in Cool) and hot (EIMD in Hot) environment followed by an exercise-heat stress (HS) test after 3-hour seated rest. Core temperature, heart rate, thermal sensation and ratings of perceived exertion were recorded throughout each exercise session. Blood and urine samples were collected at immediately before (pre-
and after (post-) EIMD and HS, and 24 hours post-EIMD (post-24h). Serum creatine kinase (CK) activity, maximal voluntary isometric contraction of the quadriceps (MVC) and perceived muscle soreness were recorded to evaluate muscle damage. Urinary neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) levels were measured to indicate acute kidney stress. **Results:** By the end of the EIMD sessions, the Hot condition induced a significantly higher core temperature (EIMD in Hot: 38.75 ± 0.35°C; EIMD in Cool: 38.27 ± 0.38°C, p = 0.04) and heart rate (EIMD in Hot: 157 ± 19 bpm; EIMD in Cool: 140 ± 15 bpm, p = 0.035). There was no difference in CK, MVC or perceived soreness between the conditions at any timepoints. Urinary NGAL level was significantly elevated in EIMD in Hot condition at post-HS comparing with pre-HS (pre-HS: 6.56 \{1.53 – 12.24\} ng/min, post-HS: 13.72 \{7.67 – 21.46\} ng/min p = 0.034). **Conclusions:** Downhill running combined with heat stress does not seem to aggravate muscle damage, compared with downhill running in a temperate condition. Nevertheless, prior EIMD in a hot environment may pose a greater risk of acute kidney injury during subsequent endurance exercise in the heat.
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SYMBOLS/ABBREVIATIONS

>: greater than
<: less than
±: plus or minus
~: approximately
°C: degrees Celsius
AKI: acute kidney injury
ANOVA: analysis of variance
BF%: body fat percentage
bpm: beats per minute
CK: creatine kinase
cm: centimeters
EHS: exertional heat stroke
EIMD: exercise-induced muscle damage
ELISA: enzyme-linked immunosorbent assay
g: gram
HS: heat stress
kg: kilogram
KIM-1: kidney injury molecule-1
mg: milligram
ml: milliliter
ml/kg/min: milliliter per kilogram per minute
MVC: maximal voluntary isometric contraction
ng: nanogram
NGAL: neutrophil gelatinase-associated lipocalin
Nm: Newton-meter
PV: plasma volume
SD: standard deviation
U/L: unit of enzyme activity per liter
VAS: visual analog scale
CHAPTER 1

Introduction

Exertional heat stroke (EHS) is a life-threatening condition that most often occurs in individuals performing strenuous physical activities, usually in a hot environment, with high incidence in military personnel, occupational workers, and athletes. EHS is characterized by hyperthermia (core body temperature > 40.5°C) and is associated with central nervous system dysfunction (L. E. Armstrong et al., 2007; Muldoon et al., 2008). If not treated, EHS can lead to multi-organ injury/dysfunction, including renal, musculoskeletal, hepatic, cardiovascular and thermoregulatory and systemic inflammatory response syndrome. In extreme cases EHS can lead to irreversible organ injury and ultimately, death. According to the Military Health System of the United States, there were 578 incident diagnoses of heat stroke among active component service members in 2018 (Maron et al., 2016). In young competitive athletes from the United States, heat stroke has been reported as the fifth most prevalent non-cardiovascular cause of death (after trauma, drugs, suicide and commotio cordis) (Maron et al., 2016).

The primary pathophysiological mechanism of EHS is the accumulation of excessive heat produced during physical exertion, usually in combination of environmental heat stress that overwhelm heat loss mechanisms (Sawka et al., 2011). Individuals that are compromised by previous adverse medical events may also be more susceptible to EHS when exercising in the heat. This “multiple-hit hypothesis” (Laitano et al., 2019) proposes that prior adverse events, which may include mild illness, gastrointestinal tract disturbance, skin allergy, medications, among others, can predispose
individuals towards EHS. Many of these “adverse events” are associated with inflammation.

Unaccustomed, prolonged and strenuous physical exercise can cause exercise-induced muscle damage (EIMD) and subsequent inflammatory responses, especially when a large portion of the exercise involves eccentric muscle contraction (i.e., lengthening of skeletal muscles while generating tension). For reference, eccentric muscle activities such as downhill running and descending stairs have been shown to cause more muscle damage than concentric or isometric contractions (R. B. Armstrong et al., 1991). The electromyography during eccentric contraction is usually lower than concentric contraction for a given external load, suggesting that less muscle fibers are being recruited during eccentric contraction. This would increase the ratio of load per fiber, which leads to more mechanical insult and muscle damage (Enoka, 1996). Damage to skeletal muscle cells causes release of intracellular contents (i.e., creatine kinase, lactate dehydrogenase, myoglobin, etc.) into the extracellular space, which can contribute to inappropriate inflammation and immune responses. Myoglobin and necrotic tissue can also precipitate within renal tubules, causing decreased renal blood flow that may progress into acute kidney injury (Kim et al., 2016).

Acute kidney injury (AKI) is a common complication occurring in about 30% of EHS patients (Abriat et al., 2014; Leon & Bouchama, 2015). It is the result of a combination of ischemic/oxidative injury and tubule obstruction (Bosch et al., 2009). Neutrophil gelatinase-associated lipocalin (NGAL) is one of the most upregulated genes in early stage of ischemic and nephrotoxic kidney injury (Devarajan, 2008). Both urinary and serum NGAL levels have a strong correlation with serum creatinine, which reflects
glomerular filtration rate (Devarajan, 2008). More importantly, NGAL protein is easily detected by western blotting and enzyme-linked immunosorbent assay (ELISA) in blood and urine shortly after AKI (Devarajan, 2008). These features make NGAL one of the most widely used biomarkers for AKI (Schlader et al., 2019). Another commonly used biomarker for AKI is Kidney Injury Molecule-1 (KIM-1), which is a transmembrane protein that has been shown to be upregulated following ischemic and toxic kidney injury (Ichimura et al., 1998). Unlike NGAL which indicates general tubular injury, KIM-1 is mostly upregulated in proximal tubules during AKI (Han et al., 2002).

EIMD has been proposed as a “prior adverse event” that predisposes EHS, particularly when performing endurance exercise in the heat (Fortes et al., 2013). Fortes and colleagues (2013) induced muscle damage with one hour of downhill running (-10% gradient) in a temperate environment (20°C at 65%VO₂max) in non-heat acclimated males. After 30 minutes or 24 hours, subjects performed the exercise heat stress tests which required them to run 40 minutes at 65%VO₂max in a hot environment (33°C and 50% relative humidity). Results showed that EIMD increased heat strain during both subsequent heat stress tests at 30 minutes or 24 hours. The authors particularly noted an increase in rectal temperature and metabolic energy expenditure, compared with the control condition (Fortes et al., 2013). Using the same protocol, Junglee et al. (2013) showed that exercise in the heat after EIMD induced higher plasma interleukin (IL)-6 and core temperature, compared with the temperate condition. Furthermore, exercise in the heat after EIMD elevated NGAL (Junglee et al., 2013). These evidences suggested that thermoregulation was impaired and acute kidney stress was increased up to 24 hours after the muscle damaging event. Based on the findings of Fortes et al. (2013) and Junglee et
al. (2013), muscle damage might be considered a risk factor for EHS and AKI when exercising in the heat.

Not only exercise can induce muscle damage, severe heat stress independently of exercise, can increase cell membrane permeability, and could result in muscle damage. A study using skeletal muscle cells reported that higher temperatures induced faster dye leakage from the cells and the increase in permeability was a function of both temperature and time exposed to the heat (Bischof et al., 1995). The duration of the stable period of fluorescence, indicating stable membrane permeability, was only 16 minutes at 40° C (Bischof et al., 1995). Therefore, it is likely that severe and prolonged heat stress may lead to muscle cell death. It should be noted that many occupational workers, firefighters and military personnel perform physical activities in the heat twice in the same day. In this scenario, they undergo the combined effect of exercise and heat stress which might aggravate the degree of muscle damage and inflammation, since both of them can independently result in muscle damage.

**Problem Statement**

Occupational workers, military personnel, firefighters, and athletes may have to perform physical exertion in a hot environment for two shifts in a day. Thus, muscle damage could be induced during the first shift in the morning and become a predisposing factor of EHS and AKI during the second shift in the afternoon. It should be noted that the EIMD protocols in both Fortes et al. (2013) and Junglee et al. (2013) are downhill running performed in temperate environment (i.e., 20°C). As of now, it remains unclear if
exercise-induced damage performed in a hot environment aggravates muscle damage. Furthermore, could EIMD and heat stress early in a day increase the risk of EHS and AKI later in the same day during endurance exercise in the heat?

**Purpose of the Study**

The purpose of the study was to investigate the effects of downhill running performed in a hot environment on muscle damage (increased serum creatine kinase and perceived soreness, and decreased maximal force production), as well as heat strain (increased core temperature) and acute kidney stress (increased urinary NGAL and KIM-1) during subsequent endurance exercise in the heat.

**Hypotheses**

The following hypotheses will be tested in the study:

Hypothesis 1: Muscle damage (indirectly assessed by serum CK, quadriceps maximal force production and perceived muscle soreness) will be worse in the EIMD in Hot than the EIMD in Cool condition.

*To date, there has been no study examining the combined effect of downhill running and heat stress on levels of muscle damage. However, since both downhill running (Fortes et al., 2013) and heat stress (Bischof et al., 1995) can independently result in muscle damage, it is hypothesized that the combination will result in a higher stress, leading to a greater level of muscle damage.*
Hypothesis 2: By the end of EIMD session, core temperature, heart rate, ratings of perceived exertion and thermal sensation will be higher in EIMD in Hot than EIMD in Cool.

*The environmental heat stress will impair heat dissipation; thus, more metabolic heat will be accumulated to raise core temperature. The cardiovascular system will need to work harder in favor of thermoregulation, resulting in a higher heart rate* (González-Alonso et al., 1999).

Hypothesis 3: Core temperature, heart rate, ratings of perceived exertion and thermal sensation during the HS session (an exercise-heat stress session performed 3 hours after the EIMD session) will be higher in the EIMD in Hot than the EIMD in Cool condition.

*To date, there has been no study examining the effect of prior downhill running in a hot environment on heat strain during subsequent exercise in the heat. However, it is speculated that downhill running in a hot environment would result in a higher level of inflammation, which could impair thermoregulation and increase cardiovascular stress in a subsequent exercise session in the heat.*

Hypothesis 4: Urinary NGAL and KIM-1 concentrations will be higher following EIMD in Hot, comparing with EIMD in Cool.

*Acute kidney injury is a result from a combination of ischemic/oxidative injury and tubule obstruction* (Bosch et al., 2009). *Prolonged exercise in the heat will result in more splanchnic hypoperfusion, resulting in a decrease of blood flow to the kidney. Thus, it is hypothesized that downhill running in a hot environment will result in higher concentrations of acute kidney injury markers.*
Scope of the Study

Subjects were ten healthy, non-heat-acclimated, physically active males aged 20 – 33 years old. Individuals who ranked lower than the 50th percentile in maximal oxygen consumption (VO$_2$ max) according to the American College of Sports Medicine (ACSM) guidelines, regularly performed downhill running, had musculoskeletal injuries or history of rhabdomyolysis or heat stress complications were excluded from the study. Using a crossover design, each subject completed an EIMD in Hot and an EIMD in Cool condition in a randomized order. A wash-out period of at least 2-weeks was given between the two conditions. During the first lab visit, subjects performed a VO$_2$ max test on a treadmill, a verification of treadmill speed at 55% and 65% VO$_2$ max at 1% gradient, and a familiarization trial for MVC test to reduce the learning effect. Treadmill speed that elicited 65% VO$_2$max was used in subsequent EIMD sessions and the speed that elicited 55% VO$_2$max was used in subsequent HS sessions. The EIMD in Cool condition included a one-hour downhill running on a -10% gradient treadmill in a temperate environment (i.e., 22°C, 30% relative humidity) and after a three-hour rest, a 40-minute HS session (1% gradient treadmill running) in a hot environment (i.e., 35°C, 30% relative humidity). The EIMD in Hot condition included a one-hour downhill running on a -10% gradient treadmill in a hot environment (i.e., 35°C, 30% relative humidity) and after a three-hour rest, a 40-minute HS session (1% gradient treadmill running) in the same hot environment. Blood draw, urine samples, perceived muscle soreness test (100-mm visual analog scale) and MVC tests were performed immediately before and after EIMD and HS, and 24 hours after EIMD. Serum CK, urinary NGAL and KIM-1 were analyzed.
Core body temperature and heart rate were monitored throughout every exercise session by a rectal thermistor integrated to a thermometer and Polar heart rate sensor, respectively. Room temperature water was provided *ad libitum* and the volume of water intake was recorded throughout the trials. During the 3-hour rest, participants were required to drink water to replace body weight loss after the EIMD session. Rating of perceived exertion (6-20 scale) and thermal sensation (0-8 scale, 0 = very cold, 8 = very hot) were recorded every ten minutes during each exercise session.

**Assumptions**

The following assumptions were identified in this study:

1. Prior to each exercise visit, participants have refrained from any resistance and eccentric based exercises for 72 hours, caffeine for 8 hours, and alcohol for 24 hours.
2. Participants put forth a maximal effort during MVC tests.
3. Participants were not heat acclimatized/acclimated.
4. Participants reported their nude body weight honestly and accurately.
5. Every time when recording urine output, participants emptied their bladders fully and entirely into the cylinder.
6. The CK measured in the blood is of skeletal muscle origin.

**Limitations**

The following limitations were identified in this study:
1. The study sample consisted of healthy, physically active males between the ages of 20 - 33 years. Therefore, the results of this study may not apply to females, individuals who are sedentary, have chronic disease, and are outside of this range for age.

2. Although at least two weeks for a washout period was given between the conditions and a randomized order design was used, participants may have had a lesser EIMD response in their second arm of the study due to the “repeated bouts effect”.

**Significance of the Study**

Heat stroke results in more than 600 deaths per year in the United States (Gaudio & Grissom, 2016). The overall crude incidence rate of heat stroke in the US military was 0.45 case per 1000 person-years. In young competitive athletes from the United States, heat stroke has been reported as the fifth non-cardiovascular cause of death, after trauma, drugs, suicide and commotio cordis (Maron et al., 2016). Military personnel and occupational workers may have to work double shifts in the same day. If it is during summertime when the ambient environment is hot, the risk of EHS and AKI could be increased with the existence of muscle damage. For outdoor sports athletes, many events such as the summer Olympics are held during the hottest season during the year. It is not uncommon that some athletes need to train two sessions or to participate in two competitive events in the same day, with a few hours of rest in between. Therefore, with muscle damage induced by the first exercise session in a day, the athletes are possibly in a compromised condition when performing the second exercise bout later the same day.
The findings of this study could be meaningful for those populations, in that it would help scientists better understand muscle damage as a predisposing factor related to EHS and AKI and further, mitigate the risk of developing heat-related illnesses.

Definition of Terms

Creatine kinase: indirect marker of muscle damage.

Eccentric exercise: lengthening muscular contraction; has been demonstrated to induce significant amounts of muscle damage.

Exercise-induced muscle damage (EIMD): physical disruption of muscle structures induced by strenuous and/or unaccustomed exercise.

Exertional heat stroke (EHS): a medical emergency with a life-threatening high body temperature and central nervous system dysfunction caused by physical activity usually, but not necessarily, performed in a hot environment.

