The Effect of Interval and Continuous Work on Markers of Acute Kidney Injury in a Hot Environment

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THE EFFECT OF INTERVAL AND CONTINUOUS WORK ON MARKERS OF ACUTE KIDNEY INJURY IN A HOT ENVIRONMENT

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ABSTRACT

Purpose: The purpose of this study was to examine the effect of high intensity interval work (HIIW) and moderate intensity continuous work (MICW) patterns on markers of acute kidney injury (AKI) and kidney damage in a hot environment. Methods: In a randomized crossover design, nine physically active males completed two hours of physical work (two bouts of 60 minutes separated by 10 minutes of passive rest) in a hot environment (40°C and ~15% relative humidity) in either HIIW [2 minutes at 80% maximum oxygen consumption (VO2max) and 3 minutes at 30% VO2max] or MICW (matched for total distance covered in HIIW trial). Blood and urine samples were collected immediately before (Pre) and after (Post-Work), one hour (1hr Post-Work), and 24 hours after (24hr Post-Work) the trials. Urine flow rate (UFR), osmolality (Uosm), and creatinine (uCreatinine), serum creatinine (sCreatinine), urinary neutrophil
gelatinase-associated lipocalin (uNGAL) and urinary kidney injury marker 1 (uKIM-1) were measured. Estimated glomerular filtration rate (eGFR) was calculated. Core temperature (Tc), heart rate (HR), thermal sensation (TS), and rating of perceived exertion (RPE) were measured during the trial. Incidence rate of AKI were assessed using sCreatinine and the Acute Kidney Injury Network criteria. **Results:** 66.7% and 22% of participants developed Stage 1 AKI Post-Work immediately after HIIW and MICW, respectively. Serum creatinine was significantly higher during HIIW compared to MICW [F(1,8)= 63.720; p< 0.01] and eGFR was significantly lower during HIIW compared to MICW [F(1,8) 37.390; p< 0.01]. Tc [F(1,7)= 12.170; p=0.01] and HR [F(1,7)= 22.833; p <0.01] were significantly higher in HIIW compared to MICW. uKIM-1 and uNGAL displayed significant effects for time [F(3,24)= 15.508; p<0.01] and [F(3,24)= 13.234; p<0.01] respectively, but not for condition. **Conclusion:** When compared to MICW, HIIW cause a higher incidence rate of Stage 1 AKI in a hot environment, however there was no difference between the markers of kidney damage. It seems that higher intensity interval work impacts acute renal function, but not molecular markers of AKI (KIM-1 and NGAL).
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Chapter 3: Research Manuscript

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SYMBOLS/ABBREVIATION

>: greater than
<: less than
±: plus or minus
~: approximately
°C: degrees Celsius
ηp²: partial Eta-squared
AKI: acute kidney injury
ANOVA: analysis of variance
BF%: body fat percentage
bpm: beats per minute
CK: creatine kinase
CKD: chronic kidney disease
cm: centimeters
eGFR: estimated glomerular filtration rate
ELISA: enzyme-linked immunosorbent assay
g: gram
Hct: hematocrit
HIIW: high intensity interval work
HR: heart rate
kg: kilogram
KIM-1: kidney injury molecule-1
mg: milligram
MICW: moderate intensity continuous work
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
RPE: rating of perceived exertion
SD: standard deviation
Tc: core temperature
TS: thermal sensation
U/L: unit of enzyme activity per liter
UFR: urine flow rate
Uosm: urine osmolality
CHAPTER 1

Introduction

Chronic kidney disease (CKD) is a major health problem associated with serious complications such as renal failure, increased risk of cardiovascular disease and increased mortality rates. The rate of CKD is increasing in prevalence across the globe (Jager & Fraser, 2017) and especially alarming in low- and middle-income countries (Vanholder et al., 2017; Vos et al., 2017). The prevalence of CKD also places a burden on the United States health care system (Bowe et al., 2018) with treatment of CKD and end-stage renal disease such as kidney transplant and dialysis procedures incurring over $114 billion dollars of Medicare spending in 2016 (Saran et al., 2019). The occurrence of CKD has been increasing among field workers, particularly those who are regularly exposed to hot environments (Correa-Rotter et al., 2014). The presence of CKD in this population has led to investigations as to why this group was developing CKD due to the absence of traditional risk factors or comorbidities such as hypertension, diabetes, cardiovascular disease, and advanced age (Meisinger et al., 2006; Michishita et al., 2017). Previous research has sought to identify occupational factors that may lead to this increase in risk of CKD without traditional risk factors, termed CKD from unknown origins (CKDu), for field workers (Correa-Rotter et al., 2014; Madero et al., 2017; Wesseling et al., 2020). One factor that may contribute to the increases in CKDu prevalence is global warming which in turn would cause those in outdoor occupations to be exposed to higher doses of heat stress (Glaser et al., 2016). One hypothesis for the development of CKDu is that repeated acute kidney injury (AKI) may result in long term or permanent damage to the
kidneys, thus impairing function (Arias-Cabrales et al., 2018; Coca et al., 2012; Schlader et al., 2019). AKI is a clinical condition marked by a rapid decrease in kidney function and is identified through measurement of serum creatinine, glomerular filtration rate, and urine output, though the exact measurement criteria differs between group initiatives (Luo et al., 2014). It is vital to understand what may contribute to AKI which is experienced by workers after shifts and potential development of CKDu in occupational workers and others who are exposed to repeated work in hot environments.

Often, AKI is reversible and transient in nature, where there is an increase in biomarkers but no prolonged health risk. Serum and urine markers of AKI that are commonly used in research are neutrophil gelatinase-associated lipocalin (NGAL) which is a marker of ischemic kidney injury representing general tubular injury (Schlader et al., 2019), kidney injury molecule-1 (KIM-1) which is often upregulated after ischemic-reperfusion injury and is associated with injury to the proximal tubules (Ichimura et al., 1998; Schrezenmeier et al., 2017), in conjunction with decreases in kidney function (eGFR, urine output) to determine the severity or lasting impact of AKI. A complete understanding of the factors that lead to AKI during physical work in the heat is lacking, but there is evidence for potential contributing factors. Thermal stress and dehydration (Chapman et al., 2020), muscle damage (Junglee et al., 2013), and duration of work (Schlader et al., 2017) are related to increases in AKI biomarkers and decreases in kidney function. These factors are thought to contribute to AKI by causing ischemia/reperfusion injury caused by changes in renal blood flow due in large part to hypovolemia (Chapman et al., 2020) and sympathetic nervous system activity through catecholamine release (Kawakami et al., 2018). The reduction in blood flow with increased heat stress
(Miyamoto, 1994; Wilson, 2017) and exercise (Freund et al., 1991; Kawakami et al., 2018) may create a hypoxic environment and reduced ATP availability in the renal circulation. This problem is exacerbated by an increase in fluid reabsorption in the distal tubule and collecting ducts which is metabolically expensive due to activation of Na+/K+ pumps. Inflammation and oxidative stress occur due to this ischemic and metabolically costly environment which can lead to damage of the tubules resulting from impaired calcium handling and increases in reactive oxygen species (Chatauret et al., 2014; Devarajan, 2005, 2006). One factor that has not been examined independently is the role of work intensity in the heat on the prevalence of AKI.

Recent research has suggested that at high exercise intensities (i.e. above lactate breakpoint) there is a marked reduction in renal blood flow (Kawakami et al., 2018). Using Doppler ultrasound, it was shown that the total cross-sectional area of kidney vasculature decreased at exercise intensities equal to 120% and 140% of lactate breakpoint, while renal blood flow was reduced at 100%, 120%, and 140%, respectively. These findings were in agreement with a previous study where renal clearance was compromised to 70% of resting values at 50% VO$_2$max and to 35-45% of resting values at heavy workloads (Grimby, 1965) and work showing a decrease in glomerular filtration rate during moderate (60%) and intense (80% VO$_2$max) exercise compared to rest (Freund et al., 1991). This reduction in renal blood flow should be a consideration for individuals performing physical work in hot environments such as field workers, military personnel, firefighters, and athletes. One study examining field workers during a day long shift found that sugarcane cutters worked at an average of 54% of their heart rate max, spending on average 4:44 hours of their workday above 50% age-predicted heart rate
max (Lucas et al., 2015). Additionally, job environments that involve piece work, where an individual’s salary is dependent upon the amount of work completed, may increase the incidence of AKI by encouraging workers to work at higher intensities and take fewer breaks (Moyce et al., 2017). This evidence suggests that the role of work intensity and work pattern, (i.e. interval or continuous) in hot environments should be examined to determine as it presents an opportunity to control and possibly limit the impact of a variable that can lead to the development of AKI.

**Problem Statement**

Individuals who must perform physical activity in the heat are exposed to multiple factors that have been shown to exacerbate the incidence rate of AKI. These factors include heat stress, dehydration, work intensity and long duration of heat exposure. These data, along with studies suggesting that workers may have to work above moderate intensities periodically throughout the day suggest that work intensity may be a contributing factor to AKI. Work intensity and pattern also may be a more manipulatable factor when compared to heat stress or dehydration by designing work breaks or job demands to decrease the intensity of physical work.

**Purpose of the Study**

The purpose of the current study is to examine the impact of working for brief periods at high intensities, deemed high intensity interval work (HIIW), compared to moderate intensity continuous work (MICW), on kidney function and markers of AKI, while controlling for work duration, heat strain, and dehydration.
Hypotheses

Hypothesis 1: Serum Creatinine levels and subsequently AKI incidence rate in the HIIW condition will be higher than in the MICW condition at one or more time points.

*Rationale: An increase in serum creatinine is expected as it is indicative of decreased kidney function which is often seen after higher intensity physical activity (Moyce et al., 2020). AKI incidence rate is expected be higher subsequent to the increase of serum creatinine, as an increase in serum creatinine is the primary measures used by the AKIN criteria (Mehta et al., 2007).*

Hypothesis 2: Estimated glomerular filtration rate (eGFR) in the HIIW condition will be reduced more than in the MICW condition at one or more time points.

*Rationale: Glomerular filtration rate, as estimated from anthropometric measures and serum creatinine, is reduced to a greater extent after high intensity physical activity compared to moderate intensity physical activity due to decreased renal blood flow (Freund et al., 1991; Kawakami et al., 2018).*

Hypothesis 3: Urinary NGAL and KIM-1 concentrations in the HIIW condition will be higher than in the MICW condition at one or more time points.

*Rationale: Due to repeated bouts of high-intensity work followed by low intensity recovery periods could potentially cause ischemic-reperfusion injury, it is predicted that both NGAL and KIM-1 will be elevated as that is often seen after ischemic kidney injury (Schlader et al., 2019).*
Hypothesis 4: Maximal core temperature will be similar during the HIIW and MICW protocols.

*Rationale: Although there is greater energy expenditure during running than walking (Hall et al., 2004), the low intensity rest periods during HIIW should blunt a substantial increase in core temperature from metabolic heat production.*

Hypothesis 5: Dehydration will be similar between HIIW and MICW.

*Rationale: Both groups will be provided water during the work trials, including an individualized amount of water after the first hour. This should allow for the amount of dehydration to be matched between trials.*

**Scope of the Study**

We recruited 9 healthy (i.e. without known renal, metabolic, or cardiovascular disease) males between the ages of 24-45 years old. Individuals were excluded who have a previous history of heat illness or who have a musculoskeletal injury which would limit treadmill exercise. Additionally, all participants ranked below the 80th percentile in maximal oxygen consumption (VO$_{2\text{max}}$) according to normative data from the American College of Sports Medicine (ACSM). Using a randomized, counterbalanced design participants completed the HIIW and MICW, with at least a one-week washout between trials.

During the first lab visit, the participants’ anthropometric data was collected, followed by a VO$_{2\text{max}}$ test on a motorized treadmill. Following the VO$_{2\text{max}}$ test, speeds
which elicited 80% and 30% of VO$_2$max were determined to be used as the work intensities during the HIIW trial. The HIIW trial consisted of a two-minute high intensity interval at the speed which elicited 80% VO$_2$max followed by a three-minute low intensity interval at the speed which elicited 30% of VO$_2$max. The total distance that was covered during the HIIW trial was calculated and a continuous speed to cover this distance over the 120-minute of MICW was calculated. The HIIW and MICW were conducted in a hot environment (40°C, 15% relative humidity) and consisted of one hour of work, a ten-minute rest period, and a second hour of work at the predetermined speeds and a 5% incline. Participants were given 200ml of water every 20 minutes during the work trials, as well as an amount of water equal to 75% of sweat loss during the rest period. Core temperature and thermal sensation were recorded every five minutes during both trials. Heart rate and rating of perceived exertion were measured every five minutes during MICW and at the end of the two-minute and three-minute interval during HIIW. Serum creatinine, urinary creatinine, urinary KIM-1, urinary NGAL, eGFR, urine flow rates, hemoglobin, and hematocrit were analyzed at four timepoints: immediately pre-work, immediately post-work, one hour post-work, and 24 hours post-work.