Maximal oxygen uptake (VO₂max): the maximal rate of oxygen consumption and utilization per minute of exercise

Maximal voluntary isometric contraction (MVC): standardized method for measurement of force production.

Rating of perceived exertion (RPE): A subjective measurement to indicate individual level of perceived physical exertion.

Thermal sensation (TS): a subjective evaluation to indicate individual feeling of the thermal environment.
CHAPTER 2

This chapter presents a review article, entitled “The role of exercise-induced muscle damage in exertional heat stroke” which has been submitted for publication in Sports Medicine. It is authored by Zidong Li, Zachary McKenna, Matthew Kuennen, Flavio de Castro Magalhaes, Christine Mermier and Fabiano Amorim.
The role of exercise-induced muscle damage in exertional heat stroke

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Abstract

Exertional heat stroke (EHS) is a life-threatening condition and affects mainly athletes, military personnel, firefighters and occupational workers. EHS is frequently observed in non-compensable conditions (where the body is unable to maintain a steady thermal balance) as a result of heavy heat stress and muscle contraction associated with prolonged and strenuous physical and occupational activities resulting in central nervous system dysfunction followed by multi-organ damage and failure. Since the pathophysiology of EHS is complex and involves multiple organs and systems, any condition that changes the interrelated systems may increase the risk for EHS. It has been suggested that exercise-induced muscle damage (EIMD) can lead to thermoregulatory impairment and systemic inflammation, which could be a potential pre-disposing factor for EHS. In this review article, we aim to 1) summarize current knowledge on the pathophysiology of EHS, 2) review the mechanisms of EIMD and its complications including inflammation, exertional rhabdomyolysis and acute kidney injury, 3) address the evidence of EIMD as a predisposing factor of EHS and 4) propose a possible mechanism of how combining stressors of muscle-damaging exercise and heat may aggravate muscle damage and subsequent risk of EHS. Such an understanding could be meaningful to minimize the risk of EHS for individuals with muscle damage due to engaging in physical work in hot environments.

Key points:

- EIMD elevates inflammatory status and impairs thermoregulation, which may play a role in predisposing EHS in subsequent exercise-heat stress.
Athletes, military personnel, firefighters and occupational workers who perform multiple shifts/training sessions in a day should be especially cautious when performing physical activities with EIMD in hot/humid environments.
List of definitions

EHS: exertional heat stroke
EIMD: exercise-induced muscle damage
AKI: acute kidney injury
SIR: systemic inflammatory response
LPS: lipopolysaccharides
TLR4: toll-like receptor 4
NO: nitric oxide
POAH: preoptic anterior hypothalamus
BBB: the blood brain barrier
MMP9: matrix metalloproteinase 9
CCL2: chemokine ligand 2
CNS: central nervous system
OVLT: organum vasculosum of the lamina terminalis
IL: interleukin
TNF: tumor necrotic factor
Mb: myoglobin
CK: creatine kinase
DOMS: delayed-onset muscle soreness
ATP: adenosine triphosphate
PGE2: prostaglandins E2
VO2max: maximal oxygen consumption
NGAL: neutrophil gelatinase-associated lipocalin
Introduction

Exertional heat stroke (EHS) is a life-threatening condition that occurs in individuals performing strenuous physical activities, usually in a hot environment, with high incidence in military personnel, occupational workers, and athletes. According to the Military Health System of the United States, there were 578 incident diagnoses of heat stroke among active component service members in 2018 [1]. In young competitive athletes from the United States, heat stroke has been reported as the fifth non-cardiovascular cause of death, after trauma, drugs, suicide and commotio cordis [2]. EHS is characterized by severe hyperthermia (core body temperature $> 40.5^\circ$C) associated with central nervous system disturbances (reduced level of consciousness, confusion, ataxia, seizures, dementia or coma) [3,4]. If not treated, EHS can lead to multi-organ injury/dysfunction, including renal, musculoskeletal, hepatic, cardiovascular and thermoregulatory and systemic inflammatory response syndrome. In extreme cases EHS can lead to irreversible organ injury and ultimately, death. During EHS, hyperthermia and muscle damage occur due to heavy heat and mechanical load. Evidence has shown that exercise-induced muscle damage (EIMD) can impair the thermoregulatory response [5] and in combination with hyperthermia can synergistically or independently lead to systemic inflammation, which could be a potential pre-disposing factor for EHS and acute kidney injury (AKI) [5,6]. Therefore, this current review aims to provide an in-depth synopsis of the mechanisms of EHS and EIMD, as well as the possible role of EIMD in predisposing EHS and AKI during subsequent exercise-heat stress.

Part 1. Exertional heat stroke
1.1 Pathophysiology of exertional heat stroke

The primary pathophysiological mechanism of EHS is the accumulation of excessive heat produced during physical exertion, usually in combination of environmental heat stress that overwhelm heat loss mechanisms (review by Sawka et al. [7]). As physical activity begins, metabolic heat production increases and core body temperature rises. To increase heat dissipation, thermoregulatory responses such as vasodilation of skin blood vessels and sweat production are initiated to maintain core temperature within safe limits. The heat produced by the active skeletal muscles is transferred from internal body areas to the skin via the circulatory system and then to the environment. Factors such as high air temperature and relative humidity and heavy clothing have been shown to reduce heat dissipation. If the rate of heat production is greater than the rate of heat dissipation, core temperature will rise continuously. In this scenario, the cardiovascular system responds by further shunting blood away from the hepatosplanchnic circulation, in favor of increased blood flow to the skin (to support thermoregulatory function) and maintained perfusion of active skeletal muscle. Consequently, there is a competition between central (i.e., viscera) and peripheral (i.e., skeletal muscle and skin) areas for blood flow. This increase in peripheral blood flow together with the reduction of total blood volume induced by sweating reduces central venous pressure. As the splanchnic blood flow decreases, less heat in visceral organs is transported to the skin and core body temperature rises more rapidly. If a person spends a prolonged period doing physical activity especially in hot and humid environments without rest, sufficient fluid intake or cooling, exertional hyperthermia (i.e., the elevation in core body temperature induced by physical work) may become significant. As a result,
systematic inflammatory responses (SIR) and multi-system dysfunction may occur - in the worst scenario, leading into the life-threatening condition of EHS [8,9].

Although exertional hyperthermia does not always result in heat illnesses and is typically asymptomatic [10], severe heat overload may still have some adverse effects on a variety of tissues and organs. First, excessive heat load and reduced splanchnic blood flow during exertional hyperthermia have been shown to affect the integrity of the intestinal barrier. Morrison et al. reported that intestinal permeability was significantly elevated after 90 minutes of exercise in a hot environment (30° C, 50% relative humidity) that caused esophageal temperature of 38.6° C in both trained and untrained individuals [11]. There is more evidence of an exertional hyperthermia-induced increase in intestinal permeability when core temperatures reach 39° C or higher [12–17]. It seems clear that core temperature levels attained during exercise are strongly and positively correlated with the lactulose-to-rhamnose ratio, which is a marker of intestinal permeability [18]. In vitro, Moseley et al. [19] showed that in canine kidney epithelial cells, tight junctions started to open significantly at a temperature of as low as 38.3° C. Moreover, reduced splanchnic blood flow can lead to gastrointestinal ischemia/hypoxia and induce local acidosis, ATP depletion, impaired ion pump activity, oxidative and nitrosative stress (i.e., overproduction of nitric oxide) [20,21]. Collectively, these responses adversely affect gut epithelial cell function, including damaged cell membrane, reduced cell viability and increased tight junction permeability.

The loss of gut epithelial cell function increases gastrointestinal barrier permeability, enabling the leakage of endotoxins into blood circulation. Lipopolysaccharides (LPS) are the major component of gram-negative bacteria cell walls
[22] present in the intestinal tract, and is considered highly pathogenic. When there is
enterocyte membrane damage and tight junction permeability increase in the gut, LPS
will be released from gut lumen into the systemic circulation [23]. It is important to note
that under normal circumstances, LPS leaked from the gut wall can be effectively
neutralized by Kupffer cells in the liver [24]. However, under severe exercise-heat stress,
reduced hepatic blood flow can cause liver function to be diminished. The leakage of
LPS may overwhelm the liver’s limited capacity of microbial neutralization. This may
lead to the spill-over of LPS from portal circulation into systemic circulation, and
elevated circulating LPS concentrations in collapsed human heat stroke patients have
been reported [25].

LPS interacts with Toll-like receptor 4 (TLR4) located in the cell surface and
induces subsequent inflammatory cytokine upregulation in a variety of tissues, such as
immune tissues including liver, spleen and lung, as well as nonimmune tissues including
cardiac and skeletal muscles, resulting in SIR [26,27]. Inflammatory cytokines, including
interleukin (IL)-1β, IL-6 and tumor necrotic factor alpha (TNF-α), are not only
pyrogenic, but also can stimulate the release of nitric oxide (NO) from endothelial cells.
Hall et al. demonstrated that NO production was elevated during hyperthermia [28] and it
seems that high concentrations of NO can increase intestinal permeability [29]. As such,
it creates a vicious cycle that aggravates the release of LPS into the systemic circulation.

Second, exertional hyperthermia and associated endotoxemia can also affect the
brain. Neurons in the preoptic anterior hypothalamus (POAH) sense hypothalamic
temperature and receive afferent inputs from thermoreceptors in the skin, major vessels
and spinal cord. The POAH integrates these sources of thermal information to evoke both
behavioral and physiological thermoregulatory responses [30]. Currently, there is no
evidence showing visible hypothalamus damage in heat stroke patients using advanced
imaging technologies, however it is hypothesized that neurochemicals and cell signaling
pathways may play important roles in thermoregulatory control and cell injury in the
brain, possibly through blood-brain barrier (BBB) disruption [31]. In normal functions,
the BBB allows selective transport of substrates into the brain (such as glucose and amino
acids) and restricts the passage of large molecules (such as pathogens). This selective
permeability is essential for maintaining neural function. Du et al. examined human brain
samples from autopsy of fatal heat stroke cases and reported that matrix
metalloproteinase 9 (MMP9) mRNA expression was positively correlated with the level
of brain edema [32]. MMP9 has been considered to have a key role in the alteration of
BBB permeability and increase of MMP9 can cause BBB disruption [33,34]. Animal
studies have also shown that passive whole body hyperthermia increases BBB
permeability in rats [35,36]. Recently, Yamaguchi et al. cultured human induced
pluripotent stem (iPS) cells-derived brain microvascular endothelial cells at 42° C for 12
hours and reported that heat stress could induce BBB disruption by decreasing claudin-5
expression [36]. In addition to hyperthermia, it was reported that endotoxemia could also
induce the BBB disruption [38]. Mice showed a clear central nervous system (CNS)
induction of the chemokine ligand 2 (CCL2) 2 hours following the intraperitoneal LPS
challenge. CCL2 has been indicated to play a role in weakening the BBB [39]. Moreover,
LPS induced significant increases in hypothalamic and hippocampal transcription of
inflammatory mRNA, including IL-1β, IL-6 and TNF-α [39]. When the BBB becomes
“leaky”, it may be associated with edema and cell injury in the hypothalamus [31]. It is
also noteworthy that in the midline of the preoptic area, there is a structure called organum vasculosum of the lamina terminalis (OVLT) which lacks a BBB and thus can detect circulating cytokines in the blood [40]. At the OVLT, cytokines induce the release of prostaglandins E₂ (PGE₂) which mediates the increase in the temperature set point and causes fever [41]. In conclusion, exertional hyperthermia and accompanying endotoxemia may negatively affect the brain, which likely impairs the CNS and thermoregulatory responses.

Third, prolonged exertional hyperthermia may result in cellular energy depletion, metabolic acidosis and direct thermal injury to the vascular endothelium. When performing prolonged, strenuous physical activities especially in a hot environment, metabolic rate is markedly elevated which could result in metabolic acidosis due to an increased reliance on non-mitochondrial ATP turnover [42] and even intracellular ATP depletion. It has been shown that acidosis can decrease firing rate of warm-sensitive hypothalamic neurons, thereby disrupting thermoregulatory responsiveness [43]. Furthermore, intracellular ATP depletion can also cause skeletal muscle breakdown, which will be discussed later in this review. The endothelium controls vascular permeability and maintains a balance between procoagulant and anticoagulant substances [44]. Evidence in human heat stroke patients has shown that endothelial cells injury is associated with heat stroke [45], Renal dysfunction, liver dysfunction and coagulopathy may develop secondary to the combined effects of thermal injury, endotoxemia and cellular energy depletion, culminating in a life-threatening condition involving multi-system dysfunction [8,46] (Figure 1).
Figure 1. The sequence of events that lead to exertional heat stroke (EHS) characterized by multi-organ dysfunction. $T_{\text{core}}$: core temperature; BF: blood flow; BBB: the blood-brain barrier; CVP: central venous pressure; NO: nitric oxide; POAH: preoptic anterior hypothalamus; ATP: adenosine triphosphate; CNS: central nervous system.
1.2 Risk factors for EHS

EHS is more likely to occur in non-compensable environments such as hot and humid environmental conditions. Thus, environmental conditions (i.e., hot, humid, lack of air movement, etc.) are the most common extrinsic risk factors for EHS. Other extrinsic factors include physical work intensity and/or duration, pressure from coaches/supervisors to go harder, lack of rest, equipment/uniform, or accessibility to fluid and shade, among others. Interestingly, Piver et al. [47] examined heat stroke cases occurred during summer months in Tokyo and suggested that air pollution, specifically exposure to elevated concentrations of nitrogen dioxide (NO₂), was also a significant extrinsic risk factor for heat stroke, possibly due to an increase in the prevalence of lower respiratory symptoms and dysfunction induced by NO₂ exposure [47,48].

On the other hand, there are intrinsic factors that may predispose EHS. It is important to note that exercise in the heat depends on the integration of different physiological processes such as sustaining central blood pressure and regulating body fluid balance, among others. Any conditions that change the interrelated system may increase the risk for EHS. Such intrinsic factors include lack of heat acclimation and poor cardiorespiratory capacity. Furthermore, EHS patients commonly reported a feeling of being ill on previous days [49]. In this scenario where the individuals are compromised or primed with previous adverse events, they would be more susceptible to EHS when exercising in the heat. This is known as the “multiple-hit hypothesis” [50], which refers to a situation where the immune system is “primed” by an initial stimulus that increases vulnerability to subsequent exercise-heat stress. The intrinsic stimuli may include mild illness, gastrointestinal tract disturbance, medications and supplements, among others.
Table 1 presents predisposing factors of EHS. Recently, EIMD has been proposed to become a novel predisposing factor for EHS, as it can acutely elevate inflammatory status along with impaired thermoregulation [5,51]. The following sections will discuss EIMD, rhabdomyolysis and their potential roles in predisposing individuals to EHS.

Table 1. Predisposing factors for exertional heat stroke. Adapted from Leon and Bouchama (2015) and Morrissey et al., (2019).

<table>
<thead>
<tr>
<th><strong>Extrinsic risk factors</strong></th>
<th><strong>Intrinsic risks factors</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>High air temperature</td>
<td>Viral/bacterial infection</td>
</tr>
<tr>
<td>High thermal radiation</td>
<td>Medication/supplement (diuretics, antihistamines, CNS stimulants)</td>
</tr>
<tr>
<td>High air humidity</td>
<td>Lack of heat acclimatization</td>
</tr>
<tr>
<td>Lack of air movement</td>
<td>Poor cardiorespiratory fitness</td>
</tr>
<tr>
<td>Clothing</td>
<td>Skin disorder (anhidrosis, psoriasis, blisters, etc.)</td>
</tr>
<tr>
<td>Exercise/work intensity</td>
<td>Gastrointestinal tract disturbance</td>
</tr>
<tr>
<td>Pressure from coach/supervisor</td>
<td>Genetic conditions</td>
</tr>
<tr>
<td>Air pollution</td>
<td></td>
</tr>
</tbody>
</table>

Part 2. EIMD, rhabdomyolysis and acute kidney injury

2.1 EIMD and inflammation

Although physical activity is necessary for maintaining skeletal muscle function, exercise can also cause damage and inflammation in skeletal muscle. The term used to describe this transient phenomenon is exercise induced muscle damage (EIMD), which can be caused by unaccustomed or intense exercise, particularly if the exercise includes extensive eccentric muscle contraction (i.e., lengthening of skeletal muscles while
generating tension). Eccentric muscle activities, including downhill running, descending stairs and unaccustomed exercise, have been well documented for causing more muscle damage compared to concentric or isometric contractions [52]. As muscles contract while lengthening, they generate more tension so that greater load is distributed among the same number of muscle fibers. This increased load per fiber ratio leads to more mechanical insult and muscle damage [53]. As a result, skeletal muscle cells likely undergo morphological structure disturbance, such as extensive sarcomere disruption [54]. This will lead to a leakage of intracellular contents, such as myoglobin (Mb) and creatine kinase (CK) from skeletal muscle into the systemic circulation.

In most cases, muscle damage induced by exercise is transient and at a very small scale. It is associated with a common symptom called delayed onset muscle soreness (DOMS), which refers to the pain, soreness and/or stiffness felt in muscles after strenuous or unaccustomed exercise. It usually occurs within 24 hours and peaks between 24 to 72 hours, then subsides within seven days post-exercise. People usually do not feel the pain when the “damaged” muscle is at rest, and only feel discomfort when the muscle is being stretched or under external pressure [55].

In addition to pain and soreness in skeletal muscle, EIMD has also been shown to reduce maximal muscular force production, where the temporary decrease in muscular strength induced by downhill running protocols can lead to 10 to 23% of strength loss [56,57]. Exercise protocols that include maximal eccentric contractions can generate as much as 50 to 65% decrease in maximal strength [58]. The time course of the force loss is similar to DOMS in that it usually reaches minimal values 24 hours after exercise, and gradually recovers within a week, depending on the severity of muscle damage [59,60].
Because of these responses after muscle-damaging exercise mentioned above, instead of directly assessing muscle damage using the biopsy technique, researchers have been quantifying serum Mb and CK, perceived muscle soreness and maximal voluntary force production to indirectly measure the levels of muscle damage. Warren et al. [61] reviewed a total of 52 studies examining skeletal muscle damage in human subjects and confirmed that perceived muscle soreness (63% of the studies), blood proteins (52% of the studies) and maximal voluntary contraction torque (50% of the studies) were the three most commonly used indirect markers.

EIMD causes an inflammatory response that initially serves to clear cellular debris from the damaged areas and later facilitates the process of tissue remodeling. As such, inflammation is a natural consequence after muscle damage [62]. Upon muscle damage, satellite cells located between the basal lamina and the fiber membrane are activated [63]. They proliferate to yield myoblasts, which fuse and terminally differentiate into new myotubes that later replace the damaged muscle [64]. Activated neutrophils are the first to arrive at areas of muscle damage [65]. Macrophages also play a role and work together with neutrophils to clear debris. Neutrophils and macrophages act to degrade damaged tissue by releasing reactive oxygen and nitrogen species, which amplify the damage, increase membrane permeability [62,66] and allow for greater release of intracellular protein (such as CK and Mb) into the systemic circulation. As a result of leukocyte accumulation, cytokines are produced at the injury site. Table 2 is a summary of the results of several studies that investigated inflammation after EIMD under temperate environmental conditions. Studies have shown that downhill running induces significant neutrophil infiltration into skeletal muscle tissue at 45 minutes.
following exercise [67], and significant changes in the systemic circulation at 1 hour following exercise [62]. Increased cytokine levels have been found after strenuous eccentric exercise. Muscle IL-1β was significantly increased immediately after downhill running [67] and remained elevated for up to 5 days [68]. Bruunsgaard et al. [69] reported a more prominent increase in plasma IL-6 level after high-intensity eccentric exercise compared with concentric exercise, suggesting that cytokine production after exercise is related to skeletal muscle damage. Hellsten et al. [70] also reported significantly elevated levels of plasma IL-6 from 90 minutes to 4 days post-eccentric exercise, which were proportional to plasma CK concentrations. Other researchers have argued that EIMD may not be an important contributor to the elevated plasma IL-6 levels after eccentric exercise, as plasma IL-6 levels had returned to pre-exercise values at 24 hours following exercise, whereas markers of muscle damage (i.e., plasma Mb and CK levels) remained significantly elevated [62,71]. On one hand, there is evidence showing that skeletal muscle IL-6 mRNA was increased after a marathon race, indicating local production of IL-6 by the damaged muscle [72]. On the other hand, the production of IL-6 by skeletal muscle may not be sufficient to account for the amount of IL-6 in the blood. Some of that increase in plasma IL-6 may be attributed to endotoxemia caused by splanchnic ischemia and subsequent intestinal wall disruption, as opposed to direct skeletal muscle damage [73]. Therefore, depending on the different modes of muscle-damaging exercise, (i.e., endurance or resistance exercise), plasma/serum IL-6 level may not be a good indicator of inflammation induced by EIMD. Despite the controversy of the role of IL-6 in EIMD and inflammatory responses, when all observations (including leukocyte accumulation, IL-1β production and sensation of DOMS) are taken together,
researchers have concluded that muscle damaging exercise likely causes inflammation [65,74,75]
Table 2. Summary of studies examining inflammation after exercise-induced muscle damage (EIMD) in normal ambient temperature.

<table>
<thead>
<tr>
<th>Study</th>
<th>Model</th>
<th>EIMD protocol</th>
<th>Evidence of inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannon et al., 1989</td>
<td>Males</td>
<td>45 minutes of -16% gradient downhill running At 75% VO₂ max</td>
<td>Muscle IL-1β was found in muscle tissue up to 5 days after exercise.</td>
</tr>
<tr>
<td>Fielding et al., 1993</td>
<td>Untrained males</td>
<td>45 minutes of -16% gradient downhill running At 70% HRmax</td>
<td>Muscle IL-1β: 135% increase immediately after exercise (p &lt; 0.05); 250% increase 5 days after exercise (p &lt; 0.05). Neutrophils: significant accumulation 45 minutes and 5 days after (p &lt; 0.05).</td>
</tr>
<tr>
<td>Hellsten et al., 1997</td>
<td>Sedentary males</td>
<td>5 bouts of strenuous one-legged eccentric exercise</td>
<td>Plasma IL-6 increased after 90 minutes (p &lt; 0.05) and remained elevated for 4 days.</td>
</tr>
<tr>
<td>Bruunsgaard et al., 1997</td>
<td>Recreationally active males</td>
<td>30 minutes of high intensity braking with reversed revolution on a cycle ergometer</td>
<td>Increased IL-6 (p &lt; 0.05) 2 hours after. Increased NK cells and CD8+ cells 20 minutes after (p &lt; 0.05)</td>
</tr>
<tr>
<td>Child et al., 1999</td>
<td>Physically active males and females</td>
<td>70 maximal voluntary eccentric isokinetic muscle contractions using knee extensors</td>
<td>Serum β-glucuronidase elevated after 7 days (p &lt; 0.001). Histological analysis showed increased Cellular infiltration between Day 4 and 7 after exercise.</td>
</tr>
<tr>
<td>Peake et al., 2005</td>
<td>Well-trained male runners</td>
<td>45 minutes of -10% gradient downhill running at 60% VO₂ max</td>
<td>Neutrophils and plasma IL-6 increased at both immediately after and 1 hour after exercise (p &lt; 0.01)</td>
</tr>
</tbody>
</table>

HR: heart rate; IL: interleukin; NK: natural killer; VO₂ max: maximal oxygen consumption.
2.2 Exertional rhabdomyolysis

Although one may suffer from an acute decrease in muscular force and DOMS, because EIMD is usually a transient phenomenon (e.g. one that resolves within one week of exposure to damaging exercise), it is unlikely to cause adverse effects on health long-term. However, in the event of intense or prolonged exercise and subsequent severe muscle damage, exertional rhabdomyolysis may occur. This pathophysiological condition is a continuum from EIMD and is characterized by pain, weakness and swelling in the skeletal muscle along with increased levels of muscle proteins (such as CK and Mb) in the bloodstream [76,77]. When Mb levels in the blood reach a range of 300 ng/ml to 2 µg/ml, they may even “spill over” from the blood into the urine. When Mb is present in the urine, the color of urine becomes darker (cola-colored urine) [78]. This could be a life-threatening condition as precipitation of Mb in the kidneys can cause renal failure and even death.

Exertional rhabdomyolysis is a complication of EHS, and it has been suggested that Ca\textsuperscript{2+} plays an important role in the pathogenesis. Continuous and high intensity muscle contraction could lead to fatigue and the depletion of intracellular ATP [79]. Under ATP-depleted conditions, ATP-dependent ion transporters (Na\textsuperscript{+}-K\textsuperscript{+} ATPase and Ca\textsuperscript{2+} ATPase) within skeletal muscle cells become dysfunctional, resulting in Ca\textsuperscript{2+} influx into the cells [79]. This increase in intracellular calcium leads to the activation of proteases and phospholipase A\textsubscript{2} [80], which could result in cell membrane damage and leakage of toxins. Furthermore, increased levels of Ca\textsuperscript{2+} in the mitochondria due to the concentration gradient between sarcoplasm and mitochondria may facilitate the
production of reactive oxygen species, which could also damage the cell membrane [81]. Therefore, researchers suggest that the increase in Ca$^{2+}$ may initiate damage and death of muscle cells during prolonged or otherwise excessive exercise [79,82]. Necrotic skeletal muscle cells release intracellular contents (i.e., CK and Mb) causing pain and swelling in the muscle, and potential liver and kidney damage [83].

When excessive muscle contraction is combined with heat stress, dehydration and subsequent declines in cardiac output may cause a reduced blood flow to the active muscles [84]. Thus, it seems plausible that heat may accelerate ATP depletion within the active muscle cells and the development of exertional rhabdomyolysis due to reduced blood delivery. Nevertheless, in endurance-trained and heat-acclimated individuals, despite of a slight blood flow reduction in active muscles, muscle oxygen delivery and consumption increase in parallel with whole body oxygen consumption during prolonged endurance exercise in the heat [84]. This is due to a continuous increase in muscle arterial-venous O$_2$ difference, indicating a better ability of oxygen extraction [84,85]. It is worth noting that Gonzalez-Alonso et al. [84] used endurance-trained and heat acclimated subjects. Whether heat stress and associating dehydration contribute to exertional rhabdomyolysis in less trained and/or non-heat-acclimated individuals remains to be elucidated.

2.3 Acute kidney injury

Among the complications of exertional rhabdomyolysis, acute kidney injury (AKI) has attracted the most attention. There is a strong correlation between AKI and death, and 10 – 50% of rhabdomyolysis cases may lead to AKI [86]. AKI is also a
common complication occurring in about 30% of EHS patients [9,87]. Myoglobinuria (i.e., presence of Mb in the urine) has been shown to be a key factor contributing to AKI. As mentioned above, exertional rhabdomyolysis causes muscle cell breakdown and leakage of Mb from muscle cells to the blood and urine. The broken down tissue and Mb precipitate and block the renal tubule lumen, resulting in the formation of casts (i.e., tiny granular particles) [88,89]. Because of the casts, renal blood flow is restricted, which can also be attributed to the increased production of vasoconstrictors such as endothelin-1, thromboxane A₂, TNF-α, and a deficit in the production of the vasodilator NO. Furthermore, fluid sequestration within damaged muscle causes intravascular volume depletion and homeostatically activates the renin-angiotensin system, sympathetic nervous system and vasopressin, all of which can lead to vasoconstriction and ischemia. Collectively, AKI results from a combination of ischemic/oxidative injury and tubule obstruction [89]. The risk of AKI due to muscle damage is usually low when CK levels are less than 20,000 U/L [78,90]. It is very common to observe a mild-to-moderate elevation of blood CK indicating EIMD after a routine practice or training in athletes and military personnel. In Clarkson et al. [91], maximal eccentric resistance contractions significantly elevated serum CK at 4-day post exercise (mean value of 7,713 U/L), which remained elevated at post 7 and 10 days. Despite the fact that muscle damage was shown, the lack of clinically significant changes in serum creatinine, blood urea nitrogen, phosphorus and uric acid suggested that kidney function was not compromised [91]. Therefore, EIMD at non-clinical levels seems unlikely to induce AKI. Nevertheless, if a person has coexisting conditions such as sepsis, dehydration and acidosis, AKI is a risk even with a CK level as low as 5,000 U/L [86]. Specifically, dehydration could play a
role in developing rhabdomyolysis-induced AKI. Exertional rhabdomyolysis often occurs during strenuous exercise in the heat, suggesting that the patients were likely dehydrated during and after the exercise [92]. This is important because in animal models, it has been reported that as long as urine flow is adequate, myoglobin does not cause renal failure [93]. A recent human study demonstrated that maintaining euhydration and/or alleviating hyperthermia during prolonged physical work in the heat equally reduces the risk of AKI [94]. These data stress the importance of maintaining adequate hydration in the effort to prevent AKI that is induced by Mb precipitation.

Part 3. Association between heat stress, EIMD and AKI

3.1 Heat stress-induced muscle damage and AKI

There seems to be an association between heat stress, muscle damage and AKI. Severe heat stress, independently of exercise, could increase cell membrane permeability, and can result in muscle damage. It has been shown in vitro that Chinese hamster lung cell membrane protein starts denaturation at a temperature above 40° C [95,96]. Another study using skeletal muscle cells reported that higher temperatures induced faster dye leakage from the cells and the increase in permeability was a function of both temperature and time exposed to the heat [97]. The duration of the stable period of fluorescence, indicating stable membrane permeability, was only 16 minutes at 40° C [97]. Therefore, it is likely that severe and prolonged heat stress may lead to muscle cell death. The degraded muscle cell membrane allows intracellular contents (i.e., CK and Mb) to leak into systemic circulation [3], eventually resulting in rhabdomyolysis. A high
concentration of Mb in the blood can increase Mb presence in the urine, causing renal tubule obstruction and AKI.

### 3.2 Is EIMD a predisposing factor for EHS and AKI?

Conversely, existing muscle damage could be a predisposing factor for exertional heat illnesses including EHS and AKI. Montain et al. [98] were the first to study whether muscle injury is a risk factor contributing to heat illness. Fifty minutes of exercise in the heat (45 – 50% VO₂ max on a treadmill, 40°C, 20% relative humidity) at 2 hours and 7 hours after lower body eccentric resistance exercise caused a small yet consistent increase in core temperature in heat acclimated subjects, compared to the control condition where no previous muscle damage was induced. Possible explanations for this added heat strain might include early inflammatory response shown by elevated circulating leukocytes numbers and plasma IL-6 concentration; or decreased walking economy after EIMD, as the subjects would have likely modified their gait due to muscle soreness and stiffness [98].