**Assumptions**

The following assumptions were made by the authors of this study:

1. Participants followed specified guidelines for avoiding heavy exercise, caffeine, and alcohol prior to each of the trials.

2. Participants kept an accurate log of food, drink, and urine output during the study.

3. Participants performed a maximal effort to volitional fatigue VO$_2$max testing.
4. Participants in the study refrained from exercise between the end of the trial and the blood draw on the following day.

5. Participants were not heat acclimated during their period of participation in the study.

Limitations

The following limitations were identified:

1. The study participants consisted of healthy, physically active males between the ages of 18-45. The results of the study may not be extrapolated to individuals who have chronic diseases, are outside of the age range, or who are highly trained or sedentary.

2. The study applies to an acute setting of heat and exercise stress and may not reflect what would happen after consecutive days of exercise in the heat.

3. Treadmill exercise and may not simulate the work done in the field by workers.

Significance of the Study

Chronic kidney disease from unknown origins (CKDu) is increasing in prevalence, a trend that might be related to global warming and will continue to increase as average yearly temperatures continue to rise (Vos et al 2017; Glaser 2016). This increase leads to more fatalities and rising health care costs for the treatment of CKDu and end-stage renal disease. One theory as to why field workers are more susceptible to the development of CKDu is that repeated acute kidney injury (AKI) events occur in this population. Many factors related to physical work in the heat have been attributed to an increased incidence
of AKI including heat strain, dehydration, muscle damage, and work duration. No study has examined the effect of work intensity as a factor on increasing the markers of AKI (i.e., reduced kidney function and increased kidney damage biomarkers). The significance of this study will be to examine whether completing a similar amount of work with a bout of high intensity work may cause more severe AKI than working at a continuous moderate intensity in a hot environment.

**Definition of Terms**

**Creatinine**: a product of creatine phosphate breakdown which is excreted by the kidneys.

**Glomerular Filtration Rate**: Rate at which blood is filtered through glomeruli in the kidneys. Commonly used marker of renal function.

**Kidney injury marker 1 (KIM-1)**: protein preferentially released from the proximal tubules of the kidney after ischemic stress.

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

**Neutrophil gelatinase-associated lipocalin (NGAL)**: commonly used biomarker of acute kidney injury. Released from kidney tubular cells following ischemic stress.

**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 titled: “Factors of physical activity contributing to acute kidney injury in endurance athletes and occupational workers” which has been submitted for publication as a review article in Kidney International Reports. It was authored by Jonathan Houck, Zachary McKenna, Christine Mermier, Michael Deyhle, Orlando Laitano, and Fabiano Amorim.
Factors of Physical Activity Contributing to Acute Kidney Injury in Endurance Athletes and Occupational Workers

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KEYWORDS: acute kidney injury, physical activity, endurance athletes, occupational workers, chronic kidney disease, kidney function

ABBREVIATIONS

AKI: acute kidney injury
CK: creatine kinase
CKD: chronic kidney disease
eGFR: estimated glomerular filtration rate
ER: exertional rhabdomyolysis
GFR: glomerular filtration rate
IGFBP7: insulin-like growth factor-binding protein 7
KIM-1: kidney injury marker-1
NGAL: neutrophil gelatinase-associated lipocalin
Abstract

Repeated incidences of acute kidney injury (AKI) caused by work related factors have been implicated as a potential cause for the rise in chronic kidney disease cases among occupational workers. During physical activity, particularly in hot and humid environments, the kidneys are challenged by several stressors such as inflammation and nephrotoxicity from hyperthermia and muscle damage, hypovolemia caused by dehydration, and ischemic oxidative stress from reduced renal blood flow. These stressors may compound and create an internal environment which is detrimental to kidney health and cause both acute and long-term decreases in renal function. One population which also undergoes similar stressors are endurance athletes. Despite undergoing similar stressors there has been no evidence of similar concerns of CKD among endurance athletes, even those who partake in ultra-endurance events. This review aims to discuss the link between risk factors related to physical activity and AKI among occupational workers and endurance athletes. Potential risk factors covered in this review are hyperthermia, dehydration, muscle damage, work duration, work intensity, and the effect of repeated bouts of physical activity. Additionally, we will discuss potential reasons why endurance athletes may not be at similar risk for development of CKD despite presenting with AKI after physical activity.
Introduction

An epidemic of chronic kidney disease (CKD) has been observed in field and manual workers, especially cane cutters, in areas with hot climates who do not possess classic risk factors or comorbidities such as hypertension, advanced age, and poor glycemic control \(^1\text{-}^3\). This phenomenon is often referred to as chronic kidney disease of unknown origins (CKDu) and has high prevalence in various regions of the world including Asia \(^4\) and Mesoamerica \(^1\). The cause of the rise in chronic kidney disease remains unknown, but one popular hypothesis is that this epidemic occurs in areas where workers are chronically exposed to high levels of heat stress, which combined with physical work, dehydration, and exertional muscle damage, can cause recurrent acute kidney injury (AKI) \(^2\text{-}^5\text{,}^6\).

Many initiatives including the Risk, Injury, Failure, Loss, End-Stage Kidney Disease (RIFLE) criteria \(^7\), Kidney Disease Improving Global Outcomes (KDIGO) \(^8\), and Acute Kidney Injury Network (AKIN) criteria \(^9\) have been created to evaluate and categorize AKI and reductions in kidney function, usually by measuring markers of kidney function such as estimated glomerular filtration rate (eGFR), urine production rate, and serum creatinine (sCr) concentrations. Additionally, kidney injury biomarkers, such as neutrophil gelatinase-associated lipocalin (NGAL), urinary kidney injury marker-1 (KIM-1), and insulin-like growth factor-binding protein 7 (IGFBP7), have been used in conjunction with AKI criteria to better understand the nature and severity of renal injury \(^10\text{,}^11\).

Studies in both field settings and controlled laboratory environments have been conducted on factors related to the development of AKI after physical activity in hot
conditions. It has been suggested that the major work-related factors associated to AKI in fieldworkers are excessive and recurrent dehydration, hyperthermia, and muscle damage. One population which may also face many of these factors are endurance sport athletes. Many habitually perform exercise many days in a row, incur muscle damage and hyperthermia, must overcome hydration challenges presented by exercise from increased sweat loss, and often exercise for extended periods of time in hot environments. Interestingly though, there is no reported CKD epidemic among those participating in endurance sports. The aim of this review is to discuss the risk factors for AKI and review previous research examining factors that may lead to AKI incidences among occupational workers and endurance sport athletes. In addition, we will discuss why endurance athletes may not be at similar risk for development of CKD despite presenting with AKI after physical activity.

**Hyperthermia**

Physical activity performed in the heat can result in substantial rises in core temperature due to both metabolic and environmental heat stress. Elevated core temperature is known to reduce renal blood flow by increasing renal sympathetic nervous system activation, circulating vasopressin levels, and renin angiotensin aldosterone system activity. This reduction in renal blood flow can cause ischemia and ATP depletion which is believed to be the primary mechanism by which hyperthermia leads to the development of AKI. Indeed, controlled experimental studies using both rodents and humans have shown that kidney injury is worsened with increases in core temperature. Importantly, the severity of kidney injury may depend on the extent of hyperthermia. For example, Chapman et al. recently demonstrated that increases in
biomarkers of AKI were blunted with the use of cooling to prevent rises in both core and skin temperature\textsuperscript{18}. While renal blood flow was not measured, their results suggest that hyperthermia is an important risk factor in the development of AKI and thus should be considered for occupational and agricultural workers as well as endurance athletes who are often exercise/work in hot environments.

Measurements of core temperature amongst agricultural workers in the field are scarce, however current evidence suggests that these populations regularly experience hyperthermia during their work shifts. Mac et al. observed high core temperatures in agricultural workers, with over half (57\%) of the workers exceeding 38°C and maintaining this elevated core temperature for an average of 79 ± 73 minutes\textsuperscript{19}. Furthermore, heat stress has been significantly related with increased risk of AKI in male agricultural workers\textsuperscript{20}. However more data is needed to establish this relationship. Aside from agricultural workers there are other occupational populations, such as firefighters, who also experience situations of elevated core temperatures. For example, 68-91\% of cadet firefighters were noted to have core temperatures exceeding 38.5°C during a structural firefighter training course. Likewise, wildland firefighters working between one and five hours had mean core body temperatures of approximately 38°C\textsuperscript{21}. Schlader et al. demonstrated that simulated firefighting work induces AKI, however this has yet to be confirmed in the field\textsuperscript{22}. Nonetheless it is clear that individuals who perform work in hot environments (both inside and outside) experience hyperthermia and may be at risk for the development of AKI.

It is well known that endurance exercise can have profound impacts on core temperature, especially when performed in hot and/or humid conditions\textsuperscript{23}. However, few
studies have examined the impact of heat stress on AKI amongst endurance athletes. Evidence of AKI has been observed in marathon runners following races in temperate conditions (ambient temperature >18°C) 24–26. Mansour et al. found that 82% of runners (n=22) presented with evidence of AKI following a race, however core temperature was not measured 24. In a later study, Mansour et al. noted that runners had substantial rises in core temperature after completing a marathon and that 55% of the runners (n=23) developed AKI during the race. Interestingly they did not find a significant relationship between core temperature and AKI development, suggesting that other factors may be at play 25. It is important to note that high core temperatures (i.e., 40°C) are not uncommon in competitive marathon runners which may warrant further investigation 25,27,28. Furthermore, more research is needed on endurance athletes who regularly compete in tropical environments as they are likely to experience greater rises in core temperature.

**Dehydration**

Another factor that is common between workers and endurance athletes is the tendency to become dehydrated during the workday or workout. It has long been understood that dehydration reduces renal blood flow 29,30, which may lead to a detrimental environment within the kidney. During periods of dehydration, hypovolemia causes increased fluid and Na⁺ reabsorption in the tubules. This, along with decreases in renal blood flow due to sympathetic nerve activity, cause a mismatch in ATP production and a higher level of oxidative stress in the kidney 31. Additionally, it has been hypothesized that increased osmolality may lead to activation of the poly-fructokinase pathway, which both decreases renal ATP concentration and produces uric acid resulting in further renal vasoconstriction and oxidative stress 31–35.
In a controlled laboratory environment, Chapman et al. examined the impact of dehydration during two hours of physical activity in a hot environment on markers of AKI. The research compared groups which received; no intervention (Control), 100% water replacement every 15 minutes (Water), or received both the water and a cooling vest interventions (Water + Cooling). Body weight was maintained in the groups that were given water and biomarkers of acute kidney injury, IGFBP7 and serum creatinine, were lower than the control group. This suggests that proper hydration may play an important role in the mitigation of AKI and particularly lessen damage to the proximal tubules, where IGFBP7 is preferentially released.