In the context of inflammation, the roles of circulating pro-inflammatory cytokines, including IL-1β, IL-6, TNF-α and interferon alpha, as endogenous pyrogens to induce fever have been reviewed by Dinarello [99]. In the classical model of pathogenesis, pyrogenic cytokines are released from damaged muscle into the bloodstream and mediate a febrile response at the thermoregulatory center in the hypothalamus [100]. As mentioned above, OVLT in the midline of the preoptic area can detect circulating cytokines which induce the release of PGE₂. PGE₂ mediates the increase in the temperature set point and causes fever [41]. In addition, muscle damage
induces local release of proinflammatory cytokines which leads to a release of phospholipase A2. Phospholipase A2 has also been suggested to have pyrogenic characteristics and stimulate PGE2 synthesis at the OVLT level [101], thus impairing thermoregulatory responses.

However, the data shown by Montain et al. [98] suggesting that cytokines induce heat strain are not convincing. First, IL-6 was the only cytokine measured in the study. Second, the authors failed to identify a relationship between IL-6 concentration and core temperature increase, as they reported that the plasma IL-6 level was only elevated 7 hours after EIMD and unchanged 2 hours after, whereas core temperature remained elevated at both 2- and 7-hour post EIMD. More recently, Fortes and colleagues [5] induced muscle damage with one hour of downhill running (-10% gradient) in a temperate environment (i.e., 20°C) at 65%VO2max in non-heat-acclimated males. After 30 minutes or 24 hours, the subjects performed exercise heat stress tests which required them to run 40 minutes at 65%VO2max in a hot environment (33°C and 50% relative humidity). Results showed that EIMD increased heat strain during both subsequent heat stress tests 30 minutes or 24 hours later, in particular, a greater increase in rectal temperature and metabolic energy expenditure, compared with the control condition. In a separate publication, the group showed that exercise in the heat after EIMD induced higher plasma IL-6 [6]. Furthermore, exercise in the heat after EIMD induced AKI, as shown by the elevated urinary neutrophil gelatinase-associated lipocalin (NGAL) concentration. Results from these studies suggest that EIMD may impair thermoregulatory response due to increased pyrogenic cytokines production, increase metabolic heat production due to DOMS and reduced exercise economy, and reduce
kidney function during a subsequent exercise bout in the heat. These altered responses might increase the risk of EHS and AKI [5,6] (Figure 2).

Figure 2. Possible mechanisms of how exercise-induced muscle damage (EIMD) predisposes exertional heat stroke (EHS) and acute kidney injury (AKI). EIMD resulting from the morning activity leads to muscle soreness/stiffness, reduced force production, leukocytes and cytokines production and rupture of muscle cell membrane. These responses may affect thermoregulation and renal function during the afternoon exercise-heat stress. First, muscle soreness/stiffness and reduced force-generating capability can lead to a reduced exercise economy and increase metabolic heat production. In addition, inflammatory cytokines are pyrogenic and may impair thermoregulatory response at hypothalamus. Together, these may increase the risk of EHS. On the other hand, damaged muscle cells release myoglobin (Mb) to the systemic circulation. Mb precipitates in the renal tubules, resulting in renal vasoconstriction and reduced kidney
function. Exercising in the heat further decreases renal blood flow and increases inflammatory cytokines production, all of which may increase the risk of AKI.

In summary, based on the existing evidence, it is suggested that acute muscle damage should be considered as a risk factor for the development of EHS especially for individuals who are not heat-acclimated.

Part 4. Does EIMD in the heat worsen muscle damage?

Prolonged or strenuous eccentric exercise can induce muscle damage, soreness and decrease in maximal force production. Similarly, there is evidence that hyperthermia can lead to increased cell membrane permeability and protein denaturation, which may culminate in necrosis of muscle cells [95–97]. Since EIMD and passive hyperthermia can both confer muscle cell damage and subsequent inflammatory responses, it seems reasonable to speculate that the combination of these two stressors during the performance of eccentric exercise in a hot environment may cause even greater levels of muscle damage (Figure 3).

Despite the logic behind this hypothesis, studies that have actually examined the effects of EIMD in the heat are quite scarce. It has been well documented that during continuous exercise in the heat, internal muscle temperature may exceed 40 °C [102,103]. Castellani et al. [104] used shortwave diathermy to locally heat elbow flexors of non-heat acclimatized individuals to about 40.3°C that was followed by two eccentric exercise trials. Results showed that there was no difference in serum CK, Mb, perceived soreness, maximal voluntary isometric contraction, inflammatory cytokines (IL-1β and IL-6) at 6h, 24h, 48h, 72h, 120h and 240h post exercise between the heat and control conditions. These data suggest that the combination of local muscle heating and eccentric exercise
did not accentuate muscle damage or inflammatory responses. It is important to note that the heat intervention in Castellani et al. [104] was passive local muscle heating using diathermy. Thus, their findings have limited application to real-world settings, where people perform exercise in a hot environment and experience whole body hyperthermia. Moreover, hyperthermia-related muscle damage is a function of both temperature and time exposed to the heat, rather than temperature alone [97]. Castellani et al. reported that muscle temperature remained elevated for only five minutes post heating, thus the effect of hyperthermia could be short-lived. Therefore, it remains to be determined whether prolonged muscle-damaging exercise in the heat might induce a greater extent of muscle damage, as compared to performing the same exercise in a temperate ambient environment. More research is needed to study the association of EIMD and heat stress as the findings could be important for military personnel, laborers and athletes who perform physical work in the heat, to mitigate potential muscle damage and its associated risk of EHS.
Figure 3. The proposed mechanism of how muscle-damaging exercise in the heat may aggravate muscle damage. ATP: adenosine triphosphate; Ca^{2+}: calcium ion; ROS: reactive oxygen species; EIMD: exercise-induced muscle damage.

Application and recommendations

When performing strenuous or prolonged exercise in the heat, there is an increased risk of EHS which could be accompanied with exertional rhabdomyolysis and AKI. Also, prior muscle damage may predispose individuals to EHS and AKI when exercising in the heat. There are specific populations that are at particular risk of EHS. For example, athletes and military personnel may be highly motivated and driven to perform, sometimes beyond their physiological capacity. Likewise, firefighters and occupational workers may be exposed to harsh environmental conditions combined with limited heat dissipation due to heavy impermeable clothing and a high humidity. Those
individuals often perform physical exertion in a hot environment for multiple shifts/exercise bouts in the same day [105]. Thus, muscle damage could be induced in the morning shift/exercise bout and be a predisposing risk factor of EHS during the afternoon shift/exercise bout. Therefore, it is important to take protective steps for those populations to mitigate the risk of EHS. There is evidence showing that training status, especially cardiovascular fitness level, affects thermoregulatory capacity and the susceptibility for EHS [106]. Therefore, more careful attention should be paid to the scenarios where athletes resume training after a period of inactivity, and for new military recruits during their first days of training. Examples of preventative methods include heat acclimation/acclimatization, training to increase cardiorespiratory fitness levels, enhancing education, decreasing intensity of exertion, taking rest periods, avoiding hot periods of the day for training/working, and maintaining good hydration [106–108].

Since Fortes et al. [5] proposed that an acute bout of EIMD likely increases the risk of EHS, Dolci et al. [109] suggested a practical strategy which incorporates a muscle-damaging bout into training to reduce the risk of EHS during subsequent strenuous exercise bouts with an eccentric component [109]. It is very well-known that a prior muscle-damaging exercise generates a protective adaptation, and when the same muscle-damaging exercise is repeated, the symptoms of EIMD (e.g., perceived muscle soreness, voluntary force production, etc.) will be blunted [110,111]. This is known as the repeated bout effect. Dolci et al. [109] had non-heat acclimated males perform 1 hour of downhill running (-10% gradient) and 40 minutes of exercise-heat stress, with 30 minutes rest in between. This protocol was repeated 14 days later. A control group performed the same protocol but at +1% gradient instead of downhill. Results showed that the prior
downhill running bout successfully induced the repeated bout effect which blunted the increase in heat strain during the latter exercise-heat stress. Nosaka et al. showed that the repeated bout effect could last for up to 6 months [112]. Therefore, it is recommended to incorporate muscle-damaging exercise into training 2 weeks to 6 months early to “prime” muscle for potential future eccentric exercises and subsequent strenuous physical activities in the heat [109].

Although the repeated bout effect of muscle-damaging exercise does not necessarily “prevent” muscle damage, evidence has shown that it attenuates muscle soreness and changes in the magnitude of maximal voluntary contraction and muscle damage markers in the blood (i.e., CK and Mb) [113]. There is no effect of the repeated bout on the inflammatory cytokines [109,113], suggesting that different levels of muscle damage do not induce significant alterations in plasma cytokine levels.

Other than the repeated bout effect, researchers have focused on nutritional interventions to prevent and alleviate EIMD. Since the repeated bout effect has been shown to be effective in the reduction of the risk of EHS associated with EIMD [109], any nutritional supplementation strategies that have been shown to be effective to attenuate muscle damage from eccentric exercise could be useful for reducing the risk of EHS associated with EIMD. Future studies on the effects of nutritional supplementation, such as branched chained amino acids, antioxidant rich foods, creatine, and omega-3 fatty acids, on the levels of muscle damage and subsequent risks for EHS when exercising in the heat are warranted.
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CHAPTER 3

This chapter presents a research manuscript, entitled “The combined effects of exercise-induced muscle damage and heat stress on acute kidney stress and heat strain during subsequent endurance exercise”. This manuscript is authored by Zidong Li, Zachary McKenna, Zachary Fennel, Roberto Nava, Andrew Wells, Jeremy Ducharme, Jonathan Houck, Christine Mermier, Flavio de Castro Magalhaes, Matthew Kuennen and Fabiano Amorim. This manuscript follows the formatting and style guidelines of the European Journal of Applied Physiology.
The combined effects of exercise-induced muscle damage and heat stress on acute kidney stress and heat strain during subsequent endurance exercise

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Abstract

**Purpose:** The purpose of this study was to investigate the combined effect of downhill running and heat stress on muscle damage, as well as on heat strain and kidney stress during subsequent endurance exercise in the heat. **Methods:** Using a crossover design and randomized order, ten non-heat acclimated, physically active males completed downhill running in a cool (EIMD in Cool) and hot (EIMD in Hot) environment followed by an exercise-heat stress (HS) test after 3-hour seated rest. Core temperature, heart rate, thermal sensation and ratings of perceived exertion were recorded throughout each exercise session. Blood and urine samples were collected at immediately before (pre-) and after (post-) EIMD and HS, and 24 hours post-EIMD (post-24h). Serum creatine kinase (CK) activity, maximal voluntary isometric contraction of the quadriceps (MVC) and perceived muscle soreness were recorded to evaluate muscle damage. Urinary neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) levels were measured to indicate acute kidney stress. **Results:** CK, MVC and perceived soreness were not different between the two conditions at any timepoints. In the EIMD in Hot condition, urinary NGAL level was significantly elevated at post-HS comparing with pre-HS (pre-HS: 6.56 {1.53 – 12.24} ng/min, post-HS: 13.72 {7.67 – 21.46} ng/min, p = 0.034). **Conclusions:** As compared with downhill running in a temperate environment, downhill running in a hot environment does not appear to aggravate muscle damage. However, elevated NGAL levels following EIMD in a hot environment suggest such exercise may increase the risk of acute kidney injury during subsequent endurance exercise in the heat.
**Key words:** exercise-induced muscle damage, exertional heat stroke, acute kidney injury, downhill running

**Declarations**

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**Conflicts of interest:** The authors declare that they have no conflicts of interest.

**Author contributions:** FA conceived the experiments. ZL, ZM, FCM, MK, CM, FA designed the experiments. ZL, ZM, ZF, RN, AW, JD, JH collected data. ZL, ZM analyzed data. ZL drafted the manuscript. All authors contributed to the final manuscript.

**Ethics approval:** This study was approved by the Institutional Review Board at the University of New Mexico Main Campus (IRBNet ID: 1520480).
Abbreviations

EHS: Exertional heat stroke
EIMD: Exercise-induced muscle damage
IL: Interleukin
NGAL: Neutrophil gelatinase-associated lipocalin
AKI: Acute kidney injury
HS: Heat stress
HR: Heart rate
RPE: Rating of perceived exertion
MVC: Maximal voluntary isometric contraction
RH: Relative humidity
CK: Creatine kinase
VAS: Visual analog scale
ELISA: Enzyme-linked immunosorbent assays
CV: Coefficient of variation
SD: Standard deviation
Introduction

Exertional heat stroke (EHS) is a life-threatening condition that usually occurs in individuals performing strenuous physical activities, usually in a hot environment, with high incidence in military personnel, occupational workers, and athletes. EHS is characterized by hyperthermia (core body temperature > 40.5°C) and is associated with central nervous system dysfunction (Armstrong et al. 2007; Muldoon et al. 2008). If not treated, EHS can lead to multi-organ injury/dysfunction, including renal, musculoskeletal, hepatic, cardiovascular and thermoregulatory, and systemic inflammatory response syndrome. In extreme cases EHS can lead to irreversible organ injury and ultimately, death. Although it is generally believed that EHS occurs when thermoregulation is overwhelmed by excessive metabolic heat production and environmental heat stress, the etiology is still not well understood.

Unaccustomed, prolonged and strenuous physical exercise can cause exercise-induced muscle damage (EIMD), especially when the exercise involves a large portion of eccentric muscle contraction. Damage to skeletal muscle cells causes release of intracellular contents (creatine kinase and myoglobin, etc.) into the extracellular space, which can contribute to inappropriate inflammation and immune responses. Myoglobin and necrotic tissue can also precipitate within renal tubules, causing decreased renal blood flow that may progress into acute renal failure (Kim et al. 2016).

Independently from exercise, severe heat stress could increase cell membrane permeability, and can result in muscle damage. A study using skeletal muscle cells reported that higher temperatures induced faster dye leakage from the cells and the increase in permeability was a function of both temperature and time exposed to the heat
The duration of the stable period of fluorescence, indicating stable membrane permeability, was only 16 minutes at 40° C (Bischof et al. 1995). Therefore, it is likely that severe and prolonged heat stress may lead to muscle cell death.

Recent evidence shows that EIMD can increase heat strain and acute kidney stress during subsequent endurance exercise in the heat, particularly noticing greater increases in body core temperature, energy expenditure, plasma interleukin (IL)-6 and neutrophil gelatinase-associated lipocalin (NGAL) levels (Junglee et al. 2013; Fortes et al. 2013). Therefore, EIMD has been proposed to be a potential risk factor for EHS and acute kidney injury (AKI) (Junglee et al. 2013; Fortes et al. 2013). However, it should be noticed that the EIMD protocols in both Fortes et al. (2013) and Junglee et al. (2013) consisted of downhill running performed in temperate environment (i.e., 20° C). As of now, no study has examined the effect of downhill running in the heat on muscle damage and thermoregulation in the following exercise. Therefore, the purpose of the present study was two-fold: to investigate 1) if downhill running performed in a hot environment aggravates muscle damage, and 2) whether the combined effect of EIMD and heat stress would increase the risk of EHS and AKI during subsequent endurance exercise in the heat.

**Methods**

**Participants**

Ten healthy, non-heat-acclimated young adult-aged males (age 26.2 ± 3.5 years, height 178.0 ± 7.1 cm, weight 72.9 ± 11.0 kg, body fat 13.1 ± 5.8%, VO2max 51.2 ± 5.3...
ml/kg/min) were recruited for the study. Body fat % was estimated using the 3-site skinfold method (Jackson and Pollock 1978). Participants were physically active (i.e., > 150 minutes of moderate to vigorous intensity aerobic activity per week for a minimum of 3 months) and did not regularly perform downhill running in their exercise routines. A health history questionnaire was given to the participants to exclude any individuals who had musculoskeletal injury, history of rhabdomyolysis and/or heat stress complications, or medication use (e.g., regular use of nonsteroidal anti-inflammatory drugs). Participants were made aware of all procedures, including the risks and benefits, before signing the informed written consent. The study was approved by the Institutional Review Board at the University of New Mexico.

**Experimental Design**

All data collection was done in the Exercise Physiology Laboratories at the University of New Mexico. Using a crossover counterbalanced design, each participant completed the EIMD in Cool and the EIMD in Hot experimental conditions in a randomized order. The order of conditions was randomized using an online software (http://www.randomizer.org; Site Statistics, Social Psychology Network). After a 3-hour rest period, both conditions were followed by a 40-minute HS session. Participants wore standardized clothing that consisted of a short-sleeved t-shirt, shorts, socks and running shoes. Core body temperature and heart rate (HR) were monitored continuously throughout exercise using a rectal thermistor (Smiths Medical ASD Inc, ER400, Minneapolis, MN) and a Polar HR chest strap sensor (Polar H1, Kempele, Finland).
Every ten minutes during exercise, thermal sensation was acquired based on a 0 – 8 scale (0 = very cold, 8 = very hot), while rating of perceived exertion (RPE) was acquired based on Borg’s 6 – 20 scale (Borg 1982). During every exercise session, participants were encouraged to drink room temperature water *ad libitum*. The volume of water intake and urine output were recorded and differences in nude body mass measurements taken immediately before and after exercise were used to calculate sweat rate. Figure 1 represents a schematic flow of the study, where participants were asked to refrain from vigorous resistance and/or aerobic exercise for 24 hours prior to each exercise trial and a minimum of 2-week of wash-out was provided between the two study conditions.

**Figure 1.** Schematic detailing flow of study. The gradient of downhill running and flat running are -10% and 1%, respectively. EIMD: exercise-induced muscle damage, RH: relative humidity, HS: heat stress.

*Biodex familiarization and maximal oxygen uptake (VO2max) testing.* On the first lab visit, participants were familiarized with how to perform maximal voluntary isometric
contraction (MVC) on a Biodex dynamometer (Biodex Medical Systems 4, Shirley, NY). The MVC test consisted of three maximal contractions of isometric knee extension using the dominant leg at 90° for five seconds, with a 10-second rest provided between contractions. Participants sat upright with the chair’s backrest inclined to 85°. As the purpose of the familiarization session was to minimize the likelihood of a learning effect in subsequent trials, no data were collected during this session. After a brief rest, VO2max was measured using an individualized continuous incremental protocol on a motor-driven treadmill (Precor, TRM885, Woodinville, WA). A five-minute warm-up at a self-selected pace was done before starting the test. The initial speed was 8 km/h and increased every 60s at an individualized rate. The initial incline was 1 % and increased 0.5% every 60s only towards the end of the test when the participants reached their preassigned individual maximal speed. All participants finished their tests at volitional fatigue within 8 to 12 minutes. During the test, a Parvomedics metabolic cart (TrueONE 2400, Sandy, UT) was used to measure and record breath-by-breath expired gases and ventilation data. VO2 data were processed using an 8-breath VO2 rolling average function to improve data interpretation.

Using the VO2 data and corresponding time and treadmill speed from the VO2max test, the speeds eliciting 55% and 65% VO2max were located. Following a 15-minute rest period, a speed verification test was conducted to identify the speeds that would evoke 55% and 65% VO2max in a participant while running at 1% gradient. Expired gas and ventilation were continuously measured during the verification test. The speed was adjusted to elicit the target VO2 until a steady state had reached (± 1 ml/kg/min of the
target VO2). The determined speeds that elicited 55% and 65% VO2 max were later used in HS session and EIMD session, respectively.

**EIMD protocol:** On a separate day, participants reported to the lab in the morning between 8 to 10 am. About 30-minute before the start of each trial, participants had a standardized breakfast consisting of two Natural Valley granular bars and bottled water (equivalent to 5ml/kg body mass). Urine specific gravity was measured using a handheld refractometer (Cole-Parmer, RSA-BR90A, Vernon Hills, IL) to ensure euhydration on the first urine sample during the trial day. Dehydration was established by a urine specific gravity greater than 1.028. Depending on the randomized order, participants performed downhill running at their verified speed to elicit 65% of VO2 max on a -10% gradient treadmill to induce muscle damage in an either Hot (i.e., 35°C, 30% RH) or Cool (i.e., 22°C, 30% RH) condition for 60 minutes.

**3-hour rest.** Following the EIMD session, participants took a 3-hour rest in a seated position in a temperate environment (i.e., 22°C, 30% RH). During this resting period, participants were required to replace nude body weight change with equivalent amount of water to ensure euhydration before the HS. Water intake beyond the required amount was ad libitum. Volume of water intake and urine output were recorded. Lunch was also provided to the participants, which consisted of a sunflower butter and strawberry jelly sandwich and a Clif Bar. The ingredients of the sandwich were two slices of white bread, two tablespoons of sunflower butter and one tablespoon of strawberry jelly. The lunch provided approximately 630 kcal (380 kcal from the sandwich and 250 kcal from the Clif Bar).
**Heat stress session.** After the 3-hour rest, participants ran on a flat treadmill (1% gradient) in a heat chamber (35°C, 30% RH) for 40 minutes at the previously determined speed that elicited 55% of VO\textsubscript{2}max.

**Blood and urine samples collection.** At each timepoint (shown in Figure 1), 10 ml of blood was drawn from the antecubital vein using a 22-gauge needle and Vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ). Hemoglobin concentration in whole blood was measured in duplicate using a Hemoglobin Reagent Set (Point Scientific, Canton, MI) following manufacturer’s instruction. A spectrophotometer (Beckman Coulter DU-520, Fullerton, CA) was used to read the absorbance at a wavelength of 540 nanometers (nm). Hemoglobin and hematocrits values were used to correct for plasma volume changes as described by Dill and Costill (Dill and Costill 1974). Serum tubes were left at room temperature for 20 minutes prior to centrifugation for 15 minutes at 1,400 g. Serum and urine aliquots were stored at -80 °C until subsequent analysis.

**Muscle damage assessments.** As an indirect blood marker of muscle damage, serum creatine kinase (CK) was measured in duplicate using a commercially available creatine kinase reagent (Pointe Scientific, Canton, MI). Absorbance was read at 340 nm. In addition, perceived muscle soreness and MVC were examined at pre-EIMD, pre-HS, post-HS and post-24h. Participants rated their leg muscle soreness using a 100-mm visual analog scale (VAS, 0 = no soreness, 100 = the worst possible soreness) while performing a wall sit (an isometric squat at 90° knee flexion with their back against a wall) (Fortes et al. 2013). Peak torque and average peak torque of the three contractions from MVC were
recorded. Same protocol as the familiarization trial was used. Verbal encouragement was
given to the participants during contractions.

Acute kidney injury assessment. To assess acute kidney injury, enzyme-linked
immunosorbent assays (ELISA) were performed for the acute kidney injury markers
using commercially based kits as per kit instructions: urinary NGAL (BioPorto Human
NGAL ELISA Kit, Hellerup, Denmark) and urinary KIM-1 (Enzo Life Sciences KIM-1
human ELISA kit, Farmingdale, NY). Urine samples were diluted 1:500 for NGAL and
1:10 for KIM-1 analyses. In addition, urinary creatinine was measured (Enzo Life
Sciences, Creatinine colorimetric detection kit, Farmingdale, NY) to correct NGAL and
KIM-1 absolute values in order to exclude the influence of urine concentration (Xin et al.
2008). Intra-assay coefficients of variation (CV) for NGAL and KIM-1 were 7.1% and
9.2%, respectively. Inter-assay CVs for NGAL were 5.3% and 6.6%, and inter-assay CVs
for KIM-1 were 5.3% and 3.7% (NGAL and KIM-1 each used two ELISA kits for all the
samples).

Statistical analysis

Dependent variables were examined for normality by Shapiro-Wilk tests. All
variables passed the normality test, with the exception of NGAL raw values, NGAL
normalized values and KIM-1 raw values. Normally distributed variables were presented
as means ± standard deviations (SD), while medians {interquartile range} were used to
present variables without a normal distribution.

For all normally distributed dependent variables, repeated measures ANOVA
(condition x time) was used to examine the interaction and the significance between
conditions and across timepoints. Post hoc tests with Bonferroni correction were performed to locate the significant comparisons. Assumptions of sphericity were tested using Mauchly's test of sphericity and violations were corrected using the Greenhouse-Geisser correction factor. Nonparametric Friedman tests followed by Dunn’s multiple comparisons were used to analyze non-normally distributed variables.

GraphPad Prism 8 (GraphPad, San Diego, CA) and JASP (University of Amsterdam, Amsterdam, Netherlands) were used to analyze the data and generate graphs. Statistical significance was set at $p < 0.05$. All F values reported are for the interaction (condition x time).

**Results**

**EIMD session.** Core temperature demonstrated an interaction ($F = 7.86$, $p < 0.001$) and post hoc analysis indicated that core temperature readings at the final timepoint (i.e., the 60th minute) was significantly higher in Hot than (38.75 ± 0.35°C vs. 38.27 ± 0.38°C, $p = 0.04$). There was trend for main effect of condition on core temperature (Hot - Cool mean difference = 0.19 ± 0.09°C, $p = 0.075$). HR was significantly higher in Hot than Cool at the final timepoint of the session (157 ± 19 vs. 140 ± 15 bpm, $p = .035$). HR also showed a main effect of condition (Hot - Cool mean difference = 11 ± 3 bpm, $p = .009$). There was also a main effect of condition on thermal sensation scores (Hot vs. Cool mean difference = 1.4 ± 0.3, $p < 0.001$), but no interaction or condition effect were shown for RPE (Figure 2). Sweat rate was significantly higher in Hot than in Cool (1,261.5 ± 247.0
ml/h vs. 889.1 ± 289.2 ml/h, p = 0.003). Similarly, water intake in Hot was significantly greater than in Cool (424.5 ± 245.8 ml vs. 245.0 ± 197.6 ml, p = 0.01).

Figure 2. Physiological variables and subjective feelings during EIMD sessions in Hot and Cool conditions. A: core temperature (°C); B: heart rate (beats per minute); C: thermal sensation score; D: ratings of perceived exertion (RPE). #: Hot is significantly higher than Cool (p < 0.05). **: main effect of time, significantly greater than Time 0 (p < 0.001).

**HS session.** Participants’ core temperature did not show a significant main effect of condition (p = 0.186) or interaction (F = 2.17, p = 0.092). Similarly, there was no difference between the conditions in HR (p = 0.725), thermal sensation score (p = 0.568)
or RPE (p = 0.119) (Figure 3). A significant interaction was demonstrated in thermal sensation score (F = 5.94, p = .011). Sweat rate was not different between the conditions (p = 0.91). Interestingly, prior EIMD in Hot may have influenced participants thirst sensation, as they demonstrated lower water intake during the HS session (EIMD in Hot vs. EIMD in Cool = 331.4 ± 236.8 ml vs. 484.5 ± 247.5 ml, p = 0.04).

![Figure 3](image)

**Figure 3.** Physiological variables and subjective feelings during HS sessions in prior EIMD in Hot and EIMD in Cool conditions. A: core temperature (° C); B: heart rate (beats per minute); C: thermal sensation score; D: ratings of perceived exertion (RPE). **: main effect of time, significantly higher than Time 0 (p < 0.001)

**INSERT TABLE 1 HERE**
Markers of muscle damage. Regardless of environmental conditions, EIMD session caused a significantly increase in serum CK (Table 2) and perceived soreness score on the VAS at pre-HS, post-HS and post-24h (p < 0.001 for both CK and perceived soreness at all three timepoints, Figure 4). However, no differences were shown between study conditions at any timepoint. Similarly, isometric peak torque demonstrated a decrease at post-HS (p = 0.016) and a decreasing trend at post-24h (p = 0.056), comparing to the baseline at pre-EIMD. Average peak torque was also significantly lower at post-HS than pre-EIMD (p = 0.013). There were significant main effects of time on both peak torque (p = 0.012) and average peak torque (p = 0.011). No difference between the conditions existed (Figure 5).

![Graph 1](image1.png)

**Figure 4.** *Left:* Serum creatine kinase fold change following exercise-induced muscle damage in cool and hot condition and subsequent exercise-heat stress. *Right:* Perceived muscle soreness scores on a 100-mm visual analog scale. **: main effect of time, significantly greater than pre-EIMD (p < 0.001).
Figure 5. Peak torque and average peak torque (Newton-meters) over the three contractions during the maximal voluntary isometric contraction (MVC) test. *: significantly different from pre-EIMD (p < 0.05).

Acute kidney injury markers. Urinary NGAL and KIM-1 concentrations are presented as raw values, corrected values based on urinary creatinine and corrected values based on urine output (where applicable, see Table 3). After a prior EIMD session in the hot condition, NGAL was significantly higher at post-HS than pre-HS (13.72 {7.67 – 21.46} ng/min vs. 6.56 {1.53 – 12.24} ng/min respectively, p = .034). Median difference (prior EIMD in Hot – prior EIMD in Cool) and 95% CI for urinary NGAL flow rate between both conditions were -3.17 [-8.79, 1.326] ng/min and 4.19 [-3.01, 17.43] ng/min at pre-HS and post-HS, respectively (Figure 6). KIM-1 flow rate increased 30% and 110% from pre-HS to post-HS in EIMD in Cool and EIMD in Hot, respectively. KIM-1 flow rate also showed a significant interaction (F = 13.74, p = 0.004), however, no main effect of condition (p = 0.438), or difference between timepoints was found (Figure 7).

INSERT TABLE 3 HERE
Figure 6. Left: urinary NGAL flow rate at pre-HS and post-HS for EIMD in Cool (shaded boxes) and EIMD in Hot (clear boxes) conditions. Data are presented as median (solid line in box), quartiles (boxes), and ranges (whiskers) given nonparametric data. The dark circle indicates an outlier. *: significantly different from pre-HS within the condition (p = 0.034). Right: Median difference and 95% confidence intervals between prior EIMD in Hot and prior EIMD in Cool for urinary NGAL at pre- and post-HS. A positive value indicates urinary NGAL is higher in the EIMD in Hot condition.

Figure 7. KIM-1 flow rate (left) and fold change (right) between pre-HS and post-HS in the EIMD in Cool and EIMD in Hot conditions.
Discussion

The purpose of the present study was to investigate the combined effect of downhill running and heat stress on the degree of muscle damage, as well as thermoregulatory strain and acute kidney stress during subsequent endurance exercise in the heat. Our data demonstrate that as compared to downhill running in a temperate environment, downhill running in the heat contributed to an elevated level of NGAL, suggesting that increased kidney stress may have been elevated during the subsequent exercise-heat stress. However, heat stress combined with downhill running did not aggravate muscle damage. To date, this is the first study examining the potential effect of prolonged muscle-damaging exercise in the heat on the risk of EHS and AKI during subsequent endurance exercise. These findings may be of benefit to firefighters, occupational workers, military personnel and athletes, all of whom may perform multiple work or exercise bouts of that involve eccentric muscle contraction in hot environments in the same day.