One study examined the hydration of sugarcane workers in Nicaragua across a work shift, ranging from approximately seven to twelve hours. At baseline, it was noted that ~20% of 168 males in the study had elevated serum creatinine and 14% of the males had estimated glomerular filtration rate which would meet KDIGO criteria for chronic kidney disease before starting their work shift. On average, workers who lost >0.5kg body weight (N=47) during the work shift and those who did not experience weight loss, defined as a loss of < 0.5kg of body weight, (N= 95) consumed approximately similar amounts of water (~0.8L/hour). However, the group that lost >0.5kg had an increase in serum osmolality and serum creatinine that was significantly different from the group that maintained body weight. Interestingly the group that lost weight also had a decrease in hematocrit ratio, compared to the group which maintained body weight. This observation, which is contradictory to expected findings, was attributed to shifts of interstitial fluid into plasma due the workers being able to drink ad libitum. Additional data from a study of Florida agricultural workers indicated that over
half (53%) of workers reported to shifts already dehydrated as measured by a urine specific gravity of >1.020 and by the post-shift timepoint 81% of workers were classified as dehydrated. It was determined that 63 of 187 (~33%) of workers experienced AKI, defined as serum creatinine increase of $\geq 0.3$ mg/dL from pre-work to post-work or serum creatinine at least 1.5 times pre-work levels on at least one day and ~6% experienced AKI on multiple days. This suggests that recurrent dehydration with incomplete recovery may be a reality for many occupational workers. A recent study examined the hydration habits and relationship to AKI of agricultural workers. The study indicated that males reported drinking 3.3L of fluid throughout the workday and females reported drinking 2.3L, however the authors noted 11% of the workers lost more than 1.5% of their body weight, which indicates dehydration by National Institute for Occupational Health and Safety (NIOSH) standards. Additionally, the authors reported that the total volume of fluids that the workers consumed was positively correlated to increased association of risk of AKI, based on changes in serum creatinine, in pooled data when analyzed as ounces per kilogram of bodyweight. The authors hypothesized that the increased AKI in the workers who consumed more water may be due to those workers performing a larger maximal workload.

Few studies have been conducted to examine the effect of dehydration and AKI during endurance exercise. A study examining Chinese 100-km ultramarathon runners noted that 22 of 26 runners (85%) met the diagnosis criteria for AKI after the race with 65% of runners experiencing moderate dehydration with no correlation between AKI and level of dehydration. Another study which was conducted on runners after a marathon in a mild ambient temperature (18°C) noted that sweat volume losses were significantly
higher in runners who developed AKI compared to those who did not (3.89L compared to 1.66L respectively)\textsuperscript{25}. A limitation of this study was that the sweat volume loss was estimated from a sweat patch worn during the first five miles of the marathon and may not truly reflect whole body sweat loss as total fluid intake, and net weight loss were not significantly different between those with and those without AKI. Additionally, Poussel et al. indicated that a very low prevalence of AKI based on eGFR (0-12.5\% based on method of eGFR calculation) after a 120km trail race, which took place in mild ambient temperatures (8.6-11.1\(^\circ\)C), in runners who were properly hydrated\textsuperscript{40}. Additionally, the authors reported an increase in urinary NGAL post-race, indicating possible tubular injury independent of eGFR changes in properly hydrated runners.

Research is mixed on the correlation of dehydration and markers of AKI and kidney injury but, more importantly, it appears that limiting dehydration may have a meaningful role in decreasing the incidence rate of AKI. A key difference between occupational workers and endurance athletes may involve the number of exposures to dehydration. It has been hypothesized after repeated dehydrative events, such as working in a hot environment, a combination of increased vasopressin release and increased uric acid production causing subsequent hyperuricemia may lead to damage of the renal vasculature and development of CKD, with proper hydration acting as a potential protective mechanism against CKD development\textsuperscript{41,42}. This hypothesis is supported by community data showing promise that increased fluid intake\textsuperscript{43} and higher urine output\textsuperscript{44} were both associated with decreased risk of CKD. These studies were not conducted on the populations of interest to the current review, however longitudinal studies on
occupational workers and athletes in regard to hydration intake and long-term kidney function are warranted.

Muscle Damage

Muscle damage and exertional rhabdomyolysis have been shown to occur in working populations \(^{45,46}\) and endurance athletes \(^{47,48}\) following bouts of physical exertion and have been implicated as a contributing factor to the development of AKI and CKD \(^{2,49-53}\). The potential for AKI after muscle damage might be driven by acute inflammatory responses to muscle damaging physical activity \(^{49}\) as well as myoglobin, released from damaged skeletal muscle, possibly causing nephrotoxicity and possible tubular obstruction \(^{39}\).

In a study of sugarcane workers during a work shift, it was found that there was a significant increase in serum creatine kinase (CK), an indicator of muscle damage \(^{54}\), in both workers who developed AKI (98 IU/l to 415 IU/l) and those who did not (120 IU/l to 358 IU/l), but there was no significant differences in CK between the groups \(^{45}\). The authors could not conclude whether muscle damage alone was enough to cause AKI or whether it was the combination of detrimental factors associated with work in a hot environment (dehydration and heat stress). Additionally, Sorensen et al. did not identify any association with increases in CK and any measures of AKI after work shifts in Guatemalan field workers \(^{46}\). Data from another study conducted on Brazilian sugarcane cutters indicated that there was a nonsignificant (p= 0.06) moderate negative correlation between resting serum CK values and GFR estimated from resting serum creatinine values (range from 119 to 361 U/L), where higher CK values were associated with lower eGFR \(^{55}\). The authors also noted that 59% of the study participants at baseline had resting
serum CK values over the reference value of 198 U/L. These findings were similar to another study on burnt sugarcane harvesters where resting CK values were elevated during the harvest season; 136.5 U/L (inter-quartile range: 108.5–216.0) \textsuperscript{56}. This study was conducted near the end of the harvest season and indicated that workers were regularly subjected to skeletal muscle damage \textsuperscript{55}.

Some studies from endurance events have suggested that muscle damage may be related to increased risk of AKI. It was indicated in a systematic review of exertional rhabdomyolysis (ER) and AKI in endurance athletes that ultra-endurance running accounted for the majority (96\%) of ER+AKI cases over 43 studies \textsuperscript{48}. ER was denoted when CK was > 5000 U/L and AKI was noted when serum creatinine was >1.88 mg/dL. The authors also noted that while there were large increases in muscle and renal injury after endurance events, the values normally returned to baseline in an average of 5.8 days \textsuperscript{48}. One study examining 100-km runners noted that an increase in plasma CK from pre-race, 146 U · L\textsuperscript{-1}, to immediately post, 2299 U · L\textsuperscript{-1}, was indicative of AKI development \textsuperscript{39}. Interestingly, in this study the authors designated the AKI as transient due to serum creatinine levels returning below AKIN criteria for stage 1 24 hours after the race, even though CK levels remained significantly elevated. Data from a study on ultramarathon runners found a significant difference in CK between those who met the criteria for AKI compared to both those who met the criteria for risk of AKI and those who met neither criteria (82,244 \textpm 69,053 U · L\textsuperscript{-1}; 29,150\textpm 27,732 U · L\textsuperscript{-1}; and 29,579\textpm 32,693 U · L\textsuperscript{-1}, respectively) \textsuperscript{57}. Another study looking at 100-km ultramarathon runners noted that although four runners developed stage II AKI defined as a 2-3 fold increase in serum creatinine after the run compared to prerace levels, \textsuperscript{9}, yet there was no significant
difference in myoglobin or CK levels between runners who did and did not develop AKI. Results from studies on marathon runners have suggested that although there is an increase in CK, there is no correlation to markers of kidney injury, suggesting that muscle damage alone may not be enough to increase the risk of AKI during a marathon.

Although the exact relationship between muscle damage and AKI is not known, many studies have noted a relationship between increased markers of muscle damage and decreases in kidney function. It appears that the differences between endurance athletes and occupational workers may arise from the severity and frequency of muscle damaging events. Ultramarathon athletes indicate CK values rise well above the threshold for exertional rhabdomyolysis, but even in severe cases, a return to baseline values happens rather quickly. The issues that workers face is not necessarily in large increases in CK and muscle damage, but rather in the duration of subclinical damage, where they do not have the opportunity to fully recover. This is noted by data showing resting baseline values of CK above expected values.

**Work Duration and Intensity**

Work duration and intensity are important considerations with impaired kidney function and AKI. In a laboratory study, Schlader et al. demonstrated that there was a larger decrease in kidney function as measured by eGFR and increased serum creatinine as well as an increase in plasma NGAL after 60 compared to 40 minutes of walking in a hot (38°C, 50% relative humidity) environment (Schlader et al., 2017). The increased risk of AKI was attributed to the higher degree of hyperthermia and dehydration accrued during the longer work protocol. These data were supported by a second laboratory study, showing that 150 minutes of exercise caused eGFR to be significantly reduced and
markers of kidney injury to be elevated when compared to 30 minutes of exercise. Participants became significantly more dehydrated, however, core temperature was not measured and the study may not have been as stressful as the study by Schlader et al., as it took place in a temperate environment (20°C)\textsuperscript{59}. It should be noted that no water was provided during either of these studies, which may have led to hypovolemia and subsequent decrease in renal function. These data suggest that work duration in both temperate and hot environments, particularly if participants become dehydrated, may cause increased incidence rates of AKI.

It has been demonstrated that renal blood flow is decreased as exercise intensity increases\textsuperscript{60–62}. This is important to consider when workers or athletes are exercising or working, especially in a hot environment. Blood flow is directed away from the kidneys during exercise in hot environments to redirect blood to the skin and working muscle. Kawakami et al. demonstrated a significant reduction in renal blood flow in a temperate environment when participants cycled at or above their lactate breakpoint\textsuperscript{61}. Glomerular filtration rate has also been shown to decrease at moderate and heavy workloads (60\% and 80\% of VO\textsubscript{2}max, respectively)\textsuperscript{60} and renal clearance was reduced to ~40\% of resting values at heavy workloads\textsuperscript{63}. These data suggest that higher work intensity may also be considered a risk factor regarding decreased renal function.

Occupational field workers who are paid on a piece work system were more likely to develop AKI than those who were paid hourly (4.24 adjusted odds ratio of AKI),\textsuperscript{20}. This may be due to the fact that workers that are paid by the piece forego breaks and work at a higher intensity than those paid by the hour to increase their productivity to receive a higher wage. Additionally, using accelerometry data, Moyce et al. found that
heavier occupational workloads and piece-rate work were both associated with an increased adjusted odds of AKI after a single work shift\textsuperscript{64}. One study comparing sugarcane farmers and cutters showed a significant increase in higher urinary NGAL in sugarcane cutters\textsuperscript{65}. The increase in NGAL had a significant correlation to the longer working hours (~9 hours compared to ~5 hours) and to the heavier workloads experienced by the cutters compared to the farmers\textsuperscript{65}.

Few studies have been done to examine the role of intensity in workers and athletes regarding AKI, however some have noted correlations between runners who sustained acute kidney injuries and faster performance times during events. One study of ultramarathon runners suggested that those who progressed to acute renal failure and had higher levels of serum creatinine were faster than those who developed rhabdomyolysis but did not progress to acute renal failure\textsuperscript{66}. Another study of ultramarathon runners noted that the slowest runners in the study were 82\% less likely to develop AKI when compared to the fastest finishers\textsuperscript{67}. The authors hypothesized that this could have been due to the greater dehydration that the fastest runners incurred\textsuperscript{67}. These results were not seen in other studies where no correlations were noted between finish time and the development of AKI in ultramarathon runners\textsuperscript{57} or marathon runners\textsuperscript{26}. Data from an observational study noted that kidney function was impaired to a greater degree after running races of 42km and 100km in length when compared to a 310km, lending support to the theory that higher intensity exercise may temporarily impair kidney function when compared to lower intensity exercise of a much longer duration\textsuperscript{68}. Conversely, data on cyclists completing long distance events competing in a hot environment (33.2 ± 5.0 °C, 38.4 ± 10.7\% RH) found a positive correlation between serum NGAL and finishing times
which was suggested to be due to a longer duration under heat stress for the slower riders.

These data suggest that higher intensity physical activity is highly associated with AKI in occupational workers and endurance athletes than duration of the activity. Conclusions should be made cautiously as there are not many well-controlled studies on the individual effects of intensity or duration of activity on AKI, particularly in field settings. Future research should closely examine the intensity of work that field workers complete and strive to control confounding factors such as dehydration and hyperthermia.

**Repeated Exposures**

Few studies have examined the effect of repeated exposures to stressful stimuli on renal function in occupational workers or athletes. Repeated bouts of physical activity, especially in hot environment could prove detrimental due to repeated dehydration and hyperthermia. Repeated endothelial injury, caused by in part by ischemic and oxidative stress as well as nephrotoxicity are thought to cause possible long-term complications by disrupting repair processes and promote a fibrosis of the kidney vasculature over time, thus reducing functionality.