Downhill running is commonly used in studies to induce muscle damage (Peake et al. 2005; Junglee et al. 2013; Fortes et al. 2013). The goal of our model was to elicit mild-to-moderate muscle damage by prolonged aerobic exercise. This level of muscle damage would moderately impair participants’ ability to complete subsequent endurance exercise in the heat after a 3-hour rest. The extent of skeletal muscle damage induced by our protocol was indirectly evaluated by post-exercise changes in serum CK concentrations, isometric force production and participants’ perceived soreness ratings. Between pre-EIMD and pre-HS, peak torque, serum CK and perceived soreness ratings all showed a time effect (peak torque mean difference = 15.5 ± 5.5 Nm, p = 0.02; CK
mean difference = 25.9 ± 3.5 U/L, p < 0.001; VAS mean difference = 28.4 ± 3.3 mm, p < 0.001), likely indicating that muscle damage had been inflicted. Moreover, the fold changes of serum CK between post-24h and pre-EIMD (2.9-fold in EIMD in Cool, 2.5-fold in EIMD in Hot) were comparable to the CK fold change (3.1-fold) reported by Fortes et al. (2013).

By the end of the EIMD session, core temperature was ~0.5° C higher and HR was 17 bpm higher in Hot than those in Cool, suggesting that the combined effect of downhill running and environmental heat inflicted greater thermoregulatory and cardiovascular stress comparing to the effect of downhill running in temperate environment. Nevertheless, the combined effect of EIMD and heat did not seem to aggravate muscle damage comparing to EIMD alone. Studies that examined the effects of EIMD in the heat are quite scarce. Castellani et al. used shortwave diathermy to locally heat elbow flexors of non-heat acclimatized individuals to about 40.3° C followed by two eccentric exercise trials. Results showed that there was no difference in serum CK, myoglobin, perceived soreness, MVC, inflammatory cytokines (IL-1β and IL-6) at 6h, 24h, 48h, 72h, 120h and 240h post exercise between the heat and control conditions (Castellani et al. 2016). These data suggest that the combination of local muscle heating and eccentric exercise did not accentuate muscle damage or inflammatory responses. It is worth noting that hyperthermia-related muscle damage is a function of both temperature and time exposed to the heat, rather than temperature alone (Bischof et al. 1995). Castellani et al. reported that muscle temperature remained elevated for only five minutes post heating, thus the effect of hyperthermia could be short-lived. In the present study, although the time exposed to the heat was much longer, the average peak core
temperature during the one hour of downhill running in Hot condition was only 38.75°C. Therefore, the added heat stress during downhill running in the present study may only have minimal effect on muscle damage.

After 3-hour rest, participants had very similar physiological responses in the two conditions during the HS. There was no difference in core temperature during the whole HS session between the conditions. Given that hydration levels in the two conditions were similar as shown by plasma volume changes, the similar responses to core temperature suggest that the inflammatory response and exercise economy after EIMD in Hot and EIMD in Cool were similar as well. It is also no surprise that HR response was similar in both conditions since participants remained euhydrated.

To exclude the influence of urine concentration on urinary AKI markers, we present not only raw values but also corrected values based on urine output, and corrected values based on urinary creatinine. This has been done to improve data interpretation, but it is worth noting that there is no “gold standard” for AKI markers normalization. Normalization using urinary creatinine is commonly used in clinical settings (Xin et al. 2008; Sirota et al. 2013; Pajek et al. 2014). It has an underlying assumption that glomerular filtration rate (GFR) is constant across and within individuals (Waikar et al. 2010). However, this assumption cannot be always met, especially in prolonged or strenuous exercise settings like the present study where GFR likely changes within individuals due to splanchnic hypoperfusion. Therefore, normalization using timed urine output is suggested to be more accurate (Waikar et al. 2010; Junglee et al. 2013). In the present study, NGAL and KIM-1 values at pre-EIMD and post-24h cannot be corrected based on urine output because there was no timed urine collection for the two timepoints.
Thus, values normalized by urine output were presented only at pre-HS and post-HS. Our data show that at post-HS, NGAL (ng/min) was significantly higher than pre-HS in the condition where a 3-hour prior EIMD session was performed in the heat, comparing with that performed in temperate temperature. KIM-1 (ng/min) at post-HS, although not statistically significant, was 2.1-fold higher than pre-HS in the EIMD in Hot condition, comparing with a 1.3-fold increase in the EIMD in Cool condition. These results may suggest that the combined effect of downhill running and heat stress may increase the risk of AKI during a subsequent endurance exercise in the heat.

Although NGAL and KIM-1 showed an increasing trend from pre-HS to post-HS and post-24h, we recognize that our median raw values are far lower that the values used to define clinical AKI. The median NGAL value in level 1 AKI individuals stratified by Acute Kidney Injury Network (AKIN) score is 79.2 ng/ml (Schinstock et al. 2013). In the present study, the highest median raw value was at post-24h in EIMD in Hot condition (28.8 ng/ml) and the highest observed individual value was 63.7 ng/ml. It is certainly not our study aim to induce AKI. However, these subclinical elevations of NGAL and KIM-1 may indicate “pre-renal” AKI in response to renal hypoperfusion (Nejat et al. 2012). It describes a mild form of structural AKI that can recover typically in 24 – 48 h.

In the present study, participants were encouraged to drink *ad libitum* during exercise. During the 3-hour rest, participants were required to drink at least the amount of water equivalent to their nude body weight loss after the EIMD session. Therefore, as shown by plasma volume changes, our participants remained euhydrated after the HS sessions. It is important because dehydration could play a role in developing muscle damage-induced AKI. It has been demonstrated that maintaining euhydration and/or
alleviating hyperthermia during prolonged physical work in the heat both reduce the risk of AKI (Chapman et al. 2020). As long as urine flow is adequate, myoglobin does not cause renal failure (Knochel 1982). This evidence stresses the importance of maintaining adequate hydration in an effort to prevent AKI that is induced by myoglobin precipitation.

Conclusion

In summary, as compared with downhill running alone, the combined effect of downhill running and heat stress does not seem to aggravate muscle damage. However, our data suggest that risk of mild AKI during subsequent endurance exercise in the heat may be elevated. The findings of the present study could be of importance to some specific populations. Firefighters and occupational workers may be exposed to hot conditions combined with limited heat dissipation due to heavy impermeable clothing. Athletes who just returned to training after a period of physical inactivity and new military recruits are more susceptible to EIMD if the exercises are unaccustomed. Those individuals often perform physical exertion in a hot environment for multiple shifts/training sessions in the same day (Scarneo et al. 2018). Thus, muscle damage could have been induced in the early shift/training session, potentially increasing the risk of AKI during the late shift/training session. Therefore, precautions such as maintaining euhydration and body cooling should be taken to mitigate the risk of AKI.
References


Table 1. Physiological variables and subjective feelings at the beginning and the end of EIMD and HS sessions.

<table>
<thead>
<tr>
<th></th>
<th>Pre-EIMD</th>
<th>Post-EIMD</th>
<th>Pre-HS</th>
<th>Post-HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core temperature (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIMD in Cool</td>
<td>37.15 ± 0.34</td>
<td>38.27 ± 0.38</td>
<td>37.23 ± 0.30</td>
<td>38.65 ± 0.40</td>
</tr>
<tr>
<td>EIMD in Hot</td>
<td>37.20 ± 0.35</td>
<td>38.75 ± 0.35 *</td>
<td>37.22 ± 0.28</td>
<td>38.41 ± 0.44</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIMD in Cool</td>
<td>85 ± 11</td>
<td>140 ± 15</td>
<td>98 ± 11</td>
<td>167 ± 19</td>
</tr>
<tr>
<td>EIMD in Hot</td>
<td>98 ± 16</td>
<td>157 ± 19 *</td>
<td>102 ± 16</td>
<td>167 ± 16</td>
</tr>
<tr>
<td>Rating of perceived exertion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIMD in Cool</td>
<td>6.0 ± 0.0</td>
<td>13.6 ± 2.2</td>
<td>6.0 ± 0.0</td>
<td>14.4 ± 2.0</td>
</tr>
<tr>
<td>EIMD in Hot</td>
<td>6.0 ± 0.0</td>
<td>14.5 ± 1.7</td>
<td>6.0 ± 0.0</td>
<td>14.2 ± 2.3</td>
</tr>
<tr>
<td>Thermal sensation score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIMD in Cool</td>
<td>3.1 ± 1.0</td>
<td>5.7 ± 1.2</td>
<td>4.6 ± 1.0</td>
<td>7.4 ± 0.7</td>
</tr>
<tr>
<td>EIMD in Hot</td>
<td>4.5 ± 0.7 *</td>
<td>7.1 ± 0.7 *</td>
<td>4.8 ± 0.8</td>
<td>6.8 ± 0.6</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. EIMD: exercise-induced muscle damage; HS: heat stress; bpm: beats per minute. *: significantly different from the EIMD in Cool condition (p < .05)
Table 2. Peak torque, average peak torque and serum creatine kinase following exercise-induced muscle damage in Cool and Hot conditions, and subsequent exercise-heat stress.

<table>
<thead>
<tr>
<th></th>
<th>Pre-EIMD</th>
<th>Pre-HS</th>
<th>Post-HS</th>
<th>Post-24h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peak torque (Nm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIMD in Cool</td>
<td>193.03 ± 57.03</td>
<td>184.23 ± 60.25</td>
<td>179.02 ± 71.26</td>
<td>181.46 ± 68.62</td>
</tr>
<tr>
<td>EIMD in Hot</td>
<td>195.12 ± 58.50</td>
<td>170.02 ± 52.18</td>
<td>170.29 ± 54.33</td>
<td>173.72 ± 47.38</td>
</tr>
<tr>
<td><strong>Average peak torque (Nm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIMD in Cool</td>
<td>181.47 ± 54.20</td>
<td>170.73 ± 55.62</td>
<td>166.23 ± 59.69</td>
<td>168.84 ± 58.26</td>
</tr>
<tr>
<td>EIMD in Hot</td>
<td>183.34 ± 54.52</td>
<td>162.08 ± 47.98</td>
<td>158.79 ± 50.24</td>
<td>164.14 ± 45.18</td>
</tr>
<tr>
<td><strong>Creatine kinase (U/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIMD in Cool</td>
<td>42.68 ± 11.43</td>
<td>69.55 ± 20.81</td>
<td>90.48 ± 22.12</td>
<td>115.20 ± 39.78</td>
</tr>
<tr>
<td>EIMD in Hot</td>
<td>45.81 ± 20.90</td>
<td>70.70 ± 28.49</td>
<td>85.20 ± 26.85</td>
<td>105.47 ± 31.04</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. EIMD: exercise-induced muscle damage; HS: heat stress; Nm: Newton-meter.
Table 3: Kidney injury markers following exercise-induced muscle damage in Cool and Hot conditions, and subsequent exercise-heat stress.

<table>
<thead>
<tr>
<th></th>
<th>Pre-EIMD</th>
<th>Pre-HS</th>
<th>Post-HS</th>
<th>Post-24h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urinary NGAL raw values (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIMD in Hot</td>
<td>12.95 (5.20 – 18.65)</td>
<td>3.05 (0.73 – 5.90)</td>
<td>7.40 (2.93 – 9.85)</td>
<td>28.80 (7.55 – 40.18)</td>
</tr>
<tr>
<td><strong>Urinary NGAL flow rate (ng/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIMD in Cool</td>
<td>N/A</td>
<td>9.40 (2.99 – 14.80)</td>
<td>8.15 (2.73 – 17.02)</td>
<td>N/A</td>
</tr>
<tr>
<td>EIMD in Hot</td>
<td>6.56 (1.53 – 12.24)</td>
<td>13.72 (7.67 – 21.46) *</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Urinary NGAL/creatinine (ng/mg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIMD in Cool</td>
<td>6.3 (0.98 – 27.65)</td>
<td>3.40 (2.55 – 8.60)</td>
<td>4.90 (2.38 – 10.00)</td>
<td>12.85 (5.45 – 63.73)</td>
</tr>
<tr>
<td>EIMD in Hot</td>
<td>11.15 (5.25 – 28.05)</td>
<td>3.45 (2.00 – 10.75)</td>
<td>6.75 (4.25 – 16.68)</td>
<td>8.85 (2.53 – 34.45)</td>
</tr>
<tr>
<td><strong>Urinary KIM-1 raw values (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIMD in Cool</td>
<td>0.54 (0.23 – 1.09)</td>
<td>0.42 (0.18 – 0.96)</td>
<td>0.76 (0.28 – 2.72)</td>
<td>0.74 (0.13 – 1.64)</td>
</tr>
<tr>
<td>EIMD in Hot</td>
<td>0.60 (0.27 – 0.86)</td>
<td>0.22 (0.08 – 0.44)</td>
<td>0.22 (0.17 – 1.05)</td>
<td>1.15 (0.32 – 1.88)</td>
</tr>
<tr>
<td><strong>Urinary KIM-1 flow rate (ng/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIMD in Cool</td>
<td>N/A</td>
<td>42.85 ± 20.20</td>
<td>50.83 ± 39.27</td>
<td>N/A</td>
</tr>
<tr>
<td>EIMD in Hot</td>
<td>34.29 ± 25.14</td>
<td>68.94 ± 59.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Urinary KIM-1/creatinine (ng/mg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIMD in Cool</td>
<td>0.71 ± 0.36</td>
<td>0.46 ± 0.18</td>
<td>0.54 ± 0.28</td>
<td>0.60 ± 0.37</td>
</tr>
<tr>
<td>EIMD in Hot</td>
<td>0.51 ± 0.20</td>
<td>0.41 ± 0.19</td>
<td>0.49 ± 0.28</td>
<td>0.47 ± 0.15</td>
</tr>
</tbody>
</table>

Data are the median (25% and 75% quartiles) or mean ± SD. EIMD: exercise-induced muscle damage, HS: heat stress, NGAL: neutrophil gelatinase-associated lipocalin, KIM-1: kidney injury molecule-1. *: Significantly different from the EIMD in Cool condition (p = .034).
Table 4. Water intake, plasma volume change and sweat rate throughout the trials.

<table>
<thead>
<tr>
<th></th>
<th>Pre-EIMD</th>
<th>EIMD session</th>
<th>Post-EIMD</th>
<th>3-hr rest</th>
<th>Pre-HS</th>
<th>HS session</th>
<th>Post-HS</th>
<th>Total in the day</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water intake (ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIMD in Cool</td>
<td></td>
<td>245.0 ± 197.6</td>
<td></td>
<td>1074.0 ± 386.7</td>
<td>484.5 ± 247.5</td>
<td></td>
<td></td>
<td>1803.5 ± 562.2</td>
</tr>
<tr>
<td>EIMD in Hot</td>
<td>N/A</td>
<td>424.5 ± 245.8**</td>
<td>N/A</td>
<td>1074.8 ± 193.3</td>
<td>N/A</td>
<td>331.4 ± 236.8 *</td>
<td>N/A</td>
<td>1830.7 ± 427.6</td>
</tr>
<tr>
<td><strong>PV change (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIMD in Cool</td>
<td>0</td>
<td>N/A</td>
<td>0.29 ± 7.37</td>
<td>N/A</td>
<td>-1.68 ± 5.97</td>
<td>N/A</td>
<td>0.22 ± 8.89</td>
<td>N/A</td>
</tr>
<tr>
<td>EIMD in Hot</td>
<td>0</td>
<td>-3.68 ± 3.94</td>
<td></td>
<td>-0.35 ± 5.52</td>
<td></td>
<td></td>
<td>0.55 ± 6.00</td>
<td></td>
</tr>
<tr>
<td><strong>Sweat rate (ml/h)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIMD in Cool</td>
<td>N/A</td>
<td>889.1 ± 289.2</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>1273.5 ± 446.7</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>EIMD in Hot</td>
<td></td>
<td>1261.5 ± 247.0**</td>
<td></td>
<td></td>
<td></td>
<td>1287.2 ± 324.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. PV: plasma volume, EIMD: exercise-induced muscle damage, HS: heat stress. N/A: not applicable.

*: significantly different from the EIMD in Cool condition (p < .05)

**: significantly different from the EIMD in Cool condition (p < .01)
CHAPTER 4

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

This study was the first to investigate the combined effect of EIMD and heat stress on muscle damage, as well as acute kidney stress and heat strain during subsequent endurance exercise in the heat in physically active males aged 20 – 33 years. Most endurance athletes and new military recruits fall into this age range. According to the US Fire Department Profile 2018, 48% of the firefighters in the United States are between 20 and 39 years old. Specifically, we investigated: 1) if downhill running in a hot environment aggravates the degree of muscle damage as measured by indirect markers including serum creatine kinase, maximal muscular force production and perceived soreness, comparing with downhill running in a temperate environment, and 2) the combined effect of EIMD and heat stress, after a 3-hour seated rest, on acute kidney stress and heat strain during subsequent endurance exercise in the heat as evaluated by urinary NGAL and KIM-1 and core body temperature. The review manuscript in Chapter 2 entitled “The role of exercise-induced muscle damage in exertional heat stroke” systematically reviewed current literature on the pathophysiology of EHS and EIMD, presented current evidence and a proposed mechanism of EIMD as a predisposing factor for EHS and AKI. This paper demonstrates that, though a prior EIMD may increase the risk of EHS and AKI, whether the muscle-damaging exercise performed in the heat further increases the risk of EHS and AKI during subsequent exercise remains unconfirmed.
The research manuscript in Chapter 3 entitled “The combined effects of exercise-induced muscle damage and heat stress on acute kidney stress and heat strain during subsequent endurance exercise” provides evidence that a downhill running in a hot environment that occurred 3 hours prior may increase the risk of AKI when performing endurance exercise in the heat, comparing with a prior downhill running in a temperate environment.

Conclusions

We conclude the following regarding our study of downhill running in a hot environment versus a cool environment performed by young adult-aged physically active males:

- Heat stress combined with downhill running does not aggravate muscle damage, comparing with downhill running in a temperate environment.
- Heat stress combined with downhill running does not increase heat strain 3 hours later during an endurance exercise in the heat, comparing with downhill running in a temperate environment.
- Heat stress combined with downhill running may increase acute kidney stress 3 hours later during an endurance exercise in the heat, comparing with downhill running in a temperate environment.

Recommendations

We did not find any difference in degrees of muscle damage, as well as heat strain during subsequent exercise between the two conditions. However, we suggest future research use an EIMD protocol that involves both aerobic and resistance exercise in the
heat, as firefighters, military personnel and occupational workers may not only perform aerobic exercise but also some upper body resistance exercise. This will likely induce a greater level of muscle damage comparing with solely downhill running and provide more insight as to the combined effect of EIMD and heat stress on the risks of EHS and AKI during subsequent exercise.

Further examination of inflammatory markers (e.g., NF-κB, COX-2, TNF-α and IL-6) within peripheral blood mononuclear cells may provide more understanding into the combined effect of downhill running and heat stress. In addition, since EIMD in the heat can induce a higher end-of-exercise core body temperature than EIMD in a cool environment, it would be good to examine gastrointestinal permeability by measuring intestinal fatty-acid binding protein, as endotoxin leakage from the gut could clearly impact thermoregulatory and inflammatory responses.

It is recommended for populations such as firefighters, athletes, military personnel and occupational workers to take protective steps to mitigate the risk of EHS and AKI induced particularly by prior muscle-damaging exercise performed in the heat. Some general preventative methods include heat acclimation/acclimatization, training to increase cardiorespiratory fitness levels, enhancing education, decreasing intensity of exertion, taking rest periods, avoiding hot periods of the day for training/working, and maintaining good hydration (Casa et al., 2015; Cleary, 2007; Périard et al., 2015). To specifically mitigate EIMD, Dolci et al. suggested a practical strategy which incorporates a muscle-damaging bout into training to reduce the risk of EHS during subsequent strenuous exercise bouts with an eccentric component (Dolci et al., 2015). It is very well-known that a prior muscle-damaging exercise generates a protective adaptation, and when
the same muscle-damaging exercise is repeated, the symptoms of EIMD (e.g., perceived muscle soreness, voluntary force production, etc.) will be blunted (Byrnes et al., 1985; McHugh et al., 1999). This is known as the “repeated bout effect”. In addition, any nutritional supplementation strategies that have been shown to be effective to attenuate muscle damage from eccentric exercise could be useful for reducing the risk of EHS and AKI associated with EIMD.
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APPENDICES

A. Informed Consent
B. Flyer
C. Health History Questionnaire
D. Data Collection Sheets
Appendix A

Informed Consent

Does Exercise-induced Muscle Damage in the Heat Exacerbate Heat Strain in Subsequent Exercise?
Consent to Participate in Research
07/30/20

Purpose of the study: You are being asked to participate in a research study that is being done by Fabiano Amorim Ph.D., who is the principal investigator and Zidong Li and their collaborators from the Department of Health, Exercise, and Sports Science at the University of New Mexico. The purpose of this study is to examine if an initial downhill running in the heat compared to the same run in a cool environment induces more muscle soreness, as well as heat strain and acute kidney stress during subsequent endurance exercise in the heat.

You are being asked to take part in this study because you are 18-40 years old, healthy and physically active (i.e., >150 minutes of moderate to vigorous intensity aerobic activity per week for a minimum of 3 months).

You are not able to participate if you are pregnant, regularly perform downhill running or are heat-acclimated, or have any lower body injuries or have history of rhabdomyolysis (i.e., the rapid breakdown of damaged skeletal muscle) or any heat stress complications.

This form will explain what to expect when joining the research, as well as the possible risks and benefits of participation. If you have any questions, please ask one of the study researchers. Your participation in this research is voluntary.
**Key information for you to consider:**

- Visit 1: Informed consent, Height, Weight, Body Composition, Cardiorespiratory Fitness (VO₂ max) Test.
- Visit 2: Urine and Blood collection, Muscular Strength Test, Downhill Running 3-hour rest, Flat Running.
- Visit 3: Blood Draw, Muscular Strength Test.
- Visit 4: Urine and Blood Collection Draw, Muscular Strength Test, Downhill Running, 3-hour rest, Flat Running.
- Visit 5: Blood Draw, Muscular Strength Test.
- The environmental condition of Downhill Running (i.e., cool or hot) in Visit 2 and 4 will be decided by a coin flip.
- You will be asked to come in two days in a row for Visits 2 and 3, Visits 4 and 5. There will be at least 2 weeks between Visit 3 and Visit 4.
  - Visit 1 will take approximately 1 hour.
  - Visit 2 and 4 will each take approximately 6 hours.
  - Visit 3 and 5 will each take approximately 20 minutes.
  - Total time estimate: 13 hours.
- Major Benefits:
  - Results of your cardiorespiratory fitness test and body fat.
- Major Risks:
  - Exercise in the heat: sweaty, hot, light-headed, fatigue and nauseous during or after the session.
  - Downhill running side effects: muscle pain and/or soreness during the next few days.
  - Blood Draw: bleeding at the site, feeling of lightheadedness when the blood is drawn, and rarely, an infection.
  - Increased risk of exposure to COVID-19 because of your participation in this study.

**What you will do in the study:** After reading the consent form and discussing the details with the research team, if you decide to participate, you will be asked to fill out a health history questionnaire and a COVID-19 Symptoms Screening Checklist that will be sent to you via email. You will complete the questionnaire and send it back to the research team via email before your first visit. You will sign the consent form during your first visit. If you never show up for Visit 1 or signs the informed consent, your completed health history questionnaire will be deleted permanently within 4 weeks of receipt.

Before every visit, a research team member will call you and inquire whether you have any COVID symptoms using the COVID-19 symptoms checklist. You will also be asked if you have been exposed to anyone with suspected or known COVID-19. You will be approved to come to the laboratory if you have no signs and symptoms of COVID-19 and if you have not been exposed to anyone who has COVID-19 symptoms or has tested positive for the virus. Prior to entering the lab, your body temperature will be measured by a no-touch forehead thermometer. If your temperature is over 37.5 °C (99.5 °F) you will not be allowed to get into the lab and the visit will be rescheduled.

**Visit 1: Height, Weight, Body Composition, Familiarization to the Muscular Strength Test, Cardiorespiratory Fitness (VO₂ max) Test**
• You will report to the Exercise Physiology Lab in the University of New Mexico in Johnson Center after being asked to: (a) avoid having a full meal within 1 hour, (b) avoid having caffeine for 4 hours, (c) avoid performing vigorous exercise for 24 hours. During the initial part of the visit, a urine pregnancy test will be administered if you are female. A positive result will exclude you from the study. Then, we will measure your body weight using a digital scale and height using a stadiometer. Your body fat will be estimated by skinfold method. A trained technician from the research team will use a caliper to measure the thickness of subcutaneous fat at three sites: chest, abdomen and thigh for males, triceps, hip and thigh for females. All the sites are from the right side of your body.

• You will be instructed to perform a familiarization trial of maximal strength test. You will perform knee extensions at 90-degree using a Biodex dynamometer. The Biodex dynamometer measures the force applied with muscle contraction. You will do three repetitions as hard as you can with your dominant leg. Each repetition will be five seconds, and you will have 10 seconds to rest between each repetition.

• Your VO2max refers to the maximum amount of oxygen you can utilize during maximal exercise which is generally considered the best indicator of cardiovascular fitness.

• Your VO2max will be measured using a maximal running exercise conducted on a treadmill. Initially you will warm up at your own pace for 3 minutes. Then you will be asked to run at a speed that you can maintain for 2 minutes. Afterwards, you will be asked to put on a nose clip and mouthpiece with a breathing valve to collect expired gases. The treadmill speed will be increased continually every 60s at an individual rate, which will be based on your exercise history questionnaire, to induce fatigue within 8 to 12 minutes. The grade will be maintained at 3%. When you feel you cannot run any longer, you will sign to the researcher to stop the treadmill. The mouthpiece and nose clip will be removed, and you will cool-down for 3 minutes at your own pace. During the test your heart rate will be measured using a strap placed on your chest and a receptor. Also, every minute we will ask what your perceived rate of exertion using a 6-20 based scale.

• You can continue participating in this study only if your measured VO2max ranks above 50th percentile based on your age and gender, according to the ACSM guidelines. Fifteen minutes after the VO2max test, based on your VO2max, you will jog/run with expired gas analyzed continuously to verify the speeds that elicit 55% and 65% of your VO2max. Each steady state will be five minutes. The treadmill speed will be adjusted every five minutes until 55% VO2max has been stably reached. This treadmill speed will be used in the following flat running sessions. Once 55% VO2max is verified, speed will be increased until 65% VO2max is stably reached. The treadmill speeds required to elicit 55% and 65% VO2max will be used in the following running sessions.

Visit 2: Urine Collection, Blood Draw, Muscular Strength Test, Downhill Running in a Cool or Hot Environment, Perceived Muscle Soreness Test, Flat Running in the Heat

• For visit 2, you will return to the Exercise Physiology Laboratory between 7 and 8 am after: (a) at least a 4-hour fast, (b) avoid having caffeine for 4 hours, (c) avoid performing resistance exercise for 72 hours. You will also keep a diet diary for 24
hours before this visit. A template of diet diary will be emailed to you at least one day before this visit. You will replicate this diet 24 hours before Visit 4.

- **Urine Collection:** You will collect a small sample of urine into a sterile specimen cup by yourself in a restroom. If the urine test shows you are dehydrated, you will be asked to drink water until you are hydrated. Nude body weight will be self-measured in a private room by an electronic scale.

- **Breakfast:** You will eat a small breakfast consisting of a cereal bar equivalent to 2 kcal/kg nude body weight and water (5 ml/kg nude body weight), provided by the research team.

- **Blood draw:** A blood draw in the seated position will be performed by a trained technician, who will inspect your lower arm for a prominent vein that will be suitable for a blood draw. The amount of blood will be approximately 10ml (2 teaspoons).

- **Muscular Strength Test:** You will first warm up on a cycle ergometer at a low exercise intensity (50 Watts) for five minutes. Then you will be seated in a Biodex dynamometer to test your dominant leg quadriceps maximal strength. Your upper and lower body will be restricted with straps to help isolate the exercising leg. You will perform 10 practice repetitions of submaximal knee extension and flexion. One minute after, you will begin the muscular strength test. You will perform maximal isometric contraction at your knee joint placed at a 90-degree angle for three times. Each attempt will be five seconds and separated by ten seconds rests.

- **Downhill Running in Temperate Environment:** Before starting the exercise, in a private room, you will self-insert a rectal thermometer roughly 10cm (4 inches) past the anal sphincter. You will also measure your nude body weight in the same private room after emptying your bladder, and report the value to a research team member. In addition, you will place a chest strap around your chest, which will be used to monitor heart rate. You will perform a one-hour downhill running (-10% gradient) on a treadmill in a cool (i.e., 20°C (68°F), 30% relative humidity) or hot (i.e., 35°C (95°F), 30% relative humidity) environment. The environmental condition (i.e., either hot or cool) will be determined by a coin flip. The treadmill speed corresponds to 65% VO2max as determined in Visit 1. During the exercise, you will be asked to report your rating of perceived exertion and thermal sensation every five minutes. You will be encouraged to drink water whenever and however amount you like. Immediately after exercise, you will sit down in a cool environment for 10 minutes before a blood draw. In a private room, you will self-collect all the urine into a sterile specimen cup, remove the rectal probe and measure your nude body weight again.

- **Three-hour rest:** You will take a three-hour rest after completion of the downhill running. If there is a weight loss after the downhill running, you will drink water equivalent to the weight loss within one hour. Any water intake beyond this required amount will be your own decision. You will also eat a lunch consisting of a Clif Bar (chocolate chips flavor) and a sunflower butter and strawberry jelly sandwich, provided by the research team. During this resting period, you will stay around the lab area and may not leave Johnson Center. You will self-collect any urine output into a sterile specimen cup. Immediately after the 3-hour resting period, blood will be drawn, and muscular strength test will be performed following the same procedures described above.
• Perceived Muscle Soreness Test: You will rate your muscle soreness on a 100-mm visual analog scale (0 = no soreness, 100 mm = the worst possible soreness) while performing a wall sit with your legs bent 90-degree.

• Flat Running in the Heat: Immediately before starting the exercise, in a private room you will self-collect any urine output into a sterile specimen cup and measure your nude body weight after emptying your bladder. You will also self-insert a rectal thermometer roughly 10 cm past the anal sphincter and place a chest strap for heart rate measurement around your chest. Then you will perform a 40-minute running on a flat treadmill (1% gradient) in a hot room (i.e., 35°C, 30% relative humidity). The treadmill speed is 55% VO2max as determined in Visit 1. During the exercise, you will be asked to report your rating of perceived exertion and thermal sensation every five minutes. You can drink water whenever and however amount you like. After the exercise, you will sit down in a cool environment for 10 minutes before a blood draw. You will remove the rectal thermometer, measure nude body weight and collect urine output in a private room. Lastly, you will rate your muscle soreness and perform the muscular strength test, following the same procedures described above.