Data from a longitudinal study on sugarcane cutters showed a 9% (10ml/min) decrease in mean eGFR and an increase in mean serum creatinine both pre-shift (0.98 vs 1.18 ng/dL) and post-shift (1.06 vs 1.26 ng/dL) after only nine weeks of sugarcane harvesting. These results are concerning as they suggest a permanent suppression of renal function after a portion of the work season has passed. Similar, but less drastic, declines in renal function were found in a study by Laws et al. on Nicaraguan sugarcane workers. This study showed a mean decrease in GFR of 3, 4.5, and 4.9 ml/min/1.73m²
in sugarcane cutters, seed cutters, and irrigators respectively after a 5-month season. After five months of work, 8 field workers, 2.8% of the study sample, had a eGFR that met the criteria for Stage 3 CKD (<60 ml/min/1.73m$^2$), compared to only one worker at pre-harvest. One other longitudinal study of Brazilian sugarcane workers did not show an increase in serum creatinine at two different time points which were taken 8 months apart, however on both occasions, a large cross-shift increase in serum creatinine was noted. Another study noted a decrease in eGFR of -14% from January to April in a group of sugarcane cutters, where it was observed that workers with more severe increases in serum creatinine, measured over a subset of 6 days in the mid-season, were more likely to endure a more severe drop in eGFR than the workers who had a more moderate serum creatinine response.

Two studies on athletes were conducted on multi-day long duration endurance events to examine the cumulative effects of repeated bouts of long duration exercise on markers of kidney injury and function. One study examined participants who were completing a six-staged ultramarathon. Stages 1-6 were held on consecutive days, with no rest days in between and measurements were taken before and after stages 1, 3, and 5 which consisted of running distances of 25 miles, 25 miles, and 40 miles in a desert environment, respectively. Serum creatinine returned to near baseline levels before the start of stage 3 and stage 5, suggesting that recovery was taking place between the start of each stage. The second study took place during a long distance walking event where participants completed either 30-km, 40-km, or 50-km on three consecutive days to examine the cumulative effects on kidney injury markers. Urinary NGAL and urinary KIM-1 were measured before the start of day one (baseline) and the end of day 1 and day...
3. A significant increase in urinary NGAL was noted after day 1, but did not increase between days 1 and day 3 \(^7\). These results suggest that during days 1 and 3 of long-distance walking, which took participants 8:10 ± 1:59 hours and 8:28 ± 1:53, respectively, hours to complete, there was no cumulative effect on markers of renal damage. Additionally, 10% of participants (n=6) developed stage 1 AKI based on AKIN criteria after day 1, but no participants developed AKI after day 3.

No longitudinal studies have been conducted on endurance athletes over the course of training cycles, which may offer insight into whether there are subtle declines in kidney function over the course of their competitive season. Most of the research on endurance athletes is conducted after a competitive event, which constitutes a small portion of an athletes training cycle and may result in a skewed perspective of the amount of AKI athletes endure. Even studies across consecutive days of competition with athletes do not show a cumulative effect of renal stress. Conversely research seems to suggest that there is a cross-harvest decline in kidney function in workers due to repeated acute renal assaults, however studies are needed to further determine the underlying pathology.
Figure 1: Risk factors for the development of chronic kidney disease (CKD) in both occupational workers and endurance athletes. Repeated instances of acute kidney injury (AKI) may lead to the potential of CKD, however the development of CKD may be blocked by adequate recovery between AKI incidences.

**Conclusions**

Studies conducted on both endurance athletes and occupational workers show that they share many of the same renal stressors including elevated increased workload, core temperature, dehydration, and muscle damage. No published papers have suggested a progression of AKI to CKD in endurance athletes, however it has been suggested that caution should be taken as they share a number of physical activity based risk factors of AKI as occupational workers. The differences in development of CKD may be driven by the lack of proper recovery and volume of exposure hours and days (Figure 1). Occupational workers do not have the same freedom as athletes to manipulate crucial components of AKI management such as easy access to hydration or shade, nor can they
easily choose their working hours to avoid the hottest portion of the days or work duration. This is combined with a pitfall of piece-rate work where workers neglect to take frequent rest breaks to boost their productivity and increase their pay. The use of portable shaded areas, increase of scheduled rests, and extra water provisions that improve the quality of the work environment of those working outside in the heat may help protect against incidences of AKI and potential long-term detriments in kidney function. Though more research is needed, it appears that the inherently repetitive and unchanging nature of field work may at least partially explain the differences we see between endurance athletes and occupational workers. Research should be conducted to further examine the potential benefits of manipulating factors such as work intensity, duration, and recovery methods during and between workdays. This should be done in order to encourage proper hydration and limit the cumulative effects of hyperthermia and muscle damage in occupational workers. Athletes should also be made aware of the risks of repetitive dehydration and performing physical activity in the heat as they are in a favorable position to manipulate facets of their training to avoid long-term health issues.

**Disclosures**

None
References


CHAPTER 3

This chapter presents a research manuscript, entitled “The effect of interval and continuous work on markers of acute kidney injury in a hot environment”. This manuscript is authored by Jonathan Houck, Zachary Mckenna, Zachary Fennel, Jeremy Ducharme, Andrew Wells, Christine Mermier, Orlando Laitano, and Fabiano Amorim. This manuscript follows the formatting and style guidelines for the European Journal of Applied Physiology.
The effect of interval and continuous work on markers of acute kidney injury in a hot environment

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Abstract

Purpose: The purpose of this study was to examine the effect of high intensity interval work (HIIW) and moderate intensity continuous work (MICW) on markers of acute kidney injury (AKI) and kidney damage in a hot environment. Methods: In a randomized crossover design, nine physically active males completed two hours of physical work (two bouts of 60 minutes separated by 10 minutes of passive rest) in a hot environment (40°C and ~15% relative humidity) in either HIIW [2 minutes at 80% maximum oxygen consumption (VO₂max) and 3 minutes at 30% VO₂max] or MICW (average intensity of HIIW). Blood and urine samples were collected immediately before (Pre) and after (Post-Work), one hour (1hr Post-Work), and 24 hours after (24hr Post-Work) the trials. Urine flow rate (UFR), osmolality (Uosm), and creatinine (uCreatinine), serum creatinine
(sCreatinine), urinary neutrophil gelatinase-associated lipocalin (uNGAL) and urinary kidney injury marker 1 (uKIM-1) were measured. Estimated glomerular filtration rate (eGFR) was calculated. Core temperature (Tc), heart rate (HR), thermal sensation (TS), and rating of perceived exertion (RPE) were measured during the trial. Incidence rate of AKI were assessed using sCreatinine and the Acute Kidney Injury Network criteria.

**Results:** 66.7% and 22% of participants developed Stage 1 AKI Post-Work developed AKI immediately after HIIW and MICW, respectively. Serum creatinine was significantly higher during HIIW compared to MICW \([F(1,8)= 63.720; p< 0.01]\) and eGFR was significantly lower during HIIW compared to MICW \([F(1,8) 37.390; p< 0.01]\). Tc \([F(1,7)= 12.170; p=0.01]\) and HR \([F(1,7)= 22.833; p <0.01]\) were significantly higher in HIIW compared to MICW. UKIM-1 and uNGAL displayed significant effects for time \([F(3,24)= 15.508; p<0.01]\) and \([F(3,24)= 13.234; p<0.01]\) respectively, but not for condition. **Conclusion:** When compared to MICW, HIIW cause a higher incidence rate of Stage 1 AKI in a hot environment, however there was no difference between the markers of kidney damage. It appears that work in a hot environment causes an increase in biomarkers of kidney injury (KIM-1 and NGAL), but high intensity interval work may have a greater impact on acute renal function.

**Key words:** acute kidney injury, high intensity work, heat stress,

**Declarations**

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Conflicts of Interest: None Declared

Author contributions: JH and FA conceived the experiment design. JH, ZM, ZF, JD, AW, FA collected data. JH, ZM, FA analyzed data. JH drafted the manuscript. All authors contributed to the final manuscript.

Ethics approval: This study was approved by the University of New Mexico Main Campus Institutional Review Board (protocol #1690849-3)

Abbreviations
\[\eta_p^2\]: Partial Eta-squared
AKI: Acute kidney injury
eGFR: Estimated glomerular filtration rate
GFR: Glomerular filtration rate
Hct: Hematocrit
HR: Heart rate
KIM-1: Kidney injury marker 1
NGAL: Neutrophil gelatinase-associated lipocalin
ELISA: Enzyme-linked immunosorbent assays
PV: Plasma volume
RH: Relative humidity
RPE: Rating of perceived exertion
Tc: Core temperature
TS: Thermal stress
UFR: Urine flow rate
Uosm: Urine osmolality
Introduction

It is known that renal function is reduced as work intensity increases due to a decrease in kidney blood flow, which results in a decreased rate of urine production (Zambraski, 1990). For example, renal clearance rates have been shown to be significantly reduced to 35-45% of resting values during heavy workloads (Grimby 1965). Additionally, glomerular filtration rate (GFR) has been shown to be reduced at 60% and 80% of maximal oxygen consumption (VO$_2$max) after 20 minutes of cycling exercise, but not at lower intensities (25% and 40% of VO$_2$max) (Freund et al. 1991). Using Doppler ultrasound, Kawakami et al., showed that that the total cross-sectional area of kidney vasculature and renal blood flow decreased at cycling exercise intensities equal to 120% and 140% of lactate breakpoint (Kawakami et al. 2018).

These reductions in renal blood flow (RBF) and GFR may lead to acute impairment of renal function and result in acute kidney injury (AKI). Individuals such as agricultural workers, military personnel, firefighters, and athletes that perform physical work in hot environments may be at risk of AKI. It has been proposed that heavy work in hot climates while under a state of dehydration reduce RBF and may lead to renal ischemia and decreased renal ATP levels which in turn may cause oxidative stress and damage to the kidney (Madero et al. 2017; Juett et al. 2020). Previous studies have shown that agricultural workers and athletes experience changes in kidney function and increased markers of AKI and these changes might be related to the work intensity. Data from one study which compared AKI risk factors in sugarcane farmers and sugarcane cutters found an increase in urinary neutrophil gelatinase-associated lipocalin (uNGAL) was significantly related to both the longer shifts and more intense workloads of the
cutters compared to farmers (Pundee et al. 2020). Additionally, agricultural jobs where workers are paid by a piece work system of compensation, whose salary is dependent upon the amount of work completed, may increase the incidence of AKI by encouraging workers to work at higher intensities and take fewer breaks (Moyce et al. 2017). In a study of ultramarathon runners, it was noted that the slowest runners were 82% less likely to develop AKI, determined by increases in serum creatinine and decreases in glomerular filtration rate, than the fastest runners (Lipman et al. 2014). These data may suggest that workers and athletes who work at a higher intensity may be more likely to incur AKI.

Molecular biomarkers of AKI such as NGAL and kidney injury molecule-1 (KIM-1) are often used in conjunction with decreases in eGFR and urine output to determine the severity or lasting impact of AKI. Often, after physical activity, AKI is reversible and transient in nature, where there is an increase in biomarkers but no prolonged health risk. A complete understanding of the factors that lead to AKI during physical work in the heat is lacking, but there is evidence of potential contributing factors. Thermal stress (high core temperature) and dehydration (Chapman et al. 2020b), muscle damage (Junglee et al. 2013), and duration of work (Schlader et al., 2017) are related to increases in AKI biomarkers and decreases in kidney function. These factors are thought to contribute to AKI by eliciting ischemia/reperfusion injury caused by changes in renal blood flow due in large part to hypovolemia and sympathetic nerve activity. The reduction in blood flow with increased heat stress (Miyamoto 1994; Wilson 2017) and physical work (Freund et al. 1991; Kawakami et al. 2018) may create a hypoxic environment and reduced ATP availability in the renal circulation. In parallel, inflammation and oxidative stress occur due to this ischemic and metabolically costly
environment which can lead to damage of the tubules resulting from impaired calcium handling and increases in reactive oxygen species (Devarajan 2005, 2006; Chatauret et al. 2014). Additionally, a second insult to the kidneys may occur upon return of regular renal blood flow caused by further oxidative stress and the potential for cell swelling and subsequent death in the renal structures (Devarajan 2006). One factor related to this hypothesis of ischemic injury that has not been examined independently is the role of work intensity in the heat on the prevalence of AKI. Particularly, based on previous data, the role of working for periods at high intensity followed by periods of lower intensity which may produce a cycle of ischemia and reperfusion to the kidney.

The purpose of this study was to examine whether there is a difference in AKI biomarkers and measures of kidney function after either interval or continuous styles of work in a hot environment when total work, as determined by distance covered, is matched.

**Methodology**

This study was approved by the University Institutional Review Board (protocol #1690849-3) and each participant signed a written informed consent document before participating in the study.

Nine healthy, non-heat-acclimated, physically active adult-aged males (<45 years old) were recruited to participate in the present study. Participants reported engaging in regular physical activity (>150 minutes of moderate to vigorous intensity aerobic activity per week for a minimum of 3 months), but were not highly fit (less than the 80th percentile for VO$_2$max based on age and sex) (American College of Sports Medicine, 2018). Individuals who had lower body injuries or history of rhabdomyolysis, kidney disease, or heat stress complications were excluded from this study. Heat acclimation was
determined as regular exposure to a hot condition, such as sauna, hot water bath, etc., or living in a hot climate for the past two months. Data collection took place between the end of March and beginning of May in the northern hemisphere, so it was unlikely that participants were heat acclimated due to local environmental temperatures during the study.

**Experimental Design**

The participants reported to the lab a total of five times during the study period (figure 1). During these visits they had anthropometric measurements taken, performed a VO$_{2\text{max}}$ test, and treadmill speed determination (visit 1), completed two experimental trials (high intensity interval work (HIIW) or moderate continuous intensity work (MICW) protocols) that were randomized and counterbalanced (Visits 2 and 4) using an online randomizer (http://www.randomizer.org; Site Statistics, Social Psychology Network) and returned for two visits 24 hours after the experimental trials (visits 2 and 4) for urine and blood collection. The experimental trials were conducted in a heat chamber set to 40°C. During all lab visits, participants wore a short-sleeve T-shirt, shorts and athletic shoes. Prior to visit 1, participants were asked to avoid having a full meal within 2 hours, caffeine for 4 hours, and performing vigorous exercise for 24 hours. Prior to visits 2 and 4 participants were asked to report to the lab after at least a 2-hour fast, and avoid having caffeine for 12 hours, performing any exercise for 24 hours, and consuming alcohol 24 hours prior to the visit. The two experimental trials were separated by at least seven days to avoid heat acclimation.

**Visit 1 - Baseline testing**
During the first visit, height (cm) and body weight (kg) were measured using a stadiometer, and scale, respectively. Body fat percentage (BF%) was measured using a 3-site skinfold for men (chest, abdomen, and thigh). These values were subsequently used to estimate body density and BF% (Jackson and Pollock 1978).

Participants performed a running VO\textsubscript{2max} test on a motorized treadmill (Precor, TRM885, Woodinville, WA). All exercise tests were performed with the same metabolic cart (TrueOne 2400, ParvoMedics, Sandy, UT) which was calibrated before each trial in accordance with the manufacturer’s guidelines. Heart rate (HR) was monitored via telemetry (Polar H1, Kempele, Finland), and breath by breath measurements were taken for expired gas analysis. Before the VO\textsubscript{2max} test, the participant was asked to perform a 5-minute self-selected warmup on the treadmill. The VO\textsubscript{2max} test consisted of an individualized protocol where the participant was asked what was the fastest speed that they could achieve on the treadmill. The protocol was then designed to achieve the indicated maximal speed at eight minutes into the test, with a 0.8 km/h increase in speed every 60 seconds. After this speed was reached, 1% incline was added every 60 seconds until the participant reached volitional fatigue. At the end of every minute the participant was asked their rating of perceived exertion (RPE) on a scale of 6-20. (Borg 1982).

During the test, heart rate data was collected at the end of each minute and at the time of test termination. After the test was completed, the participant was given 20 minutes of recovery where they were free to stand or walk around the laboratory. VO\textsubscript{2max} was assessed via an 11-breath rolling average and 80% and 30% of this value were calculated.

After the 20 minutes of recovery, the participants again donned the gas collection equipment and the grade of the treadmill was increased to 6% incline, which would be
used during the trials and the speed was modified to determine the speed to elicit 80% of VO2max. After this, a speed which elicited 30% VO2max was determined. The treadmill remained at one speed for 3 minutes and if needed, the research team member adjusted the speed accordingly, until values equaling 80% and 30% of the previously measured VO2max were determined. These speeds were used to determine the workload during the exercise trials, with the speed eliciting 80% of VO2max used during the 2-minute intervals and the speed eliciting 30% VO2max used for the 3-minute intervals. The MICW was matched for total workload (distance covered) and set at a constant velocity.

Visits 2 and 4 (Experimental Trials)

At least 72 hours after the first visit, participants were asked to report to the lab euhydrated. The participants were asked to void their bladder exactly one hour before arriving at the laboratory, noting the time that this occurred. After one hour had passed they were asked to void their bladder at the laboratory, into a provided container for a urine sample collection. The urine sample was used to assess urine specific gravity (USG) using a handheld refractometer (Cole-Parmer, RSA-BR90A, Vernon Hills, IL) and the participant was considered euhydrated at a USG <1.020 (American College of Sports Medicine et al. 2007). If the participants were deemed to be dehydrated, they were then provided 500mL of water and asked to wait in the laboratory for one hour after which they were asked to void their bladder again into a urine specimen cup so that USG could be reassessed. After the urine sample were collected and USG measured, the participants assumed a seated position so that a blood draw could be performed. Blood was drawn from the antecubital vein and a maximum of 10mL were collected to obtain serum and plasma samples. The participants were then asked to obtain a nude body
weight, insert a rectal thermistor (Level 1 esophageal/rectal temperature probe, Smiths Medical, Minneapolis, MN, USA), and don a heart rate monitor (Polar H1, Kempele, Finland). Participants measured nude body weight using a calibrated scale in a private room then reported their body weight to the researchers. The participants then completed either the HIIW or MICW protocol in the heat chamber (≈40°C). For the HIIW protocol the participants alternated between the speeds and grades which elicited 30% VO₂max and 80% VO₂max for 60 minutes (two minutes at 80% VO₂max and 3 minutes at 30% VO₂max) followed by the same 10 minutes of seated recovery as MICW and a final 60-minute HIIW exercise bout. During the 10-minute rest, the participants obtained a nude body weight and water was provided equal to 75% weight lost. The 75% is based on unpublished research with field workers and is representative of the average sweat loss to water intake of field workers in a hot environment. Additionally, the participants were provided 200ml of water every 20 minutes during both work bout sessions (i.e. 20 minutes and 40 minutes into each of the work bouts). The MICW protocol consisted of a total of 120 minutes of treadmill exercise at the average speed calculated for the HIIW protocol (Equation 1).

Equation 1: Calculation of moderate intensity continuous trial speed. Where maximal aerobic capacity (VO₂max) was measured during an incremental treadmill test.

\[
\frac{(Speed \ at \ 30\% \ VO_{2\max} \times 3) + (Speed \ at \ 80\% \ VO_{2\max} \times 2)}{5}
\]

Participants were given a ten-minute break inside the chamber after the first 60 minutes of exercise followed by a second 60-minute bout of treadmill walking identical to the first half of exercise. Core temperature (Tc), heart rate (HR), thermal sensation
(TS) using a scale of 0-8 (0= very cold, 8= very hot), and rating of perceived exertion (RPE) were recorded every 5 minutes during the MICW trial, while heart rate and RPE were measured at the end of the three-minute interval and at the end of the two-minute interval during the HIIW trial (Equation 2). The trials were terminated if a participant reached a measured core temperature of 39.0°C. Immediately after exercise the participants exited the heat chamber, were asked to void their bladder into a collection container so that the volume could be measured and samples collected, removed the rectal thermistor and heart rate monitor and obtained a nude body weight. Participants were asked to assume a seated position for 10 minutes, after which a blood draw was performed to collect serum and plasma samples. Next, the participants were provided with an amount of water equivalent to 75% of their weight lost. A one-hour wait occurred after which the participants were asked to provide another urine sample to calculate one-hour urine flow rate and another serum sample was collected to analyze serum creatinine. Total sweat rate was calculated as the participant’s post trial weight subtracted from pre-trial weight, corrected for amount of water given during the trial. A diet diary template was emailed to the participant at least one day before this visit. They were asked to record their diet over the 24-hour period before arriving at the laboratory before visit 2 and to replicate this diet 24 hours before visit 4.

Equation 2: Calculation of average heart rate (HR) and rating of perceived exertion (RPE) over each five-minute interval during the high-intensity interval work (HIIW).

\[(HR \text{ or RPE during low intensity interval} \times 3) + (HR \text{ or RPE during high intensity interval} \times 2)\]
Visits 3 and 5 (Blood and Urine Collection)

Twenty-four hours after the end of visits 2 and 4, participants reported back to the laboratory for a blood and urine sample collection. The participants were asked to void their bladder exactly one hour prior to coming the laboratory. Participants completed another 24-hour diet log where they recorded their food and beverage intake prior to visit 3, which they were then asked to replicate prior to visit 5. Upon arrival they were asked to void their bladder for urine analysis and assume a seated position for 10 minutes before serum and plasma blood samples (10mL) were collected from the antecubital vein.

Blood and Urine Analysis

Blood and urine samples were collected at four timepoints (Figure 1). An aliquot of urine and whole blood were set aside for immediate analysis of hematocrit (Hct), urine osmolality (Uosm), and hemoglobin concentrations. Serum samples were left at room temperature for 20 minutes prior to being centrifuged. Serum and plasma samples were centrifuged at 2400rpm and 4°C for 15 minutes. Urine, serum and plasma aliquots were then stored until further analysis at -80°C.

Hematocrit was measured in triplicate by transferring blood into microcapillary tubes and centrifuging (Microcentrifuge Model C-MH30, UNICO, Dayton, NJ) for 5-minutes. The microcapillary tubes were then measured using a (Micro-Capillary Reader No. 2201, International Equipment Company, Needham Heights, MA). Hemoglobin concentrations were measured in duplicate within 24 hours of each blood draw using a Hemoglobin Reagent Set (Pointe Scientific, Canton, MI) following manufacturer specifications. Absorbance was read at 540 nanometers (Beckman Coulter DU-520, Fullerton, CA) and a triplicate measurement was performed if the coefficient of variance
(CV) was larger than 5% between the two measures. The hemoglobin and Hct measures were used to calculate plasma volume changes at Post, 1hr Post, and 24hr Post compared to baseline measures using a standard equation (Dill and Costill 1974). Uosm were measured in duplicate using a freezing point depression osmometer (Model 303, Advanced Instrument, Inc., Norwood, MA).

To assess acute kidney injury, NGAL (BioPorto Diagnostics, Hellerup, Denmark) and KIM-1 (Enzo Life Sciences, Farmingdale, NY) concentrations in urine were measured using enzyme-linked immunosorbent assays. Additionally, kidney function was assessed through the evaluation of serum creatinine (Cayman Chemical, Ann Arbor, MI) and urine creatinine (R&D Systems, Minneapolis, MN) concentrations using colorimetric assays. A modified CockCroft-Gault equation was used to calculate eGFR and correcting for body surface area (Rostoker et al. 2007). Urine flow rate was recorded over one-hour periods of time prior to, immediately after, and 24 hours after the exercise trials. Acute kidney injury markers were assessed using the Acute Kidney Injury Network (AKIN) criteria (Table 1) (Mehta et al. 2007). NGAL and KIM-1 were normalized to urine flow rate, urine osmolality, and urine creatinine.
Figure 2: Schematic of the study design. Work on the treadmill was performed at a 6% incline and speeds individualized based on participants' maximal aerobic capacity (VO\textsubscript{2}max). HIIW: high-intensity interval work, MICW: moderate-intensity continuous work, RH: relative humidity.

Statistical analysis

The *a priori* sample size was calculated using G-power software with an alpha level of 0.05 and power (1 - beta) of 0.81, and the number of participants required to make a valid analysis was n = 8. A study using similar methodology (changes in urinary NGAL) was used to guide the power analysis (Chapman et al. 2019). A Shapiro-Wilk test was used to examine normality for all dependent variable data. Uncorrected uNGAL, uKIM-1, and sCreatinine violated the assumption of normality and thus were log transformed prior to being analyzed. Mauchly’s test for sphericity was utilized and if this assumption was violated, a Greenhouse-Geisser correction was used. All values are stated as mean ± standard deviation (SD). To compare the differences between the two conditions (HIIW and MICW) and four time points (Pre, Post, 1hr-Post, 24hr-Post) or
nine timepoints for measures taken during the work trial (Pre, 15min, 30min, 45min, 60min, 85min, 100min, 115min, and End) repeated measures ANOVA (conditions vs. time points) with Bonferroni adjustment was used. Maximal Tc and total sweat rate were assessed using paired sample t-tests. Data for variable collected at Pre, Post, 1hr-Post, and 24hr-Post have individual values graphed along with the mean for each time point. Effect sizes are reported as partial Eta² (η²). All data were analyzed using SPSS version 19 (SPSS, IBM, Chicago, IL) and figures created using Prism GraphPad version 9 (GraphPad, San Diego, CA). Statistical differences were accepted as significant at \( p \leq 0.05 \).