Visit 3: Blood draw, Perceived Muscle Soreness Test, Muscular Strength Test
• Twenty-four hours after the completion of the downhill running in Visit 2, you will report to the lab for a blood draw, perceived muscle soreness test and muscular strength test, following the same procedures described above.

Visit 4: Urine Collection, Blood Draw, Muscular Strength Test, Downhill Running in a Cool or Hot Environment, Perceived Muscle Soreness Test, Flat Running in the Heat
• In the day before Visit 4, you will use the food diary you made for Visit 2 to eat exactly the same foods before Visit 4.
• This visit is at least two weeks apart from Visit 2. Everything will be repeated from Visit 2, except for the environmental condition of Downhill Running Protocol, which will be the opposite from what you had in Visit 2. For example, if you did the downhill running in a cool environment in Visit 2, you will perform the downhill running in a hot environment during this visit. Vice versa.

Visit 5: Blood draw, Perceived Muscle Soreness Test, Muscular Strength Test
• You will repeat the same procedures described in Visit 3.

Risks:

COVID-19 exposure risks

There is risk of COVID-19 exposure due to your participation in this study as the visits involve face-to-face interaction with research personnel. In order to minimize the risk, several manipulations will be implemented. You and research personnel must follow social distancing requirements (6 ft.) except for when it is necessary to collect data (e.g. to draw blood and to place equipment on you). Research personnel will be screened for symptoms or exposure to COVID-19 positive individuals before they will be allowed to work with you. The lab area will be cleaned and disinfected regularly and between participants. Hand sanitizer will be available in the lab. All research personnel have been trained on any new procedures adopted to prevent exposure to COVID-19. There will be
no more than 2 research team members working with you at a time. They will wear face masks at all time in the lab. You are also required to wear a mask in the lab except when you are exercising. When it is necessary for a research team member to touch your skin, such as skinfolds measurements research personnel will wear disposable gloves. For the blood draw, research personnel will wear disposable gloves, a mask, eye protection and lab coat. If you or a research team member reports exposure to, develops symptoms possibly associated with, or tests positive for COVID-19 within 14-days of a visit, the study will be paused and you will start a self-quarantine for at least 14 days. You will not be allowed to continue participating in this study unless you show no symptoms and test negative after the quarantine.

**VO₂max test risks**

There are risks associated with maximal graded exercise test including the following: muscle soreness, fatigue, nausea, or dizziness during or after completion of exercise. The incidence of risk of fatal and nonfatal events during maximal exercise testing are very low, approximately <0.8 per 10,000 tests or 1 per 10,000 hours of testing. We will minimize these risks by checking your medical history questionnaire for any medical conditions or history that could increase your risk, and by using trained personnel to conduct your testing. The occurrence of injury will result in immediate termination of the exercise test. The exercise laboratory is equipped with emergency medical equipment and emergency procedures in place. All researchers assisting in the trial have worked extensively with individuals performing high intensity exercise. Risk to you regarding exposure to COVID-19 during this test will be low. One research team member is required to stand closer to you than 6 ft in order to change the treadmill speed. They will wear a mask and will be posted in a position where they will be as far from you as possible. We will schedule at least one hour between participants in order to give time for the air in the room to recirculate several times after each exercise test. We will sanitize the treadmill and all areas of the room you will come into contact with.

**Blood draw risks**

There are risks involved in drawing blood from an arm vein which may include, but are not limited to, momentary discomfort at the site of the blood draw, possible bruising, redness, and swelling around the site, bleeding at the site, feeling of lightheadedness when the blood is drawn, and rarely, an infection at the site of the blood draw. Every attempt will be made to draw the blood sample while ensuring safety and comfort to you. If you have had symptoms or fainting with blood draws in the past, we will ask you to lay down while we obtain a sample. A researcher will stay with you for at least 5 minutes to ensure you are symptom free. The team member who draws your blood will wear a mask, disposable gloves, eye protection and a lab coat.

**Exercise in the heat risks**

During exercise in the heat, there is a risk of feeling hot, sweaty, uncomfortable, thirsty, tired, light-headed, and nauseous. Your core body temperature will be monitored continuously. If your core temperature reaches ≥40°C (104 degrees Fahrenheit), exercise will be terminated, and the participant will be removed from the heat chamber. If this happens, cooling procedures will be implemented (cold water immersion). To reduce the risk of COVID-19 contamination, the researcher will be located at the outside area of the room observing you through a window in the door. Every 5 minutes the researcher will
access the room to collect the physiological variables (i.e. core temperature) and check how you are feeling. You may ask to leave the hot room at any time. In addition, the exercise science research laboratory is equipped with an automated external defibrillator (AED), and all researchers are cardiopulmonary resuscitation (CPR) certified and are aware of the signs and symptoms of heat illness.

**Rectal probe risks**

There will be minimal risks related to the small, flexible rectal thermistor. You will be provided with privacy and as much time as needed to place the probe by yourself. In addition, lubricant will be provided to reduce discomfort. After each exercise trial, you will remove the thermistor and clean it by yourself. A disinfectant solution (Cidex glutaraldehyde, Johnson & Johnson) will be provided. You will reuse your own probe for all exercise trials.

**Muscle damage risks**

There is risk of skeletal muscle damage due to exercise. Especially, you may feel sore and/or pain in your quadriceps after downhill running. Such muscle soreness may occur 12 – 24 hours after the downhill running and disappear within 5 - 7 days. If you notice that the color of your urine turns darker toward “iced-tea” color, let the research team know and you will be opted out for any future research trials.

In the very unlikely case of an emergency, standard procedures will be followed: these include calling 911 and monitoring the participant. All investigators are certified in CPR and AED use. One of the Exercise Physiology Laboratories physicians on-call, Christopher Bossart, MD or Jacob Christensen, MD, also would be notified immediately. The average time it takes for ambulance services to reach the Laboratory is approximately 5-8 minutes, and for a physician from the Student Health Center, less than 5 min.

**Research related injury**: There is a risk that you might need to be quarantined for 14 days if a research team member you interacted with test positive for COVID-19. If you are injured or become sick as a result of this study, any emergency treatment will be at your cost. UNM makes no commitment to provide free medical care or money for injuries to participants in this study.

It is important for you to tell the Principal Investigator immediately if you have been injured or become sick because of taking part in this study. If you have any questions about these issues, or believe that you have been treated carelessly in the study, please contact the Office of the IRB at (505) 277-2644 for more information.
Benefits: We will provide you with the results of your VO2\textsubscript{max} test. Knowledge of VO2\textsubscript{max} is of benefit in that they are indicative of aerobic fitness which can be helpful in directing and developing an individual exercise program. Results of this study will inform the risks of muscle damage in those who perform physical exertion in hot environments, including military personnel, firefighters, workers and athletes.

Confidentiality of your information: To protect your information, you will receive a participant number with no link to your name on any study material, including the COVID-19 screening sheets. Only the research team will know what you do or say in this study. All information obtained during your participation in this study will be viewed only by the research team and kept in a locked cabinet and on a password protected computer in Fabiano Amorim’s office. The University of New Mexico Institutional Review Board (IRB) that oversees human subject research may be permitted to access your records. Your name will not be used in any published reports about this study. All identifiable information (e.g., your name) will be removed from the information or samples collected in this project. After we remove all identifiers, the information or samples may be used for future research or shared with other researchers without your additional informed consent.

Payment: In return for your time and the inconvenience of participating in this study, you will be paid $8 for each visit. If you complete the entire program, you will receive a maximum of $40 in the form of a gift card.

Right to withdraw from the study: Your participation in this research is completely voluntary. You have the right to choose not to participate or withdraw your participation at any time without penalty. In addition, the research team will stop your participation in the study if 1) your core temperature reaches 40 °C during any exercise trials, or 2) the color of your collected urine turns darker toward “iced-tea” color, or 3) you are not willing to wear a mask when required or follow other COVID-safe practices. If you have any questions, concerns, or complaints about the research, or contract COVID-19 (including showing symptoms or testing positive) within 14 days of a visit to the lab, please contact the principal investigator: Fabiano Amorim, Ph.D., Department of Health, Exercise & Sport Sciences, 1 University of New Mexico, Albuquerque, NM, 87131. He may be reached Monday-Friday 8:00 a.m. – 5:00 p.m. at (505) 277-3795, or anytime via email at amorim@unm.edu.

If you have questions regarding your rights as a research participant, or about what you should do in case of any research-related harm to you, or if you want to obtain information or offer input, please contact the IRB. The IRB is a group of people from UNM and the community who provide independent oversight of safety and ethical issues related to research involving people:

UNM Office of the IRB, (505) 277-2644, irbmaincampus@unm.edu. Website: http://irb.unm.edu/

CONSENT
You are making a decision whether to participate in this study. Your signature below indicates that you have read this form (or the form was read to you) and that all questions have been answered to your satisfaction. By signing this consent form, you are not waiving any of your legal rights as a research participant. A copy of this consent form will be provided to you.

I agree to participate in this study.

Name of Adult Participant                  Signature of Adult Participant                  Date

Researcher Signature (to be completed at time of informed consent)

I have explained the research to the participant and answered all of his/her questions. I believe that he/she understands the information described in this consent form and freely consents to participate.

Name of Research Team Member                  Signature of Research Team Member                  Date
APPENDIX B

Flyer

Downhill Running Study

Healthy and physically active men and women age 18-40 needed for research project

What is the study about? This research is to examine the effects of downhill running in a hot environment on muscle soreness, as well as heat strain and kidney stress.

Who can participate? Healthy, physically active men and women between ages of 18 and 40 years. Individuals who are heat-acclimated, have a history of heat stress complications or rhabdomyolysis, or accustomed to downhill running cannot participate.

What will I be asked to do if I participate? You will perform a cardiorespiratory fitness test (VO2 max test), 2 downhill running sessions and 2 flat running sessions. You will also have blood draws.

Total time required: 13 hours over a 20-day period (5 visits).

What will I receive if I participate? You will be given a up to $40 gift card for completion of the study. Two breakfasts and two lunches will also be provided.

Who can I contact for more information?

Zidong Li, (618) 525-1756
zidongli1991@unm.edu
APPENDIX C

Health History Questionnaire

HEALTH & PHYSICAL ACTIVITY QUESTIONNAIRE

Family history questions are included because certain conditions of your first degree relatives can incur risk to you during maximal exercise.

Subject #________________________ Date___/___/___     Phone (H or cell)___________________

Age____   Gender_____    Ethnicity_______

Emergency contact (name, phone #)______________________________________________

MEDICAL HISTORY

Physical injuries (muscle, joint, other):____________________________________________
(use back if needed)
Limitations_______________________________________________________________

Have you ever had any of the following cardiovascular problems? Please check all that apply.

Heart attack/Myocardial Infarction____  Heart surgery    ____       Valve problems____
Chest pain or pressure                    ____        Swollen ankles____       Dizziness_____
Arrhythmias/Palpitations ____          Heart murmur____      Shortness of breath____
Congestive heart failure____

Have you ever had any of the following? Please check all that apply.

High blood pressure _____
Asthma                  ____       Total cholesterol >200 mg/dl _____
Diabetes (specify type)____       HDL cholesterol <35 mg/dl _____
Emphysema               ____
Stroke                   ____       LDL cholesterol >135 mg/dl _____
Rhabdomyolysis          ____       Triglycerides>150 mg/dl _____
Heat illness/stroke ______
Are you being under the active care of a physician? Yes or No.

Do immediate blood relatives (biological parents & siblings only) have any of the conditions listed above? If yes, list the problem, and family member age at diagnosis.

Do you currently have any other medical condition not listed (metabolic, viral, kidney, liver disease, orthopedic injuries)?
Details

Indicate level of your overall health. Excellent ____ Good ____ Fair ____ Poor____

Are you taking any medications, vitamins or dietary supplements now? Y N
If yes, what are they?_____________________________________________________

Are you allergic to latex? Y N

Have you ever experienced any adverse effects during or after exercise (fainting, vomiting, shock, palpitations, hyperventilation)? Y N If yes, elaborate._____

LIFESTYLE FACTORS
Do you now or have you ever used tobacco? Y N If yes: type ________________
How long?______ Quantity____/day Years since quitting______________
Have you lived in a “summer” place during the past two months? Y N
Do you go sauna or hot water bathing regularly during the past two months? Y N
How often? ______________________________________________________________
(Females only) Are you pregnant? Y N

EXERCISE
Aerobic/”cardio” training
Times per week (circle one): 2-3 3-5 6-8
Minutes/Day (circle one): 30-60 min 60-90 min 90-120 min
Training background (circle one): 1-2 yr 3-5 yr 5-15 yr

Resistance training
Times per week (circle one): 2-3 3-5 6-8
Minutes/Day (circle one): 30-60 min 60-90 min 90-120 min
Training background (circle one): 1-2 yr  3-5 yr  5-15 yr
Experience with free weight exercises deadlift and/or squat: 6 month-1 yr  1-3 yr  >3 yr

**Circuit training**
Do you have previous experience in circuit weight training? (circle)  Yes / No
If so, times per week (circle one):  2-3  3-5  6-8

Do you participate in other sports? (describe) ____________________________________________

Do you specifically train downhill running?  Y  N
APPENDIX D
Data Collection Sheets

Subject # ______    Date __________   USG ________

Pre-EIMD

MVC

Peak torque _____________
Average peak torque ______________

Perceived soreness (100-mm VAS) ________________

Visual analog scale (VAS) for perceived soreness measurement

Directions:
Considering the overall severity of soreness in your legs upon performing a wall sit, draw an intersecting line across the continuum line extending from 0 – 100 mm. This mark will indicate your level of soreness (left end = no soreness, right end = extreme soreness). The distance of each mark will be measured from zero and the measurement utilized as the perceived soreness level.

No sore                                                                                                          Worst possible soreness

Hematocrit hemoglobin ______________
Subject #_______  Date/Time _______________  Experimental condition ____________

Pre NBW _____________  Treadmill speed/grade _______________

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Dry T.</th>
<th>Wet T.</th>
<th>Core T.</th>
<th>HR</th>
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Post NBW _____________  Water intake __________  Urine output _____________

Hematocrit hemoglobin _______________
3-hr rest       Starting time ______________

Required water intake to replace weight loss ______________

Extra water intake ______________

Urine output ______________

Pre Exercise-heat stress       Time _______________

MVC

Peak torque __________

Average peak torque __________

Perceived soreness (100-mm VAS) ____________

Visual analog scale (VAS) for perceived soreness measurement

No sore .............................................................................................................. Worst possible soreness

Hematocrit hemoglobin ________________
Exercise-heat stress test

Subject #_______  Date/Time _______________  

Pre NBW ___________  USG _______  Treadmill speed/grade _______________

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<th>Time (min)</th>
<th>Dry T.</th>
<th>Wet T.</th>
<th>Core T.</th>
<th>HR</th>
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Post NBW ___________  Water intake ________  Urine output ___________
Post Exercise-heat stress  Date/Time ______________

MVC

Peak torque ____________

Average peak torque ____________

Perceived soreness (100-mm VAS) ______________

Visual analog scale (VAS) for perceived soreness measurement

No sore                                                                 Worst possible soreness

Hematocrit hemoglobin ___________________
Subject # ____  Date ________________

Post 24h-EIMD

MVC

Peak torque ____________
Average peak torque ____________

Perceived soreness (100-mm VAS) ________________

Visual analog scale (VAS) for perceived soreness measurement

No sore ________________________________________ Worst possible soreness

Hematocrit hemoglobin ________________