**Table 1**: Acute Kidney Injury Network (AKIN) criteria for acute kidney injury.

<table>
<thead>
<tr>
<th>Stage 1</th>
<th>Serum Creatinine</th>
<th>Urine Output</th>
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<tbody>
<tr>
<td></td>
<td>Increase ( \geq 0.3 \text{ mg/dL} ) or increase of ( \geq 1.5-2.0 ) fold from baseline</td>
<td>( &lt;0.5 \text{ mL/kg/h for 6h} )</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Increase of ( &gt;2.0-3.0 ) fold from baseline</td>
<td>( &lt;0.5 \text{ mL/kg/h for 12h} )</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Increase ( &gt;3.0 ) fold from baseline or serum creatinine of ( \geq 4.0 \text{ mg/dL} ) with an acute increase of ( \geq 0.5 \text{ mg/dL} ) or need for renal replacement therapy</td>
<td>( &lt;0.3 \text{ mL/kg/h for 24h or anuria for 12h} )</td>
</tr>
</tbody>
</table>

**Results**

Participants in the current study were 30.1 \( \pm \) 6.2 years old, height 179.9 \( \pm \) 5.5cm, weight 77.7 \( \pm \) 9.2, body fat percentage (15.2 \( \pm \) 2.7%) and had good cardiovascular fitness (VO\(_{2\text{max}}\) = 48.7 \( \pm \) 5.2 ml/kg/min). Nine participants completed the exercise trials, however one participant was forced to stop early (75 minutes) due to reaching the core temperature cut off. The participant was not indicated as an outlier for any of the measures and therefore were kept in the analysis. Due to this, variables collected during the work trial are reported as n=8 for the 85-minute, 100-minute, and 115-minute
timepoints. There was no effect for trial order for any of the measured variables.

Environmental conditions were similar between HIIW (40.09 ± 0.12°C; relative humidity 15.24 ± 1.32%), and MICW (40.07 ± 0.1°C; relative humidity 14.68 ± 0.96%).

**Physiologic variables**

The main effects for condition \([F(1,7)= 12.170; p=0.01]\), and time \([F(8,56)= 128.698; p<0.01]\) were noted for Tc, however the interaction effect (condition*time) failed to reach significance \([F(8,56)=1.738; p=0.11]\). The HIIW group had a significantly higher mean Tc than the MICW group during the trial (38.05°C ± 0.052 compared to 37.90°C ± 0.066). HIIW and MICW had similar starting Tc; 37.0 ± 0.26°C and 36.95 ± 0.35°C respectively \((p=0.52)\), however HIIW had a significantly higher maximum Tc \((38.75°C ± 0.26 vs 38.44°C ± 0.22, respectively; p<0.01)\) (figure 2B). Similarly, main effects for condition \([F(1,7)= 22.833; p <0.01]\), and time \([F(8,56)= 115.783; p<0.01]\) were noted for HR (figure 2A). Mean HR for HIIW was 10.9bpm higher than MICW over the course of the trial. however the interaction effect (condition*time) failed to reach significance \([F(8,56)= 0.546; p= 0.817]\). RPE (figure 2D) and TS (figure 2C) displayed increases over time \([F(8,56)= 136.502; p< 0.01]\) and \([F(8,56)= 80.07; p<0.01]\) respectively, but there were not significantly difference between conditions at any timepoint.
Figure 3: Heart rate (a), core temperature (b), thermal sensation (c), and rating of perceived exertion (d) presented at multiple timepoints (min) over two hours of interval style (HIIW) or continuous style (MICW) work in a hot environment. ** denotes a significant effect for time. Significant effects for condition (HIIW higher than MICW) for mean heart rate and mean core temperature over the course of the two-hour trial signified by ***. Statistical significance indicated at p< 0.05.

Hydration

Total sweat loss, as measured by change in body weight and corrected by urine output, was similar (p= 0.23) between HIIW and MICW (2.56 ± 0.77 L and 2.30 ± 0.71 L, respectively). Uosm displayed an main effect for time [F(3,24)= 4.390; p= 0.013], increasing significantly from Pre to Post-Work and remaining elevated at the 1hr Post-Work timepoint, before returning to baseline values at 24hrs Post-Work (Figure 3B). PV was not significantly different from Pre at Post-Work, 1hr Post-Work, or 24hr Post Work after HIIW (-0.68 ± 2.7%; 0.15 ± 2.8%; -0.38 ± 4.3% respectively) or MICW (-0.43 ± 3.1%; -0.8 ± 3.2%; -0.93 ± 3.7% respectively).
Figure 4: (A) Urine flow rate, (B) urine osmolality, and (C) urine creatinine (uCreatinine); before (Pre), immediately after (Post-Work), 1hr after the completion of (1hr Post), and 24hrs after the completion of (24hr Post) two hours of either interval style (HIIW) or continuous style (MICW) work in a hot environment. Individual results have been displayed as open circles for HIIW and open triangles for MICW. Mean results at each time point have been plotted. * Denotes a significant difference from Pre. # Denotes a significant difference from Post-Work. Statistical significance indicated at p< 0.05.

Markers of Renal Function and Injury
UFR displayed a main effect for time \([F(3,24)= 7.460, p< 0.01; \eta^2_p = 0.483]\) significantly decreasing from Pre to Post-Work, before returning to baseline values at 24hr Post-Work (Figure 3A), but no difference between conditions \([F(1,8)= 0.411, p= 0.539; \eta^2_p = 0.049]\). HIIW induced a significantly lower mean eGFR \([F(1,8)= 37.390, p< 0.01; \eta^2_p = 0.449; \text{mean difference 20.37 ml/min per 1.73m}^2]\). Additionally, there was a main effect for time \([F(3,24)= 6.525, p< 0.01; \eta^2_p = 0.449]\) where Post-Work eGFR was significantly lower than 24hr-Post work (Figure 7). A main effect for condition signified HIIW had a higher mean sCreatinine than MICW \([F(1,8)= 63.720; p<0.01; \eta^2_p = 0.888]\) and a main effect for time \([F(3,24)= 6.928; p<0.01; \eta^2_p = 0.464]\) between Post-Work and 1hr Post-Work as well as between Post-Work and 24hr Post-Work \((p= 0.03; p<0.001\) respectively) (Figure 6). Additionally, uCreatinine was significantly increased at Post-Work and 1hr Post-Work, before returning to baseline levels at 24hr Post-Work (Figure 3C). Log uNGAL (Figure 4A) was significantly elevated Post-Work compared to Pre \((p< 0.05)\). When corrected for UFR (Figure 4D), uNGAL was significantly higher than Pre at both Post-Work and 1hr Post-Work, values were significantly lower at 1hr Post-Work and 24hrs Post-Work \((p< 0.05)\). No timepoints were significantly different when uNGAL was corrected for Uosm or uCreatinine (Figure 4B-C). Log uKIM-1 (Figure 5A) as well as uKIM-1 corrected for uCreatinine, Uosm, and UFR (Figure 5B-D) were all significantly higher Post-Work and 1-hr post compared to Pre \((p< 0.05)\).
Figure 5: Urinary NGAL (uNGAL) before (Pre), immediately after (Post), 1hr after the completion of (1hr Post), and 24hrs after the completion of (24hr Post) two hours of either interval style (HIIW) or continuous style (MICW) work in a hot environment. Presented as (a) log of NGAL in ng/ml, (b) uNGAL corrected for urinary creatinine (mg/dL), (c) urine osmolality (mOsm), and (d) urine flow rate (ml/min/kg). Individual results have been displayed as open circles for HIIW and open triangles for MICW. Mean results at each time point have been plotted. * Signifies a significant difference from Pre timepoint; # denoted a significant difference from Post timepoint. Statistical significance indicated at p< 0.05.
Figure 6: Urinary KIM-1 (uKIM-1) before (Pre), immediately after (Post), 1hr after the completion of (1hr Post), and 24hrs after the completion of (24hr Post) two hours of either interval style (HIIW) or continuous style (MICW) work in a hot environment. Presented as (a) log of KIM-1 in ng/ml, (b) uKIM-1 corrected for urinary creatinine (mg/dL), (c) urine osmolality (mOSM), and (d) urine flow rate (ml/min/kg). Individual results have been displayed as open circles for HIIW and open triangles for MICW. Mean results at each time point have been plotted. * Signifies a significant difference from Pre timepoint; # denoted a significant difference from Post timepoint. Statistical significance indicated at p< 0.05.
Figure 7: Serum creatinine (sCreatinine) expressed as (log) before (Pre), immediately after (Post), 1hr after the completion of (1hr Post), and 24hrs after the completion of (24hr Post) two hours of either interval style (HIIW) or continuous style (MICW) work in a hot environment. Individual results have been displayed as open circles for HIIW and open triangles for MICW. Mean results at each time point have been plotted. # Denotes a significant difference from Post. *** denotes a significant difference between groups where HIIW is significantly lower than MICW. Statistical significance indicated at p< 0.05.

Figure 8: Estimated glomerular filtration rate (eGFR) before (Pre), immediately after (Post), 1hr after the completion of (1hr Post), and 24hrs after the completion of (24hr Post) two hours of either interval style (HIIW) or continuous style (MICW) work in a hot environment. Individual results have been displayed as open circles for HIIW and open triangles for MICW. Mean results at each time point have been plotted. # Denotes a significant difference from Pre. *** denotes a significant difference between groups where HIIW is significantly lower than MICW. Statistical significance indicated at p< 0.05.

Prevalence of AKI
Using AKIN criteria (table 1) for identifying AKI using changes in sCreatinine, six of the nine participants (66.7%) developed Stage 1 AKI after the HIIW trial. Two of the participants remained in the Stage 1 category for all measured time points after the HIIW trial. Conversely, two participants (22.2%) developed Stage 1 AKI after the MICW trial, with only one participant remaining in this classification at the 1hr-post and 24hr-post timepoints.

**Discussion**

The main purpose of this study was to examine whether there is a difference in AKI biomarkers and measures of kidney function after two hours of matched work performed on high intensity interval or continuous at moderate intensity in a hot environment. The main findings of the present study were: (1) more participants were categorized as Stage 1 AKI based on AKIN criteria after HIIW compared to MICW; (2) there was higher mean sCreatinine levels in the HIIW compared to the MICW trials; (3) HIIW had a lower mean eGFR compared to the MICW trials; (4) there were no significant differences between the groups for uNGAL or uKIM-1, however both markers were significantly increased after both trials; and (5) the HIIW appeared to be more physiologically stressful on the participants, as there was a main effect for condition in both Tc and HR.

To our knowledge this is the first report to directly examine the effect of two different work intensity styles (continuous and interval) in a hot environment on kidney function and markers of acute kidney injury. In the present study, markers of renal function decreased (eGFR, UFR) and molecular markers of acute kidney injury (sCreatinine, NGAL and KIM-1) increased after both experimental conditions (HIIW and
MICW). This indicates our work protocols were sufficient to induce renal stress. Similar results were noted after exercise in the heat in a study by Junglee et al. (2013), where uNGAL and plasma NGAL were elevated after a 40-minute run in a hot (33°C) environment. Additionally, Junglee et al., noted that half of their participants which underwent a prior muscle damaging exercise met AKIN guideline criteria for stage 1 AKI after running in heat (Junglee et al. 2013). It was also shown that eGFR was decreased while sCreatinine and plasma NGAL levels increased after three 20-minute bouts of exercise in a hot environment (38°C, 50% relative humidity). Regarding work intensity, the results observed in the present study indicates that HIIW induced a higher prevalence of AKI as measured by eGFR and serum creatinine level. This is supported by studies on field workers by Moyce et al. where it was noted that heavier occupational workloads, measured using accelerometry, and piece-rate work were both associated with higher adjusted odds of AKI, as assessed by increases in sCreatinine, after a single shift of agricultural field work (Moyce et al. 2020).

This increase in transient AKI with higher intensity exercise aligned with previous studies that demonstrated a decrease in kidney function immediately after high intensity exercise (Grimby 1965; Kawakami et al. 2018). It is speculated that a reduction in renal blood flow with high intensity work may have promoted an ischemic environment which could have caused stress to renal structures and potential maladaptation after repeated exposures (Madero et al. 2017). It well described that exercise cause upregulation of the renin-angiotensin-aldosterone system, increased renal sympathetic nerve activity, and increased catecholamine release (Bie 1980; Tidgren et al. 1991; Schneider et al. 2000; Kawakami et al. 2018) which cause reduction in RBF. These
reductions are normal physiological functions to aid in the conservation of fluid during exercise by limiting urine production and excretion (Mack and Nadel 2011; Schlader et al. 2019) and are elicited during work or exercise performed at high relative intensity, but creatinine clearance, and UFR have been shown to be augmented at low or moderate intensity exercise (Virvidakis et al. 1986). Although the timeframe of these reductions is not yet known, it has been suggested that these decrease happen rapidly after the onset of high intensity exercise (Chapman et al. 2020a). This reduction in renal blood flow may create an environment prone to damage of the renal structures due to an imbalance in ATP supply and demand from the increased fluid and sodium reabsorption leading to an increase in reactive oxygen species and finally oxidative stress (Devarajan 2006; Chatauret et al. 2014; Chapman et al. 2020a).

Although we observed decrease in markers of renal function (eGFR, UFR and serum creatinine) and higher prevalence of AKI after HIIW, NGAL and KIM-1 were not different between the experimental conditions. While increases in sCreatinine and decreases in eGFR are commonly used markers of AKI severity, these responses are components of normal integrative physiological systems during work in a hot environment. Therefore, it is useful to combine these typical AKI markers with biomarkers of kidney damage to differentiate what may be functional AKI and what may be actual damage to the renal structures (Parikh and Coca 2010; Schlader et al. 2019). When there is a presence of both decreased kidney function and an increase of AKI biomarkers, more certainty may be applied to the analysis of whether AKI occurred or which participants are at a higher risk to develop AKI (Schlader et al. 2019). Both uNGAL and uKIM-1 increased significantly from Pre-Work to Post-Work and both
remained significantly higher when corrected for UFR meaning that independent of urine production, uNGAL and uKIM-1 are upregulated after two hours of work in a hot environment. UKIM was also significantly increased when corrected for Uosm and uCreatinine, while there was no significant change in uNGAL when corrected for either marker of urine concentration. Correcting for uCreatinine should be interpreted with caution after physical activity due to the inconsistency of eGFR and creatinine production after intense exercise (Philippou et al. 2009; Waikar et al. 2010; Junglee et al. 2012, 2013), whereas UFR may be a more reliable correction factor to assess biomarkers. NGAL is released at low levels from multiple organs including the kidneys, liver, and heart while at rest (Schlader et al. 2019). Increased uNGAL is a marker of ischemic kidney injury and may represent general tubular injury, particularly from the thick ascending loop of Henle (Schrezenmeier et al. 2017; Schlader et al. 2019). Similarly, KIM-1 is expressed at low levels during rest, but increases can occur after renal stress. KIM-1 is preferentially released from the proximal tubules and is often increased after ischemic-reperfusion injury (Ichimura et al. 1998; Schrezenmeier et al. 2017) and has been shown to have a role in kidney recovery in mouse models (Ichimura et al. 2008). Given the increase in both uKIM-1 and uNGAL in the current study, as well as increases in sCreatinine and decreases in eGFR, we hypothesize that the likelihood that transient AKI occurred due to both work intensity patterns is high, with HIIW potentially decreasing renal functionality to a greater extent.

HIIW elicited a higher mean Tc over the two hours of work in the heat compared to MICW and a higher maximum Tc. Although the two protocols resulted in similar work performed (or distance covered), it is possible that the HIIW resulted in higher heat
production compared to MICW as the participants had to shift from a walking to running gait to complete the high intensity interval. It is known that running has a greater energy cost than walking for the same distance covered (Hall et al. 2004) which may also explain why HR and Tc were higher during HIIW. This difference in Tc may have resulted in different stress to the kidney as hyperthermia has been shown to be an independent instigator of AKI and decreases in kidney function. It was reported in a previous study that after work in a hot environment, plasma NGAL, uNGAL, and urinary insulin-like growth factor binding protein 7 (IGFBP7) were all significantly elevated in a control group when compared to groups where either water or cooling vests were administered which alleviated core temperature increase (Chapman et al. 2020b). In one study of marathon runners, it was shown that there was no difference in mean core temperature (38.4°C and 38.8°C respectively) or maximum core temperature (39.4 °C and 39.0 °C respectively) between runners who incurred AKI and those who did not (Mansour et al. 2019). Additionally, there was no significant difference between uNGAL values in runners who did or did not get classified as having AKI (Mansour et al. 2019). Junglee reported significantly higher core temperatures after muscle damaging exercise compared to a control condition (mean difference of 0.67°C) and an increase of uNGAL and plasma creatinine levels (Junglee et al. 2013). It is unclear whether the differences in Tc between HIIW and MICW were likely to cause the increase of stage 1 AKI instances, however it seems unlikely due to the relatively small difference in both mean Tc over the trials and maximum Tc in this study. The increase in core temperature needed to cause further kidney damage or AKI in humans is not currently known nor is the correlation that core temperature may have with AKI.
Both groups experienced similar levels of dehydration during the two hours of work, as measured by sweat loss and urine osmolality. Dehydration has been shown to exacerbate AKI severity and increase prevalence after physical activity in hot environments (Chapman et al. 2020b). Participants were given 200ml of water every 20 minutes during exercise, as well as 75% of sweat loss replacement during the rest period at one hour into the work and at the end of exercise. It has been shown that fluid replacement between 32% and 100% of sweat loss may help to lessen reductions in creatinine clearance after exercise in hot environments (Otani et al. 2013; Chapman et al. 2020a). Dehydration in the current study may have contributed to the similar elevation in both NGAL and KIM-1.

There are limitations that should be considered in regard to interpretation and extrapolation of the data beyond this study. First, this study examined only an acute bout of physical work in the heat and therefore it is not possible to determine how repeated bouts of either HIIW or MICW on consecutive days may impact kidney function or risk of AKI. Second, RBF was not measured during or immediately after the work sessions, which would provide insight into the amount of RBF reduction and possible ischemia occurring. Third, the participants were all physically active males between the ages of 24 and 45 with no history of renal complications. These data may not accurately represent the response to similar work in a hot environment with more heterogenous groups. Fourth, the duration of the protocol may not accurately reflect the stressors of occupational workers who often have shifts that last between four and eight hours, meaning the results should not be used to assess the differences in work styles over longer work shifts. Additionally, it should be noted that the current study took place in a
hot (40°C), but not humid (RH=15%), which is drier than the conditions faced by many field workers. Finally, it is known that GFR and sCreatinine are impacted by physical activity. This is problematic when analyzing GFR after physical activity, as it is assumed that GFR is constant (Waikar et al. 2010). Decreases in GFR with physical activity increase may make interpretation sCreatinine difficult (Schlader et al. 2019).

Conclusion

HIIW appeared to induce transient kidney injury at a higher rate than MICW when measured by increases in sCreatinine and decreases in eGFR, however uNGAL and uKIM-1, were not different between groups but did increase after two hours of work in a hot environment. Future research on the topic of work intensity and AKI should attempt to isolate the effects of intensity further by conducting trials in a thermoneutral environment and provide full hydration to lessen the additive effects of dehydration and hyperthermia. Additionally, measuring RBF during interval work may offer insight into the time course of ischemic injury risk.
References


Summary

In the present study we were interested in the contributing factors to Acute Kidney Injury. First, we reviewed the literature on AKI and relevant risk factors including hyperthermia, muscle damage, dehydration, and work intensity and duration as they pertain to athletes and occupational workers. We reviewed the available studies that investigated factors that may cause acute kidney injury in individuals that perform work in the heat. Additionally, we discussed potential reasons why endurance athletes may not be at similar risk for development of CKD despite often presenting with AKI after physical activity. This work constituted Chapter 2 titled “Factors of Physical Activity Contributing to Acute Kidney Injury in Endurance Athletes and Occupational Workers”.

To test the hypothesis that work intensity is a contributing factor to acute kidney disease, we performed an experimental study investigating the effects of two hours of work in a hot environment, completed either as HIIW or MICW, on markers of acute kidney injury. The results suggest that both styles of work may result in an increase in kidney injury biomarkers, however HIIW may lead to a higher incidence rate of transient stage 1 AKI. This study is presented in Chapter 3 and entitled “The effect of interval and continuous exercise on markers of acute kidney injury during physical activity in a hot environment”.

Conclusions
The conclusions from our study on the effects of two hours of HIIW compared to MICW in a hot environment on markers of renal damage and function are:

- HIIW and MICW caused similar increases in uNGAL immediately post-work and uKIM-1 immediately post-work and 1hr post-work.

- HIIW caused greater mean decreases in kidney function as measured by increases in sCreatinine and decreases in eGFR compared to MICW over the course of two hours of work in the heat and 24 hours of recovery.

- HIIW caused a higher incidence rate of stage 1 AKI compared to MICW as calculated using Acute Kidney Injury Network criteria. Most incidences were transient in nature, disappearing by 1hr Post-Work.

- HIIW was more physiologically stressful than MICW as measured by a higher mean core temperature and higher mean heart rate over the course of two hours of work in the heat.

**Recommendations**

The current study did not note any differences in kidney damage markers between HIIW and MCIW, however there was a higher prevalence of stage 1 AKI after HIIW compared to MICW. Future research should work to verify these findings and include direct measurements of renal blood flow (RBF). The time course of RBF reduction with increased work intensity is not known and taking these measurements, particularly during physical activity on a treadmill, is difficult. Therefore, performing interval work on a cycle ergometer may be the preferred modality to determine this time course. The current study was designed to examine the incidence and severity of AKI after a single work bout, which is not reflective of the stress on the renal system that athletes and
occupational workers face on consecutive days. Additional research is needed to
determine whether kidney injury biomarkers and kidney function are affected by
consecutive days of work and in states of complete or partial rehydration.

Additionally, the work protocols used in the current study may not be
representative of the stress commonly experienced by occupational workers, as the
duration is shorter and the average intensity higher. The incorporation of accelerometers,
heart rate, and core temperature during work shifts in studies examining AKI in
occupational workers may give a better idea of whether there is a relationship between
work patterns and AKI in the workplace.

Additional biomarkers of acute kidney injury, such as insulin-like growth factor-
binidng protein 7 (IGFBP7) and tissue inhibitor of metalloproteinase 2 (TIMP-2) should
also be examined to better determine where the location of renal damage may be
occurring (Schlader et al., 2019). Other contributing factors such as muscle damage, as
measured by an increase in serum creatine kinase, should be incorporated to determine
whether one style of work has a greater capacity to cause muscle damage and subsequent
elevations in sCreatinine.

Due to the multitude of factors that contribute to the development of AKI,
research on the effect of individual variables during physical activity is a difficult task.
Understanding the role that work intensity has in the development of AKI is important in
helping limit the detrimental effects of working in the heat on occupational workers and
athletes. Incorporation of rest breaks and proper rehydration into work shifts has been
suggested to help mitigate potential AKI (Wegman et al., 2018). Additionally, workers
paid for piece work may have a higher incidence rate of AKI which may be due to them
choosing to work at a higher intensity and neglect taking rest breaks in order to accomplish more work during the work day (Moyce et al., 2017). Proper understanding of workload distribution and intensity on renal damage could help protect workers and encourage better work practices.
BIBLIOGRAPHY


Science in Sports and Exercise, 36(12), 2128–2134.

https://doi.org/10.1249/01.mss.0000147584.87788.0e


https://doi.org/10.1074/jbc.273.7.4135


https://doi.org/10.1152/ajprenal.00091.2013


APPENDICES

A. Informed Consent

B. Health History Questionnaire

C. COVID-19 Clearance Form

D. Data Collection Sheets
Appendix A

Informed Consent

Effect of exercise intensity on magnitude of acute kidney injury during physical activity in a hot environment

Consent to Participate in Research
3/4/2021

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 Jonathan Houck 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 the impact of interval exercise compared to moderate continuous exercise in the heat on acute kidney injury markers.

You are being asked to take part in this study because you are 18-45 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, 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. You will not be able to continue in the study if you are deemed very aerobically fit as measured by the treadmill test.

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, Interval or Moderate Continuous Intensity treadmill exercise (2 hours), Urine and Blood collection one-hour post-exercise.
- Visit 4: Urine and Blood Collection Draw, Interval or Moderate Continuous Intensity treadmill exercise (2 hours), Urine and Blood collection one-hour post-exercise.
- Visit 5: Blood Draw, Urine Collection.
- The exercise intensity condition of the treadmill exercise, Interval Exercise (IT) or Moderate Continuous Exercise (MOD) in Visit 2 and 4 will be decided via an online randomizer.
- You will be asked to come in two days in a row for Visits 2 and 3 and Visits 4 and 5. There will be at least 1 week between Visit 3 and Visit 4.
  - Visit 1 will take approximately 60 minutes.
  - Visit 2 and 4 will each take approximately 4 hours.
  - Visit 3 and 5 will each take approximately 30 minutes.
  - Total time estimate: 11 hours.
- Benefit:
  - Results of your cardiorespiratory fitness test and body composition.
- Major Risks:
  - Exercise in the heat: sweaty, hot, light-headed, fatigue and nauseous during or after the session.
  - 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 do not come to for Visit 1 or sign 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 come into the lab and the visit will be rescheduled.
Visit 1: Height, Weight, Body Composition, Cardiorespiratory Fitness (VO$_{2\text{max}}$) 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 2 hours, (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 and height. 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 will be on the right side of your body.
- Your VO$_{2\text{max}}$ refers to the maximum amount of oxygen you can utilize during maximal exercise which is generally considered the best indicator of cardiovascular fitness.
- Your VO$_{2\text{max}}$ will be measured using a maximal running exercise conducted on a treadmill. Initially you will warm up at your own pace for 5 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 a nose clip and mouthpiece with a breathing valve to collect expired gases. The treadmill speed or grade 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. 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 receiver. Also, every minute we will ask what your rating of perceived exertion using a 6-20 based scale. After the conclusion of the VO$_{2\text{max}}$ test you will be given a 10-minute rest. You will then be asked to put the mouthpiece and nose clip on again and the treadmill grade will be set to 6% (the incline used during the exercise trials). You will be asked to walk at different speeds on the treadmill as the research team members will attempt to identify the pace which would account for 80% of your previously measured VO$_{2\text{max}}$. The pace will be adjusted to one that will elicit 30% of your previously measured VO$_{2\text{max}}$ will be determined. These speeds will be used to set the workload for the IT and MOD exercise sessions.

Visit 2: Urine Collection, Blood Draw, Interval or Moderate Intensity Treadmill Running

- For visit 2, you will return to the Exercise Physiology Laboratory after: (a) at least a 2-hour fast, (b) avoid having caffeine for 12 hours, (c) avoid performing any exercise for 24 hours, (d) avoid consuming alcohol 24 hours prior to the visit. 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. You will be asked to void your bladder exactly one hour before arriving at the Laboratory.
- Urine Collection: You will collect a small sample of urine into a sterile specimen cup by yourself in a restroom immediately upon arrival at the laboratory, one-hour after you voided your bladder prior to arrival. If the urine test shows you are dehydrated, you will be provided with 500mL of water to drink until you are hydrated and asked to wait for one hour before being reassessed. Nude body weight will be self-measured in a private room.
• 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).

• Treadmill Exercise in a Hot 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 be given gloves and lubricant to ease insertion. 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 of treadmill exercise followed by a 10-minute seated break. After the 10-minute break, you will perform a second bout of treadmill exercise identical to the first hour. You will be provided with 200ml (6.7 fl oz) of water every 20 minutes during exercise. Additionally, during the 10-minute break you will be asked to take a nude body weight. This weight will be compared to your pre-exercise weight and you will be provided with 75% of the weight difference in water. The exercise and rest will take place in a hot environment (i.e., 40°C (104°F), 30% relative humidity) environment. The exercise intensity condition (i.e. IT or MOD) will be determined by an online randomizer. The treadmill speed during IT will be set to the velocity which elicited 80% of VO$_{2\text{max}}$ for two minutes and 30% of VO$_{2\text{max}}$ for three minutes, until one hour of exercise has been completed. The treadmill speed for MOD will be calculated to ensure an identical amount of work (i.e. distance covered) is performed compared to IT. During the exercise, you will be asked to report your rating of perceived exertion and thermal sensation every five minutes. Immediately after exercise, you will sit down in a cool environment for 10 minutes before a blood draw (10ml; 2 teaspoons). In a private room, you will self-collect all your urine into a sterile specimen cup, remove the rectal probe and measure your nude body weight again. You will then be provided water equal to 75% of sweat loss during the exercise trial. One hour after the post-exercise urine sample is collected, you will be asked to provide another urine sample, this will be used to estimate kidney filtration rate.

Visit 3: Blood draw and Urine collection
• Twenty-four hours after the completion of exercise session in Visit 2, you will report to the lab for a blood draw and urine collection following the same procedures described above. You will be asked to void your bladder exactly one hour before arriving at the laboratory. When you arrive, after one hour from the time you last voided your bladder, you will be asked to void your bladder again so that a one-hour urine flow rate may be collected.

Visit 4: Urine Collection, Blood Draw, Interval or Moderate Intensity Treadmill Running
• In the day before Visit 4, you will use the food diary you made for Visit 2 to eat exactly the same foods and beverages before Visit 4.
• This visit is at least one week apart from Visit 2. Everything will be repeated from Visit 2, except for the exercise intensity condition of treadmill exercise, which will be the opposite from what you had in Visit 2. For example, if you did the Interval exercise (IT) exercise during Visit 2, you will perform the Moderate Intensity (MOD) exercise during this visit and vice versa.

Visit 5: Blood draw and Urine Collection
• 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 the 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 ≥39.5°C (103.1 degrees Fahrenheit), exercise will be terminated, and the participant will be removed from the heat chamber. If this happens, cooling procedures will be implemented; the participants will be removed from the heat chamber and seated or reclined in front of a fan, given wet towels, and given a refrigerated bottle of water to drink. 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 using 75% ethyl alcohol. A research team member will further disinfectant the rectal probe using a disinfectant solution (Cidex glutaraldehyde, Johnson & Johnson). You will reuse your own probe for both exercise trials unless it is damaged or you request a new one.

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 VO$_{2\text{max}}$ test. Knowledge of VO$_{2\text{max}}$ is of benefit in that they are indicative of aerobic fitness which can be helpful in directing and developing an individual exercise program. We will also provide you with your body composition results. These results may be beneficial to understand your health classifications or risk factors based on body fat percentage. 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:** There is no financial compensation for participating in the research study.

**Future use of biospecimens:** Your biospecimens (serum, plasma, and urine) will be labeled only with your subject number. The biospecimens will be used to analyze markers of acute kidney injury and will be stored in the laboratory -80°C freezer until analysis is complete. After analysis is completed any remaining sample will be destroyed. No genome sequencing or commercial usage of the samples will occur.

**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 39.5 °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
HEALTH & PHYSICAL ACTIVITY QUESTIONNAIRE

Family history questions are included because certain conditions of your first degree relatives (mother, father, brothers, and sisters) can incur risk to you during maximal exercise.

Participant #_____________________________ Date___/___/___

Phone (H or cell)___________________

Age____ Sex____ Ethnicity_______

Emergency contact (First name only, phone #)_________________________________________

MEDICAL HISTORY

Physical injuries (muscle, joint, other):_______________________________________________________

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

- Arrhythmias/Palpitations
- Heart murmur
- Shortness of breath
- Congestive heart failure

- 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. If yes, list reason(s)

________________________________________________________________________

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 to a sauna or engage in 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
Minutes/session (circle one):  30-60 min  60-90 min  90-120 min  120-150 min  >150 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  6-15 yr  >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)
Appendix C

COVID-19 Clearance Questionnaire

COVID-19 Symptom Screening Checklist

This checklist has been developed by Safety & Risk Services (SRS) with the guidance from the State of New Mexico and approval by the Office of the Vice President of Research. This document should be filled out every day before a research personnel and participant report to the lab.

Name/Participant #: _____________________________  Date:

<table>
<thead>
<tr>
<th>In the last 14 days have you had any of the following:</th>
<th>Yes</th>
<th>No</th>
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<td>Cough</td>
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<td>Fever</td>
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<td>Muscle Pain</td>
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<td>Sore Throat</td>
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<td>New Loss of Taste or Smell</td>
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<td>Nausea, Vomiting, or Diarrhea</td>
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<td>Close contact with individuals diagnosed with COVID-19</td>
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If you answered yes to any of the above

1. Do not come to the lab
2. Go home and self-isolate
3. Please contact the principal investigator Dr. Fabiano Amorim (amorim@unm.edu)
4. Please contact the New Mexico Department of Health for testing by calling 855-600-3453 or visiting https://cv.nmhealth.org/
5. Report your symptoms or diagnosis to UNM here: http://www.unm.edu/coronavirus/
Appendix D

Data Collection Sheets

**VO₂max Data Sheet**

Circle or enter data:

Consent Form __________ (check)

No exercise for 24 hrs. Y N

No caffeine for 12 hrs.? Y N

No alcohol for 24 hrs.? Y N

Pregnancy test negative? Y N/A

No eating for 2 hrs.? Y N

Height _____________ cm

Weight _____________ kg

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<th><strong>Skinfold</strong></th>
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11-breath average VO₂max ___________
Target VO₂ 30% VO₂ max __________ / Speed _______ mph

Target VO₂ for 80% VO₂ max __________ / Speed _______ mph
24-hour Food Log

Participant #:  
Date:  

<table>
<thead>
<tr>
<th>Time</th>
<th>Food/Beverage</th>
<th>Serving Size/Amount</th>
<th>Additional Comments</th>
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</thead>
<tbody>
<tr>
<td>Example: 8am</td>
<td>Oatmeal</td>
<td>1.5 cups</td>
<td>Added cinnamon</td>
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Data Collection: AKI and Exercise Intensity

Participant number________________ Date________________
Technician(s)____________________________

Trial day: IT MOD

Circle or enter data:

Chamber turned on _________ (check)
Consent Form __________ (check)
No exercise for 24 hrs. Y N
No caffeine for 12 hrs.? Y N
No alcohol for 24 hrs.? Y N N/A
Pregnancy test negative? Y N N/A
No eating for 2 hrs.? Y N
Thermistor ___________ (check) Heart Rate Monitor __________ (check)
Height _____________ cm
USG ____________ Pre-Exercise Nude weight ____________

**Pre-Exercise Blood Draw:** _______ plasma x1; serum x1; hep x1
Hematocrit: _______ / _______ / _______ Hemoglobin _______ / _______ / _______

Urine Volume pre: _________ ml Time since last void: _________ minutes Urine osm
______/______/______

**Exercise Bout** __________________________ (Speeds)

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<tr>
<th></th>
<th>HR (bpm)</th>
<th>Body Temp (°C)</th>
<th>RPE (6-20)</th>
<th>Thermal (0-8)</th>
<th>WBT (°C)</th>
<th>Dry Bulb (°C)</th>
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**Post-exercise Blood Draw:** ___________ plasma x1  serum x1 hep x1

Post-Exercise Urine Output _________________ ml  Time since last void: ___________ minutes

Post-Exercise Nude weight ____________kg  Total Weight Loss ___________kg  Water provided: ___________ ml

Hematocrit: _______ / _______ / _______  Hemoglobin _______ / _______ / _______

Urine osm _______ / _______ / _______

**1-hour wait**

Post-1hr Urine Output _________________ ml  Time since last void: ___________ minutes

Post-1hr Blood Draw ______________ plasma x1  serum x1 hep x1

Hematocrit: _______ / _______ / _______  Hemoglobin _______ / _______ / _______

Urine osm _______ / _______ / _______

**24-hour follow-up**
Urine Volume: ___________ ml Time since last void: _________ minutes

Hematocrit: _______/ _______/ _______ Hemoglobin _______/ _______/ _______

Urine_{osm} _______/ _______/ _______

Post 24-hour urine output _______________ plasma x1 / serum x1